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001

Iron chelators inhibit VCAM-1 induction in dermal endothelial cells by acting as hypoxia mimetics

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Previous studies have demonstrated profound inhibitory effects of iron chelators on NF-kB-dependent, cytokine-mediated induction of cell adhesion molecules (CAMs) in human dermal endothelial cells (HDMEC). We hypothesized that iron chelators inhibit induction of CAMs such as vascular cell adhesion molecule-1 (VCAM-1) via induction of hypoxia inducible factors (HIF)- 1α and/or 2α, thus acting as hypoxia mimetics. Treatment of HDMEC with the iron chelator 2,2-dipyridyl resulted in inhibition of VCAM-1 induction by TNFα. DP treatment resulted in concomitant robust induction and stabilization of HIF-2α protein expression as detected by western blot, consistent with the required presence of intracellular iron for the constitutive function of the HIF-2α destabilizing enzyme prolyl hydroxylase (PHD) in HDMEC. Since PHD activity requires three cofactors-iron, α-ketoglutarate (α-KG), and oxygen-to mediate HIF-2α degradation, we predicted that α-KG antagonists, like iron chelators, should also induce HIF-2α protein expression and inhibit TNFαmediated induction of VCAM-1 expression. Treatment of HDMEC with dimethyloxalylglycine (DMOG), an α-KG antagonist and PHD inhibitor, induced HIF-2α expression. In addition, pretreatment of HDMEC with DMOG inhibited TNFα-mediated induction of VCAM-1 protein and mRNA in HDMEC in a time- and concentration-dependent fashion. Similarly, pretreatment of HDMEC with hypoxia prior to TNFα stimulation inhibited TNFα induction of VCAM-1, but inhibition required continuous hypoxia during cytokine stimulation, which is also required for the continuous stabilization of the labile oxygen sensitive factor HIF-2α. These data provide novel evidence that that hypoxia- induced regulatory protein HIF-2α may inhibit proinflammatory responses by inhibition of NF-kB dependent gene expression. Thus, although stabilization of HIF proteins may stimulate proinflammatory responses through VEGF expression, they may also provide a negative feedback loop through inhibition of expression of NF-kB dependent genes such as VCAM-1.

002

The chemokine expression of human dermal microvascular endothelial cells and umbilical vein endothelial cells by tumor necrosis factor-alpha and interferon-gamma

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Activation of endothelial cells (ECs) plays a critical role in the pathogenesis of inflammatory skin diseases, such as psoriasis. Adhesion molecules and cytokines are induced and promote binding to activated leukocytes and homing into the inflammatory sites from the circulation. However, little is known about the skin site-specific recruitment of leukocytes. The objectives were to characterize alterations of chemokine regulation by cytokines at the gene expression level in human dermal micro- versus macrovascular ECs. Human dermal blood vessel endothelial cells (BEC) were isolated from neonatal human foreskins and purified using anti-PECAM-1 affinity beads and followed by negative selection for a lymphatic specific marker. Human umbilical vein endothelial cells (HUVEC) were used as macrovascular endothelial cells. BEC and HUVEC were stimulated with tumor necrosis factor-alpha (TNF-α) and/or interferon-gamma (IFN-γ) for 4 hours and analyzed with DNA microarray comprised of 54,000 probe sets including 38,500 known genes. Unstimulated BEC expressed microvascular markers, such as C17. About 9%, 6% and 15% of probe sets underwent statistically significant change with treatment by TNF-α, IFN-γ and both, respectively. Upon stimulation with TNF-α and/or IFN-γ, the chemokine gene expression of BEC was quite similar to that of HUVEC. CCL20, IL8 and GRO3 were up-regulated by TNF-α, but not IFN-γ. MIG was induced by IFN-γ, but not TNF-α. The combination with TNF-α and IFN-γ induced strong RANTES and MCP-2 expression indicating a synergy. Some gene up-regulation was slightly different between BEC and HUVEC. GCP-2 was more up-regulated by TNF-α in HUVEC than in BEC. MCP-2 and Fractalkine expression levels induced by IFN-γ were higher in BEC than in HUVEC. Our data indicate that chemokine gene up-regulation by activated dermal microvascular ECs is quite similar to HUVEC, but dermal microvascular ECs have different baseline gene expression and responses to IFN-γ.

003

Simultaneous inhibition of VEGFR1 and VEGFR2 signaling is required for suppression of experimental melanoma metastases

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Formation of blood-borne metastases depends on a series of events, including survival and initial proliferation of cancer cells after extravasation from the capillary bed of secondary organs. Accumulating evidence suggests that the host microenvironment as critical determinant of the proliferative behavior of tumor cells is affected by bone marrow-derived hematopoietic and endothelial stem/progenitor cells. In addition, incorporation of stem/progenitor cells may facilitate angiogenesis-dependent tumor growth. We herein show that experimental lung metastasis formation (B16 melanoma cells) induces proliferation and mobilization of VEGF receptor (VEGFR)-1+ hematopoietic and VEGFR-2+ endothelial progenitor cells. In transplantation studies, in which mice were reconstituted with bone marrow from LacZ-overexpressing animals to trace bone marrow-derived cells in experimental lung metastasis, the majority of incorporated bone marrow-derived cells displayed a monomyelocytic phenotype. In addition, lacZ-positivity was frequently detected in tumor-associated fibrin clots and thrombi, also suggesting mobilization of megakaryocytic cells and platelets from the bone marrow. In approaches to determine the potential significance of VEGFR-1- and VEGFR-2-dependent signaling in blood-borne metastasis formation, the impact of anti-VEGFR antibodies on experimental lung melanoma metastasis was analyzed. These studies showed that both VEGFR-2 and VEGFR-1 signaling were functionally important for hematogenous metastasis, as only simultaneous antibody-mediated inhibition of VEGFR-1 and VEGFR-2 signaling suppressed lung melanoma metastasis formation. Hence, both VEGFR-1 and VEGFR-2 signaling appear to be critically involved in blood-borne melanoma lung metastasis.

004

IL-6-induced basic fibroblast growth factor 2 dependent angiogenesis in basal cell carcinoma cells (BCC) via STAT3 and PI3-Kinase/Akt Pathway

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We have previously demonstrated the xenograft of IL-6 over-expressing basal cell carcinoma cells (BCC/IL-6) into nude mice developed tumor with high vasculature. Here we asked whether IL-6 could induce angiogenic activity in BCC cells by using in vivo chorioallantoic membrane (CAM) and Matrigel plug assay. Conditioned medium (CM) from IL-6-overexpressing BCC cells (BCC/IL-6 and BCC/IL-6/8D) exhibited much stronger angiogenic activities in either CAM or Matrigel plug assay when compared to CM from vector control or parental BCC cells. The increase of bFGF-2 mRNA and secreted bFGF-2 protein in IL-6-overexpressing cells and in BCC cells treated with recombinant IL-6 suggesting that IL-6 can up-regulate bFGF-2 expression in BCC cells. Using anti-bFGF antibody to block bFGF-2 function significantly inhibited CM-induced HUVEC tube formation as well as Matrigel plug formation. To dissect the mechanism by which IL-6 up-regulates bFGF-2 in BCC cells, we using various pharmacological inhibitors to examine the signaling pathways involved. The experimental results show that AG490 (JAK inhibitor) and LY294002 (PI3-kinase inhibitor) inhibit IL-6-mediated transcriptional up-regulation of bFGF-2 mRNA and secretion of bFGF-2 protein. Meanwhile, blocking STAT3 pathway with dominant negative mutants, STAT3F and STAT3D, all effectively abolished IL-6-mediated expression of bFGF-2 mRNA and secreted protein. Similar inhibition on IL-6-induced up-regulation of bFGF-2 was observed when BCC cells were transfected with dominant negative Akt (dnAkt). Together, our data suggest that bFGF-2 is a functional downstream effector of IL-6-induced angiogenesis in BCC cells. The IL-6-mediated bFGF-2 up-regulation is through activation of STAT3 and PI3-Kinase/Akt pathways.

005

Overexpression of vascular endothelian growth factor receptors in epidermis of psoriasis vulgaris

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To study the expression of vascular endothelial growth factor receptors (FLT-1 and KDR) in psoriatic lesions.Tissue sections from 28 psoriasis patients were examined by immunohistochemical and in situ hybridization methods for the expression of the two VEGF receptors. Microvessal density (MVD) was counted through immunohistochemical staining by anti-CD34 antibody monoclonal antibody.

Two receptors are expressed on papillary dermal endothelial cells and their expression is positively correlated with the MVD in lesional skin. The hyperplastic epidermis expresses high amounts of the receptors, which is far more abundant than those on the dermal endothelial cells. The receptor distribution pattern on epidermis of psoriatic lesion is quite different from that of normal skin, although there was no significant difference between the total expression level of the receptors in psoriatic epidermis and that of the normal controls. It is also found that KDR/FLT-1 was expressed casually on inflammatory cells, fibroblasts, sebaceous glands and hair follicles.

The up-regulation expression of FLT-1/KDR on the lesional papillary dermal microvessels may play an important role in pathogenesis of psoriasis. For the first time we have demonstrated that KDR/FLT-1 is strongly expressed on human keratinocytes by an autocrine mechanism. The abnormal distribution of VEGFR on keratinocytes could be related to the disturbance of inflammatory cascade on psoriatic lesions. The mechanism concerning KDR/FLT-1 expression on keratinocytes, inflammatory cells, fibroblasts, sebaceous glands and hair follicles are still unknown, further explorations are needed.

006

Topical application of *ex vivo* expanded endothelial progenitor cells promotes wound healing in diabetic mice

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Impaired wound healing, leading to skin ulceration, is one of the most serious complications of diabetes. Defective angiogenesis is considered to be a major contributing factor. Endothelial progenitor cells (EPCs) have been shown to augment neovascularization in ischemic tissue. Accordingly, we performed experiments to test the hypothesis that locally administered EPCs can promote wound healing in diabetes. In genetically diabetic *db /db* mice, full-thickness skin wounds (0.8 cm) were created on the dorsum using a skin biopsy punch tool. EPCs were obtained from bone marrow mononuclear cells after 7 days culture. $5X10^4$ EPCs were directly applied to the wound immediately after surgery. For EPC tracking, cells were marked with red fluorescent carbocyanine DiI-acetylated LDL. PBS and non-selective bone marrow mononuclear cells were used as control. Wounds were covered with an occlusive dressing. Wound size was measured at day 5, 10, or 14 after the treatment followed by resection, histological analysis, and quantification of vascularity. Topical application of *ex vivo* expanded EPCs significantly promoted wound healing as assessed by closure rate and wound vascurality. Immunostaining and Western blot analyses revealed that transplanted EPCs resulted in increased local expression of vascular endothelial growth factor and basic fibroblast growth factor in the wounds. Few DiI labeled EPCs were observed in the neovasculature, assessed by *in vivo* staining of the functional vasculature with FITC-labeled Bandeiraea simplicifolia lectin I. *Ex vivo* expanded EPCs promote wound healing in diabetic mice via mechanisms involving increased local cytokine expression and enhanced neovascularization of the wound. This strategy, exploiting the therapeutic capabilities of autologously derived EPCs represents a novel approach to diabetic skin repair.

007

Ultrastructural evidence of dermal microvascular alterations in an animal model of atopic dermatitis

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We previously established an animal model of atopic dermatitis, a chronic inflammatory skin disease, by transgenic expression of IL-4 to the epidermis. To elucidate the microvascular changes associated with this inflammatory disease, transmission electron microscopy (TEM) was performed on ear dermal blood vessels in four conditions: non-transgenic (non-Tg) mice, Tg mice before onset (BO), acute (AL) and chronic disease (CL) states (n= 5 each). The number of vessels examined in these conditions ranges from 13 to 27 (capillaries) and from 7 to 23 (post-capillary venules (PCV)). Compared to non-Tg mice, the microvasculature of Tg mice BO showed no statistically significant changes, except for an increase in the number of endothelial junctional clefts (EJC) in PCV ($p<0.0001$). The most significant changes were documented in the PCV of the AL and the newly formed capillaries of the CL. In particular, TEM features that are characteristic of leaky microvasculature were found in the AL, with some reaching statistical significance when compared to non-Tg mice: red blood cell congestion, disruption of EJC, presence of fenestrations, and increases in number of parajunctional complexes of plasmalemmal vesicles, convoluted clefts ($p<0.0001$), and length of EJC ($p=0.0017$). In the CL, TEM features characteristic of angiogenesis were revealed, with some reaching statistical significance when compared with non-Tg mice: sprouting, transcapillary pillars of intussusception, presence of hypertrophic nuclei (none of these present in capillaries of non-Tg mice), increases of rough endoplasmic reticulum, ribosomes, endothelial cell thickness, number of EJC ($p=0.0045$), and decrease of the length ratio of inter-endothelial tight junction to EJC ($p<0.0001$). Our data provided evidence of dermal microvascular leakage and angiogenesis in this animal model of AD. We suggest that microvascular leakage may play a role in the pathogenesis of the cutaneous migration of inflammatory cells and that this inflammatory disease is associated with angiogenesis.

008

Topical pimecrolimus transiently induces neuropeptide release and mast cell degranulation in murine skin

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Pimecrolimus (SDZ ASM 981) has been demonstrated to be effective and safe for the treatment of AD. The only side effect reported is an initial transient burning. In order to understand the underlying mechanism of this effect, healthy Balb/c mice were treated on the shaved back twice daily with 1% pimecrolimus cream for 2, 4 and 8 days, respectively. Untreated or vehicle-treated mice served as controls. Skin specimens were investigated by light, immunofluorescence and electron microscopy. Following pimecrolimus application, mast cells were found to be degranulated following 8 days after pimecrolimus application. Immunofluorescence revealed a reduced staining for SP and CGRP in papillary nerve fibers. At the ultrastructural level, nerve fibers were found to be regular without signs of degeneration or inflammation. In addition, pimecrolimus cream (1%) alone, cream vehicle, and the topical irritant benzalkonium chloride followed by pimecrolimus tretment after 6 hours were applied on right mouse ears. Mouse ears were harvested for ELISA (SP, CGRP) after 30 min. Expression of neurokinin receptors were quantified from mouse ears 2-8 h after treatment by quantitative RT PCR. Pimecrolimus cream induces enhanced release of SP (275 % +/- 41 SEM) and CGRP (114 % +/- 56 SEM) in the early phase after pimecrolimus treatment. Protein concentrations for SP were slightly enhanced in the contact dermatitis model indicating higher release of SP in inflammatory skin as compared to controls. In contrast, no statistically significant changes on neurokinin receptor expression were observed after treatment at all time points investigated. Thus, transient early side effects of pimecrolimus such as burning and itching may be due to mast cell degranulation leading to release of neuropeptides from sensory nerve fibers. Whether this is a direct or indirect effect and whether long treatment with pimecrolimus results in a decreased release of cutaneous neuropeptides is currently under investigation.

009

VEGF-A induced psoriatic skin inflammation and the associated lymphangiogenic response are ameliorated by systemic blockage of VEGF receptor-1 and -2

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Previous studies have shown that the expression of VEGF-A and its receptors is elevated in patients with chronic inflammatory diseases including psoriasis. To directly investigate the biological role of VEGF in inflammation, we induced experimental delayed-type hypersensitivity reactions in the ear skin of mice that overexpress VEGF-A in the epidermis and in wild-type controls. Whereas in control mice, inflammation returned to baseline levels within one week, in VEGF-A overexpressing mice inflammation persisted for more than one month and displayed hallmarks of human psoriasis including epidermal hyperplasia and accumulation of dermal CD4-positive and epidermal CD8-positive lymphocytes. Double immunofluorescence stains for the lymphatic specific marker podoplanin and the proliferation associated antigen Ki67 revealed active lymphatic endothelial cell proliferation and persistently enlarged lymphatic vessels exclusively in the chronic inflamed skin of VEGF-A overexpressing mice. In striking similarity, skin biopsies obtained from human psoriatic lesions showed highly enlarged lymphatic vessels whereas in adjacent, clinically non-involved skin, lymphatic vessels were collapsed. Inhibition of VEGF-A signaling through systemic application of blocking antibodies against VEGFR-1 and VEGFR-2 significantly reduced experimental skin inflammation and lymphatic enlargement. Our findings identify VEGF-A as a novel target for the treatment of inflammatory skin diseases.

010

Mouse epidermal progenitor cells have unique proangiogenic properties compared to differentiated cells

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We have previously shown that mouse epidermal progenitor cells (EPC) accelerate the restoration of blood flow in the ischemic limbs of diabetic mice and that some of them incorporate into the vasculature. However the transient amplifying (TA) cells (daughters of the EPC) did not have a significant effect on the regeneration of the vasculature. These findings suggest that EPC may secrete proangiogenic factors at a greater level than the TA cells. In order to test our hypothesis we performed migration assays using the membrane invasion culture system (MICS). Conditioned medium from EPC, TA cells and control medium were put in the lower compartment of the MICS then covered by a 10um polycarbonate membrane. Above the membrane human umbilical vein endothelial cells (HUVEC) were added. After six hours media from the bottom of the well was collected and the number of HUVEC cells that migrated across the membrane to the bottom wells were counted. Data showed that the percent invasion of HUVEC cells in the EPC conditioned medium was 3 to 20 times greater than TA or the control medium. To determine the nature of these factors, we evaluated the level of vascular endothelial growth factor (VEGF) in the different media. EPC-conditioned medium contained 4 to 6 times more VEGF compared to TA-conditioned medium. Results from these experiments indicate that EPC secrete medium with greater chemoattractive properties for HUVEC cells than the TA- and control-conditioned medium, and that some of this property can be attributed to a higher level of VEGF. This suggests that within the epidermis, the chemoattractive property and VEGF production are dominated by EPC. Thus, EPC may be proangiogenic, a unique characteristic that could be used to treat ischemia.

011

SOCS 1 is a negative regulator of the MyD88-independent signaling pathway of the LPS-induced TLR4 natural immune response in HDMEC

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Recently, several groups have reported that LPS causes endotoxin shock via toll-like receptor (TLR)4, a central part of the natural immune response. However, these studies examined lymphocytes, macrophages, and dendritic cells. Human dermal microvascular endothelial cells (HDMEC) are also damaged in endotoxin shock. Nevertheless, LPS-induced TLR4 activation in HDMEC has not been examined. First, we confirmed the expression of TLR4 in HDMEC using RT-PCR. In general, LPS-induced TLR4 stimulation results in the activation of two intracellular signaling pathways: the MyD88-dependent and -independent pathways. The typical downstream molecule of the MyD88-dependent pathway is IκBα, while that of the MyD88-independent pathway is STAT1. Therefore, we studied whether IκBα and SATA1 are phosphorylated in HDMEC after LPS stimulation. LPS induced the phosphorylation ofIκBα, with a maximum at 1 h. LPS induced the phosphorylation of STAT1 optimally at 3 h. The SOCS family is implicated in the negative regulation of the STAT signaling pathway. It is of interest to determine whether the SOCS family regulates the LPS-induced MyD88-independent signaling pathway. To investigate the expression of the SOCS family, we performed real-time PCR. SOCS1 is induced by LPS stimulation in HDMEC. Western blot analysis confirmed the increase in SOCS1 by LPS. Next, the role of SOCS1 in the LPS-induced HDMEC reaction was studied using an adenovirus vector expressing wild-type SOCS1 (Adex-SOCS1). Transfection of Adex-SOCS1 abolished the LPS-induced phosphorylation of STAT1, while the phosphorylation of IκBα was not inhibited. Since IL-6 production in LPS-stimulated HDMEC is reported to be MyD88-independent, the effect of SOCS1 on LPS-induced IL-6 production in HDMEC was examined. Transfection of Adex-SOCS1 reduced LPS-induced IL-6 production in HDMEC markedly. Combined, these data demonstrate that SOCS1 is a negative regulator of the MyD88-independent signaling pathway of the LPS-induced TLR4 natural immune response in HDMEC.

012

The curative effect of histamine on cutaneous wound healing process

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During cutaneous repair, a new tissue formation starts with reepithelialization and is followed by a granulation tissue formation, including neutrophils and macrophage accumulation, fibroblast proliferation, matrix deposition, and angiogenesis. We analyzed roles of histamine for skin wound healing using our histidine decarboxylase gene-knockout mice (HDC-KO (-/-) mice), which were deficient in histamine production. Our study disclosed that the impaired histamine production in HDC (-/-) mice resulted in the delayed wound healing and that exogenous histamine compensation normalized healing process. While keratinocyte migration in HDC (-/-) mice was similar to that in HDC (/) mice, macrophage recruitment and angiogenesis at the wound edge were markedly impaired in HDC (-/-) mice, suggesting insufficiency in macrophages and endothelial cells in the HDC-KO mice. In a few days after skin wounding, the amount of bFGF was higher in HDC (/) mice than that detected in HDC (-/-) mice. Single topical administration of SU5402, an inhibitor of bFGF, reduced new vessel formation at the wound edge and delayed wound closure of HDC (-/-) mice. From the present analysis, deficient angiogenesis in HDC-KO mice caused the delay in the wound closure probably due to the reduction in bFGF production and macrophage accumulation. We can conclude that histamine is an accelerator in cutaneous repair.

013

Functional analysis of human vascular endothelial growth factor using a transgenic approach: the possible implication in establishing psoriasis model mouse

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Vascular endothelial growth factor (VEGF) has a crucial role in angiogenesis and leukocyte recruitment to the sites of inflamed skin. A transgenic study using murine VEGF has recently demonstrated that increasing microvascular density and enhanced leukocyte rolling and adhesion in the skin; however, only a little knowledge for human counterpart are yet available. In this study, we first generated the transgenic mice carrying the keratin 14 promoter-human VEGF transgene, to investigate the skin biology associated with the interrelationship between epidermis and dermal epithelium. The VEGF transgenic mice showed mild swelling of the ear auricle and hyper vascularity in the skin. Histological examination revealed a marked increase of dermal blood vessels and infiltrates as compared to the wild type. The vessel walls were immunostained with VEGF, and its cell surface receptors. Moreover, skin injury resulted in histologically acanthosis, parakeratosis and rete ridge formation with lymphocytic infiltration, resembling psoriatic skin. These results suggest that transgenic mice for human VEGF may provide a new insight not only for underlying disease pathogenesis of psoriasis but also for a potential candidate as therapeutic targets in this common inflammatory skin disease.

014

Genetic control of cellular production of vascular endothelial growth factor in psoriasis

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Vascular endothelial growth factor (VEGF) promotes angiogenesis, is produced by epidermal keratinocytes (KCs) and PBMCs and elevated levels are found in plaques of psoriasis. We have shown significant associations between VEGF +405 CC and the C allele and severe, early onset psoriasis; and between +405 and VEGF production in healthy subjects. +405 is close to the functional AP-1 site (+419) through which retinoids may block production of VEGF. Retinoids are an established treatment for severe psoriasis. We used cell culture to investigate whether KC and PBMC production of VEGF in patients with psoriasis exhibit the same polymorphic control. 14 patients with early onset psoriasis (7 +405 CC and 7 +405 GG genotypes) were recruited. Freshly-isolated PBMCs and KCs (passage 1) were cultured with all-trans retinoic acid (RA), lipopolysaccharide (LPS) or phorbol ester (PMA). Experiments were performed in triplicate. VEGF protein was measured using ELISA. BMCs from patients with CC genotype demonstrated significantly (all p=0.04) increased VEGF production at baseline and after stimulation with LPS or RA as compared to patients with GG genotype. PBMC incubation with PMA resulted in no significant VEGF production for either genotype group. It was only possible to stimulate production of VEGF from KCs of the CC genotype (with PMA). In marked contrast to PBMCs, RA reduced VEGF production for both genotype groups (even after stimulation of VEGF production with PMA for the CC group; p=0.04). In summary, PBMCs and KCs from patients with chronic plaque psoriasis demonstrate significant genotype dependent production of VEGF; furthermore regulation of VEGF production is different for both cell types. This is the first analysis of differential cellular control of VEGF gene expression suggesting that the +405 polymorphism dictates VEGF expression in psoriasis and that retinoids may exert an antiangiogenic effect in psoriasis via modulation of VEGF production.

015

An animal model of atopic dermatitis: morphological, serological, and molecular biological evidence of angiogenesis in dermal microvasculature

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To investigate the dermal microvasculature underlying the inflammatory process of a keratin 14-IL-4-transgenic (Tg) mouse model of atopic dermatitis, morphological, serological, and molecular biological methods were employed on samples from 4 groups: non-Tg mice (NT), Tg mice before disease onset (BO), acute disease (AL), and chronic disease (CL). Immunofluorescence microscopy using anti-CD31 on ear samples followed by computer-assisted photometric analysis showed increases in the following parameters as disease progresses: percent area occupied by vessels (NT 11±4%, BO 12±3%, AL 20±3%, and CL 27±11%); number of vessels per mm skin length (NT 36±6.5, BO 44±10, AL 63±17, CL 72±31); diameter (μm) of vessels (NT 2.2±0.4, BO 5.9±2.2, AL 9.3±1.9, CL 6.9±2.1). Skin stained with anti-VEGFR2 showed increased vascular expression: percent area with expression (NT 1.6±0.4%, CL 6±4.2%); VEFGR2+ vessel number per mm skin length (NT 23±3.1, CL 79±29). ELISA analysis of serum VEGF demonstrates increases of protein concentration (pg/ml) as the disease progresses (NT 367±291, BO 459±457, AL 1096±771, CL 1430±892), correlated with increased VEGF protein in skin extract by Western blot. Reverse transcription real-time PCR was performed on skin extract to quantify known angiogenic factors: VEGF, VEGFR1, VEGFR2, CD31, Cadherin (Cad), Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2), Tie-1, and Tie-2. Increased mRNAs were detected in CL group when compared with NT group: VEGF (1.2 fold), VEGFR2 (3 fold), CD31 (5 fold), Cad (4 fold), Ang-1(4 fold), and Ang-2 (5 fold). Similarly VEGFR2 mRNA was increased by 5 fold in the AL group. Statistically significant differences ($p<0.02$ to $p<0.0001$) were found between CL and NT groups in the following parameters: area/number/diameter of vessels, endothelial VEGFR2 expression, serum VEGF concentration, and angiogenic factor mRNAs (VEGFR2, CD31, Cad, Ang-1, Ang-2). Together, these changes in diseased mice support the occurrence of angiogenesis and suggest that angiogenesis may play a role in the pathogenesis.

016

Skin specific chemokines and cell recruitment by skin microvascular endothelial cells

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As demonstrated (Kieda, 2002, Endothelium, 9, 247-61), the endothelium is highly selective organ, which reflects the biological state of the tissue and microenvironment. The cell recruitment and the molecular targeting strategies can take advantage of such a specificity to design organ directed therapies. The skin model which consists of cocultures of the main cellular partners interact in normal situation as well as injured or activated situations. In such conditions the critical role for reparation is played by endothelial cells which are able to recruit competent cells and necessary molecules in a microenvironment and specific receptor way. Chemokines are desicive molecules which could be presented in a restricted way to help make endothelial cells to be very strictly selective. Here, we demonstrate the restricted activity of two chemokines :fractalkine (neurotactine) and especially CTACK towards endothelial cells from skin as opposed to endothelial cells from other non related organs. Furthermore, other chemokines as 6Ckine which is lymph node selective, was not able to activate skin endothelial cells in functional assays as adhesion (flow cytometry) and circulating cell rolling.

017

Keratinocyte- and sebocyte-derived factors modify UVB activity on endothelial cells: a possible mechanism for the development of vascular changes in rosacea

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Rosacea, a chronic inflammatory skin disorder and vascular dilatation, a major clinical sign, have not been adequately explained. We have raised and investigated the hypothesis that soluble factors may mediate inflammation and vascular dilatation in rosacea. In this part of the study we evaluated the paracrine influence of epithelial skin cells and/or UVB irradiation on the release of the major angiogenic factor VEGF and of IL8 by dermal microvascular endothelial cells (HMEC-1). We demonstrated by ELISA that VEGF protein levels were upregulated and pro-inflammatory IL8 levels were down-regulated in HMEC-1 supernatants after irradiation with a physiological UVB dosis (7.5 mJ/cm^2). In contrast, secretion of both VEGF and IL8 by keratinocytes (human foreskin cells) and sebocytes (SZ95 cell line) was reduced under identical conditions. VEGF reduction was annulled by increasing sebocyte density. The relatively high levels of VEGF in sebocyte supernatants remained unchanged even under high UVB doses (30-90 mJ/cm^2). IL8 release was reduced after UVB irradiation not depended to the cell density but in proportion to UVB dosis. Paracrine regulation was adressed by maintaining HMEC-1 in keratinocyte or sebocyte supernatants and subsequent UVB irradiation. The UVB effect on VEGF release by HMEC-1 in sebocyte conditioned medium was negligible and independent of the irradiation dosis (7.5-50 mJ/cm^2), but IL8 secretion was inhibited by UVB at a dosis of 15 mJ/cm^2. Maintained in keratinocyte conditioned medium, HMEC-1 released 25% less VEGF under UVB. IL8 was strongly upregulated but could be reduced with increasing UVB dosis. These data let us assume that UVB may directly induce vascular dilatation, e.g. in rosacea, but sebocytes and possibly keratinocytes produce factors which have a protective role in the development of vascular dilatation in UV irradiated skin.

018

Processing of proopiomelanocortin peptides by dermal microvascular endothelial cell extracellular peptidases: implication for endothelial cell biologic functions and skin inflammation

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Dermal microvascular endothelial cells (EC) are both source and target of the proopiomelanocortin (POMC) peptides ACTH and α-melanocyte-stimulating hormone (α-MSH). The availability of neuropeptides as important modulators of innate and adaptive immune responses is controlled by neuropeptide-specific Zinc metalloproteases such as neutral endopeptidase (NEP) or angiotensin-converting enzyme (ACE). In this study, we have tested the possibility that NEP or ACE expressed by EC may influence the local bioavailability of POMC peptides. Incubation of $ACTH_{1-39}$ with cell membranes prepared from the high NEP/low ACE expressing microvascular EC line 1 (HMEC-1) or from low NEP/high ACE expressing primary human dermal EC (HDMEC) for 30 - 480 min resulted in a decrease in ACTH immunoreactivity (IR) over time in membrane supernatants that could be partially blocked with NEP inhibitors as detected by radioimmunoassay. In parallel, α-MSH IR temporarily increased peaking after 60 min. Functionally, incubation of EC membranes with ACTH altered its ability to induce interleukin 10 release from U937 monocytes. Fragments generated by incubation of HMEC-1 or HDMEC membranes with $ACTH_{1-39}$, $ACTH_{1-24}$ or α-MSH for 1 - 120 min were further analyzed by mass spectroscopy. $ACTH_{1-39}$ and $ACTH_{1-24}$, but not $ACTH_{18-39}$ was rapidly bound to HMEC-1 and HDMEC membranes. HMEC-1 membranes generated peptide products, which could be altered by inhibition of NEP, but not ACE. Likewise, HDMEC membranes fragmented ACTH similar to HMEC-1 membranes in the presence of NEP inhibitors. Some of the proteins can be assigned to regular proteolytic cleavage while others seem to be modified. Thus, these novel data suggest that EC proteolytic peptidases locally control the bioavailability of stress hormones and anti-inflammatory peptides such as ACTH or α-MSH to melanocortin receptors, which may be important in controlling cutaneous inflammation

019

Gene gun-mediated delivery of full-length cDNA encoding the human lymphatic endothelial cell selective marker, LYVE-1, to skin yields function-blocking mouse antibodies that recognize native structure for flow cytometry and immunohistochemistry

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LYVE-1 is a surface bound receptor for hyaluronic acid (HA) that is preferentially expressed by lymphatic (vs. blood vascular) endothelial cells. The biology of lymphatic endothelial cells is poorly understood relative to blood vascular endothelial cells, in part, because of the paucity of reagents that specifically recognize lymphatic endothelial cell proteins. Moreover, while several anti-LYVE-1-specific antibodies have been reported, none have been shown to recognize native structure or block function. To circumvent this problem, cDNA encoding the full-length sequence of human LYVE-1 was coated on gold particles that were subsequently delivered via a helium-driven, biolistic-propulsion system (gene gun) into the skin of 10 Balb/C mice every 3 weeks. After 3 immunizations, mouse sera were collected for analysis. Specificity of the antisera for LYVE-1 was demonstrated by positive staining of LYVE-1-transfected 293T cells compared with mock-transfected 293T controls by flow cytometry (FACS). In addition, 70-90% of heterologous, cultured human dermal microvascular endothelial cells (HDMEC) derived from neonatal foreskin showed LYVE-1 staining by FACS. Antisera from one mouse completely inhibited binding of FITC-HA to HDMEC at 20 deg. C. By immunohistochemistry, LYVE-1 antisera detected lymphatic channels in both normal and psoriatic skin sections. Lymphatic vessels were generally increased in psoriasis and were weakly CD31 positive compared to blood vascular endothelial cells. LYVE-1 antisera did not react with SDS-denatured LYVE-1 in Western blots. In summary, function-blocking antisera that recognizes native human LYVE-1 was obtained after repeated gene gun-mediated, cutaneous immunization of mice with full-length cDNA encoding LYVE-1. The LYVE-1 antisera may be useful for studies of lymphatic dysregulation in human skin disease and for studies of the potential biologic roles of LYVE-1 in immune cell trafficking.

020

Thrombospondin 1 modulates inflammation and vascular remodeling during experimental delayed-type hypersensitivity reactions

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Thrombospondin 1 (TSP-1) is a potent endogenous angiogenesis inhibitor that is thought to play an important role in maintaining cutaneous vascular quiescence. However, TSP-1 has been detected in inflammatory diseases such as arthritis and has been described as pro-angiogenic and pro-inflammatory in some experimental models. To elucidate the function of TSP-1 in a cutaneous inflammation, we studied the expression of TSP-1 in human allergic contact dermatitis (ACD) induced by patch testing. 72 hours after the onset of the inflammation, both TSP-1 protein and mRNA expression were potently upregulated in the inflamed lesions when compared to normal skin taken from the same patient. In-situ hybridization revealed that keratinocytes and endothelial cells were the main source of TSP-1 in ACD although some inflammatory cells were also positive. To functionally characterize the role of TSP-1 in inflammation, we induced delayed-type hypersensitivity reactions by topical application of oxazolone to the skin of mice with targeted epidermal TSP-1 overexpression, in TSP-1-deficient mice and in wildtype mice. We found decreased edema formation in the inflamed ears of TSP-1 transgenic mice associated with a significant decrease in the number of enlarged blood vessels when compared to wildtype littermates. Conversely, TSP-1-deficient mice exhibited a persistent reaction, characterized by a delayed resolution of the inflammation, when compared to wildtype mice. Our data indicate that TSP-1 suppressed vascular remodeling during an ACD and thereby decreased the extent of the experimental cutaneous inflammation, probably by downmodulating the effects of pro-angiogenic factors such as vascular endothelial growth factor A and by aiding in the resolution phase of the inflammation.

021

Vimentin intermediate filaments and focal contacts in endothelial cells

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In human dermal microvascular endothelial cells (HMVEC), matrix adhesion mediated by focal contacts (FCs) plays a crucial role in processes leading to vasculogenesis and angiogenesis. FCs, rich in $\alpha v\beta 3$ integrin heterodimers, typically interact with the actin cytoskeleton. Remarkably, vimentin intermediate filaments also associate with 50-60% of FCs in HMVECs. Moreover, when endothelial cells are subjected to shear stress (flowing medium at 12 dynes/cm2), large bundles of vimentin establish connection to FCs. Using vimentin "knock-down" RNA interference (RNAi) methodology, we have shown that vimentin regulates FC size and adhesive function in endothelial cells subjected to flow. Moreover, knock-down of vimentin expression in endothelial cells leads to a collapse of cytoplasmic staining of plectin, a member of the plakin family of cytolinker proteins. Plectin has been implicated in vimentin-FC association. Hence, we tested the possibility that vimentin-FC interaction is mediated by plectin by using a plectin RNAi strategy. Cells transfected with plectin siRNA were fixed and processed for immunofluorescence and cell extracts were prepared for immunoblotting. The latter reveals that cells expressing the plectin siRNA show a significant knock-down in plectin expression. The knock-down cells show no changes in their overall vimentin, microtubule and microfilament cytoskeleton networks. However, although FCs in cells deficient in plectin are smaller than those treated with a control siRNA, there is no obvious change in the percentage of FCs showing an association with vimentin. Furthermore, the plectin knock-down cells show no abnormalities with respect to control transfected cells in their adhesion to various substrates, spreading or motile behavior. When subjected to shear stress, plectin knock-down cells, unlike their vimentin knock-down counterparts, fail to show decreased substratum adhesion. In summary, the vimentin cytoskeleton plays a role in FC structure/function but its association with FCs is not dependent on plectin.

022

ICAM-1 as a basis for vascular leak induced by cytokine-mediated endothelial cell activation

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Cytokine-based immunotherapy for treatment of skin malignancy (e.g., melanoma or cutaneous T cell lymphoma) has proven efficacy but is limited by capillary leak syndrome, a form of vascular leak resulting in hypotension, tachycardia, and edema. Vascular leak may arise from damage to endothelial cells (EC) or from cytokine (e.g., TNF)-related EC activation. In our hands TNF treatment (24-72 h) of cultured human dermal microvascular EC causes cell elongation, actin reorganization from dense peripheral bands to longitudinal stress fibers and a decrease in monolayer electrical resistance. These changes in EC morphology and barrier function are accompanied by TNF-induced upregulation of E-selectin and ICAM-1 protein expression. Similar changes occur without any requirement for TNF treatment in EC transduced to express ICAM-1, but not in EC expressing E-selectin. To determine whether morphological changes induced by TNF are mediated by ICAM-1, we inhibited TNF-induced ICAM-1 expression by transfection with two different siRNA constructs. Remarkably, TNF-induced cell elongation and actin reorganization were each reduced proportionately with the extent of siRNA-mediated inhibition of ICAM-1 expression in comparison to a scrambled control siRNA. Based on nuclear morphology the reduction in TNF effects was not due to apoptosis. These observations suggest that ICAM-1 up-regulation followed by cytoskeletal reorganization may contribute to the type of vascular leak induced by cytokine-mediated endothelial cell activation.

023

Kaposi's sarcoma-associated herpesvirus (KSHV) infection of human dermal microvascular endothelial cells (HDMEC) causes increased cellular proliferation, decreased cellular adhesion, and marked up-regulation of angiogenic growth factors

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KSHV is an oncogenic γ-herpesvirus associated with all clinical forms of KS. Histologically, KS is characterized by proliferation of KSHV-infected tumor spindle cells of endothelial origin and formation of slit-like vascular spaces, both features of dysregulated angiogenesis. In order to gain further insight into how KSHV cause KS, we studied the biology and function of HDMEC following *in vitro* infection by KSHV. Placed in basal medium with low serum (2% FBS) and no supplemental growth factors, KSHV-infected HDMEC survived and proliferated significantly better than uninfected cells for 1 week following infection. Infected HDMEC (compared to uninfected cells) also demonstrated decreased adhesion to extracellular matrix proteins, including fibronectin, vitronectin, and collagen IV. Using real-time RT-PCR to detect and quantify mRNA, we detected up-regulation of numerous angiogenic growth factors and growth factor receptors in KSHV-infected HDMEC, including VEGF-B, PlGF-1, VEGFR-1, -2, -3, Ang-2, Tie-1, -2, and bFGF. VEGFR-1 and Ang-2 mRNA levels were increased most dramatically (>10-fold). In summary, we found that KSHV infection of HDMEC caused increased cellular proliferation, decreased cellular adhesion, and marked up-regulation of angiogenic growth factor mRNA expression. These results strongly suggest that KSHV directly causes dysregulated angiogenesis in KS tumors by causing infected cells to proliferate, survive well, and produce and respond to pro-angiogenic environmental signals.

024

Enhanced acute photosensitivity and chronic UVB-induced cutaneous photodamage in VEGF transgenic mice

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Acute ultraviolet B (UVB) irradiation of the skin results in erythema, vasodilation, enhanced vascular permability and edema formation. Previous studies have found that UVB irradiation promotes the expression of vascular endothelial growth factor (VEGF) by epidermal keratinocytes in vivo and in vitro. To directly investigate the biological importance of VEGF for the cutaneous UVB response, we subjected 8-week-old male VEGF overexpressing transgenic mice (n=5) and their wild-type littermates (n=5) to acute UVB irradiation. VEGF overexpressing mice were characterized by a two-fold lower minimal erythema dose (MED = 3.6x10-2 J/cm2), as compared with wild-type mice (MED = 7.2x10-2 J/cm2), after a single UVB exposure. Acute UVB irradiation with 3.6x10-2 J/cm2 induced proliferation of epidermal keratinocytes and endothelial cells, dermal edema, vascular dilation, and inflammatory cell infiltration in the skin of VEGF transgenic mice but not in wild-type mice. Systemic treatment with an anti-VEGF blocking antibody or with blocking antibodies against VEGF receptors-1 and -2 reduced the sensitivity of 8-week-old male wild-type mice to acute UVB irradiation. In additional studies, 8-week-old male mice were subjected to 10 weeks (3 times/week) of chronic UVB irradiation with 1.8x10-2 J/cm2. Whereas no major changes were observed in wild-type mice (n=10) subjected to this regimen, VEGF transgenic mice (n=10) showed formation of wrinkles after 10 weeks, associated with pronounced neo-vascularization and matrix degradation in the skin. Our results indicate that VEGF promotes cutaneous photo-damage and that the VEGF/VEGF receptor pathway might serve as a novel target for the prevention of photo-aging of the skin.

025

Lack of thrombospondin-1 (TSP-1) and thrombospondin-2 (TSP-2) dramatically enhances skin carcinogenesis and lymph node metastasis in double TSP-1/TSP-2-null mice

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It is thought that the angiogenic switch during tumorigenesis is induced by a change in the balance of proangiogenic and anti-angiogenic molecules. To investigate the individual and the combined role of the endogenous angiogenesis inhibitors TSP-1 and TSP-2 in skin cancer development and progression, we subjected TSP-1 (n=26), TSP-2 (n=33) and TSP-1/TSP-2 (n=25) deficient mice, as well as wild-type mice (n=25) to a two-step chemical skin carcinogenesis regimen. For initiation, all mice were treated once with 25 ug DMBA, applied to the dorsal skin, followed by 20 weekly topical applications of 5 ug of the tumor promoter PMA. Mice deficient in TSP-2 and mice deficient in TSP-1 and TSP-2 exhibited accelerated papilloma and squamous cell carcinoma (SCC) development, compared to wild-type mice. Surprisingly, TSP-1 deficiency had no major effect on either papilloma or SCC development. At 29 weeks after tumor initiation, TSP-1/TSP-2 double deficient mice exhibited a 3-fold increase and TSP-2 deficient mice exhibited 2-fold increase in the number of SSC/mouse, as compared to TSP-1 deficient and wild-type mice. TSP-1/TSP-2 deficient mice (75%) and TSP-2 deficient mice (78%) also developed significantly more clinically visible metastases to regional lymph nodes, compared to the wild-type mice (25%). Our results indicate that TSP-2 plays a more important protective role against tumor development than TSP-1 in multistep skin carcinogenesis.

026

***In vivo* perfusion of human skin equivalents with circulating endothelial progenitor cells**

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The performance of engineered skin substitutes is likely limited by inadequate perfusion in the post transplantation period. We have accelerated vascularization of human skin equivalents in vivo by incorporating endothelial cells (EC) differentiated from umbilical cord or adult peripheral blood derived endothelial progenitor cells (EPC). Circulating CD34+ EPC were isolated using MACS and cultured using selective media. After 14 days, the EPC were expanded and differentiated into EC as assessed by morphology and flow cytometric detection of CD31, VEGFR-2, Tie-2, and TNF-induced E-selectin expression. Skin equivalents were constructed by first seeding keratinocytes derived from neonatal foreskins onto the epidermal side of split thickness decellularized human cadaveric dermis and inducing stratification. EPC-derived EC were then seeded on the underside of the dermis and allowed to invade residual vessel channels. The resultant skin equivalents were orthotopically grafted onto the backs of SCID/beige mice. Grafts (4 seeded with cord blood- and 6 seeded with adult blood derived EPC) harvested 2 weeks after implantation demonstrated numerous dermal vascular profiles that contained erythrocytes and were reactive with anti-human CD31 antibodies and UEA-1 lectin, indicating these vessels were lined with human EC and inosculated with the mouse circulation. Anti-smooth muscleα-actin antibody reactivity with these vessels showed smooth muscle/pericyte investiture. Restriction of anti-murine CD31 reactivity to the edges of the grafts indicated an ineffective murine angiogenic response by 2 weeks. Human EC lined vessels were also observed to persist in 4 cord- and 2 adult blood derived EPC seeded grafts harvested at 4 weeks. These data demonstrate that circulating human EPC can be used to enhance early perfusion of human skin equivalents and describe a strategy for vascularizing engineered tissues with a source of EC autologous to the recipient that can be obtained non-invasively.

027

An *in vivo* model of venous malformation induced by Tie-2 activation in human endothelial cells

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Signaling through the Tie-2 receptor is known to play a critical role in human physiologic and pathologic vascular remodeling. However, the relative importance of specific signaling pathways downstream of Tie-2 (such as the PI-3 kinase-Akt pathway) are not fully understood. We have previously shown that human umbilical vein endothelial cells (HUVEC) retrovirally-transduced to overexpress Akt, suspended in collagen/fibronectin gels, and implanted subcutaneously in SCID/beige mice form a human EC-lined microvascular network of dilated, highly-branched vessels with disorganized smooth muscle/pericyte investment. In contrast, control-transduced EC produce a sparse bed of narrow vessels with little branching. Since these Akt-HUVEC-lined vessels resembled hereditary venous malformations induced by activating mutations in *Tie-2*, we sought to determine if Tie-2 activation could reproduce the Akt phenotype. To do this, we transduced primary HUVEC with retroviral constructs expressing either wild-type Tie-2 or Tie-2 R849W, an activating mutation present in some familial venous malformations. *In vitro*, the HUVEC overexpressing either wild-type or mutant Tie-2 contained increased levels of phospho-Akt after serum withdrawal, and were resistant to apoptosis induced by serum starvation or C6-ceramide. After 14-21 days *in vivo*, both Tie-2-wt-HUVEC and Tie-2 R849W-HUVEC gave rise to dense networks of dilated, highly-branched vascular structures with irregular investment by smooth muscle cells/pericytes. These vessels appeared similar to Akt-HUVEC, suggesting that signaling through Akt alone is sufficient to mediate the pathologic effects of Tie-2 over-activation. Therefore, we have generated a humanized *in vivo* model of venous malformations/vascular tumors, which should be useful in studying signaling through the angiopoietin-Tie-2 pathway. We are currently investigating the contribution of upstream (Ang-1/2) and downstream (eNOS) components of this pathway to these phenotypes.

028

Sequence analysis of immunoglobulin E isotype switch in scleroderma skin tissue

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The involvement of mast cell, which is activated by immunoglobulin E (IgE), has been reported in the formation of SSc abnormality. IgE is generated with isotype switch. During isotype switch, switch circles resulting from direct μ-ε or from sequential μ-γ-ε switching with be created, and thus the circles can be used to assess the presence of isotype switch. We studied whether switch circular DNAs are excised via looping out and deletional recombination in SSc. We used nested polymerase chain reaction to analyze the S fragments from switch circles. Fifty-two patients with SSc, and 62 healthy women were studied. The occurrence rate of direct class switch (3/52) and sequential switch (2/52) in SSc skin tissue showed no significant difference with that of normal skin tissue (0/62) and with that of SSc whole blood cells (0/42), respectively. The occurrence rate of IgE isotype switch in SSc skin tissue (5/52) was significantly elevated compared with normal skin tissue (0/62) (P<0.02) and with that of SSc whole blood cells (0/42) (P<0.02). DNA sequencing confirmed the results. These results demonstrated that isotype switch to the ? locus achieved by direct and/or sequential switch are involved in the formation of SSc skin tissue abnormality.

029

Beneficial effects of Pimecrolimus in the Mrl/lpr mouse lupus model

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Clinical studies with oral Pimecrolimus (ASM) treatment for up to 3 months in psoriasis and atopic dermatitis patients have demonstrated high efficacy and safety. In addition, results from studies in animal models of arthritis and inflammatory bowel disease have shown that ASM may also have therapeutic potential in chronic inflammatory conditions beyond dermatology. Here we report on the effect of oral ASM and cyclophosphamide (CP) on amelioration of symptoms in a MRL/MpJ-$Tnfrsf6^{lpr}$ (MRL/lpr) mouse prophylactic model of systemic lupus erythematosus (SLE). Data from 2 independent studies where mice were given 30 (ASM 30) or 50 (ASM 50) mg/kg/d ASM orally during an 8 week period (between 3 and 5 months of age) when disease symptoms develop, suggest that both ASM and CP are equally effective in reducing disease progression and severity. Proteinuria scores were significantly reduced at ASM 50 (p⟨0.01) and ASM 30 (p⟨0.05) doses compared with placebo treated control scores. Glomerulopathology severity scores were similarly reduced: ASM 50 (p⟨0.001) and ASM 30 (p⟨0.05). In MRL/lpr treated mice both CP and ASM 50 significantly decreased (p⟨0.001) the lymph node population of CD4-CD8-CD3+, double negative T cells which is an abnormal T cell subset that accumulates over time due to the *lpr* mutation. The reduction in the generalized hypergammaglobulinemia characteristic of MRL/lpr mice which was greatly reduced by CP compared to placebo controls (p⟨0.001) was seen as a trend with ASM 50 as was the production of anti-ds DNA antibodies. A trend to lower levels of Rheumatoid Factor was evident in the ASM 50 and CP groups versus placebo treated controls. Thus, Pimecrolimus can effectively inhibit disease in a dose-dependent manner in our MRL/lpr model and hence, could be useful for primary or adjunct therapy of human SLE.

030

IL-5 in human bullous pemphigoid blisters is more significant than IL-8 and neutrophil elastase, but is unrelated to serum IgG antibodies to NC16A and tissue eosinophil major basic protein

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It is believed that the NC16A domain of type XVII collagen comprises the most important autoepitopes in pathogenesis of bullous pemphigoid (BP). The majority of rodent and some human studies suggest that neutrophils, but not eosinophils, are key cells in BP. The aim of this study was to compare the involvement of IL-5, IL-8, eosinophil-derived major basic protein (MPB) and neutrophil elastase (NE) in human BP at the tissue level in relation to the level of serum IgG antibodies to NC16A (IgG anti-NC16A). Altogether 48 BP cases and 77 control patients with other autoimmune and non-autoimmune bullous diseases were studied. The MBP in fresh blister-containing skin was detected by immunohistochemical streptavidin/peroxidase technique. Blister fluid levels of IL-5, IL-8 and NE, serum level of IL-5 and level of IgG anti-NC16A were examined by ELISA. The MBP deposits were detected in blistering skin in 55% of BP cases, but in only 13% of control cases. The level of IL-5 in blister fluid was significantly higher than that in serum in BP cases. The level of IL-5 in blister fluid in BP cases was significantly higher than that in blister fluid in controls, whereas levels of IL-8 and NE in blister fluid in BP cases did not differ significantly from those in blister fluid in controls. There were no statistically significant associations between the presence of MBP in lesional BP skin and levels of IL-5 in BP blister fluid and IgG anti-NC16A. There were no statistically significant correlations between BP blister fluid level of NE and levels of IL-8 in BP blister fluid and IgG anti-NC16A. There were no statistically significant correlations between IgG anti-NC16A and levels of IL-5 and IL-8 in BP blister fluid. Thus, in human BP tissue the actions of IL-5 and MBP seem to be more disease-specific/significant than those of IL-8 and NE. However, the action of IL-5 appears to be unrelated to IgG anti-NC16A and presence of MBP in lesional BP skin.

031

The role of intra-molecular epitope spreading in the development and transition of pemphigus vulgaris

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Pemphigus vulgaris (PV) is an immunobullous disease. Patients at the earlier stage (mPV) display only autoimmunity to desmoglein (Dsg) 3 and blisters in the mucosal tissues, while patients at the later stage (mcPV) exhibit response to both Dsg3 and Dsg1 and blisters in mucosal and cutaneous tissues. Recently, we found that autoantibodies from these two subsets of patients exhibit distinct tissue staining and antigenic profiles, indicating that epitope spreading may play a role in the disease transition from mPV to mcPV. To examine this hypothesis, domain swapped Dsg3/Dsg1 proteins were used in immunoprecipitation (IP), indirect immunofluorescence (IF) and ELISA. Using IP, we discovered that mPV patients (12 out of 14) recognize an epitope located at Dsg3:87-566 but not an epitope located at the first 87 amino acids. By competition Dsg3 ELISA and inhibitory indirect IF, we showed that the anti-Dsg3 activity to Dsg3:88-566 was blocked by $Dsg1^{1-87}/Dsg3^{88-566}$, but not by $Dsg3^{1-88}/Dsg1^{89-496}$. A longitudinal analysis of sera from patients who transitioned from mPV to mcPV (n=5) revealed that anti-Dsg3 from mPV recognize an epitope within Dsg3:87-566, while autoantibodies from the mcPV stage recognize an epitope located in the first 87 amino acids of Dsg3. To clarify the epitope recognized by anti-Dsg3 from mcPV patients, a competition Dsg3 ELISA was employed. Here, we showed that the anti-Dsg3 activity in mcPV patients was blocked by $Dsg3^{1-88}/Dsg1^{89-496}$ but not by $Dsg1^{1-87}/Dsg3^{88-566}$, suggesting that anti-Dsg3 from mcPV patients recognize an epitope within the first 87 amino acids of Dsg3. In summary, our results suggest that a Dsg3 intramolecular epitope spreading event is associated with the transition of disease from mPV to mcPV and the production of antibodies to $Dsg3^{1-87}$ is critical for developing cutaneous lesions in PV. Identification of detailed epitopes recognized by anti-Dsg3 at both disease stages may reveal the relationship of epitope spreading with the development of cutaneous lesions in PV.

032

Analysis of autoimmune T cell repertoire in an active pemphigus vulgaris animal model

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Pemphigus vulgaris (PV) is a bullous disease characterized by autoimmunity against desmoglein-3 (Dsg3). Previously, we have demonstrated that Dsg3-specific T cells play an essential role in the development of this disease. Dsg3-specific T cells expressing a CD4 memory T cell phenotype and secreting a Th2 cytokine profile, are critical in promoting the production of pathogenic anti-Dsg3. These T cells were shown to preferentially express certain T cell receptor (TCR) genes. It is difficult to fully assess the autoimmune T cell repertoire in human patients due to the complexity of their therapeutic regimens. To better characterize Dsg3-specific T cells, we recently established an active animal model of PV by transferring splenocytes from Dsg3 knockout mice (Dsg3KO) previously challenged with skin grafts from gender-matched Dsg3+ siblings (skin immunization) to Rag2-/- mice. This modified method avoids the potential immune skewing effect caused by adjuvant in the previously reported animal model and ensures that the primary and secondary immune response to Dsg3 are established in a physiological condition in vivo. In this study, we found that Rag2-/- mice developed clinical, immunological, and histological features of PV 5-14 days after receiving splenocytes from skin immunized Dsg3KO. PV phenotype development in our animal model is dependent on the number of cells transferred and the titer of anti-Dsg3 in the skin immunized Dsg3KO. To investigate the characteristics of disease-related autoimmune T lymphocytes, cells from the spleen and lymph nodes of Rag2-/- mice developing PV were harvested and RNA purified. Subsequently, cDNA was prepared and used as template with specific murine TCR beta chain primer sets in PCR and their spectratypes were analyzed. Interestingly, we observed that there is a significant oligoclonal expansion of T cells expressing BV5.2 and BV8.3 genes in these diseased mice. These results suggest that these two T cell populations may respond to Dsg3 and participate in the development of PV in this active animal model.

033

Blocking-type anti-melanin-concentrating hormone receptor antibodies in patients with vitiligo

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Vitiligo is a common depigmenting skin disorder resulting from the loss of melanocytes in the cutaneous epidermis. Although the cause of the disease remains obscure, autoimmune mechanisms are thought to be involved. Recently, our group has been identified the melanin-concentrating hormone receptor (MCHR) as an autoantigen in 16.4% of vitiligo patients using a radiobinding assay. In the present study, we aimed to determine if IgG samples from vitiligo patients were able to inhibit MCHR function. Briefly, a stable CHO-K1 cell line expressing the MCHR receptor was isolated by transfection of CHO-K1 cells with MCHR cDNA. Expression of MCHR in the stable cell line was analysed by flow cytometry and also by measuring intracellular Ca2+ levels in response to MCH addition using fluorimetry. The transfected cell line exhibited elevated levels of intracellular Ca2+ following MCH addition, compared with untransfected cells, indicating receptor expression and flow cytometry also demonstrated MCHR expression on the isolated cell line. In order to analyse for the presence of MCHR autoantibodies that block functioning of the receptor, cells expressing MCHR were incubated with either control IgG (n=20), MCHR antibody-positive (in a radiobinding assay) vitiligo patient IgG (n=9), MCHR1 antibody-negative (in a radiobinding assay) vitiligo patient IgG (n=9) and SLE IgG (n=10). Our results showed that none of the control IgG samples blocked receptor function. In contrast, seven out of nine MCHR antibody-positive vitiligo patient IgG samples and three out of nine MCHR antibody-negative vitiligo patient IgG samples inhibited the function of the receptor. Incubation of the stable cell line with SLE IgG did not demonstrate blocking of MCHR activity. The results indicate that MCHR function blocking antibodies are specific to patients with vitiligo.

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Evaluation of histamine-releasing activity in the sera of patients with chronic urticaria

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Chronic urticaria has a spectrum of clinical presentations but less well specified causes. Some of patients with this disease have detectable histamine-releasing autoantibodies in their sera, which have been considered as the cause of intolerance of nonsteroidal anti-inflammatory drugs. The term autoimmune urticaria is increasingly being accepted for this subgroup of patients, in whom immunosuppressive therapies may be useful when conventional treatments are unsuccessful. To evaluate the histamine-releasing activity in the sera of patients with chronic urticaria and to further explore the pathogenesis of chronic urticaria, we studied 62 chronic urticaria patients by in vivo skin test with autologous serum and in vitro histamine release assay using human cutaneous mast cells incubated with the sera of patients. Our results demonstrated that among those tested twenty-four patients showed a weal response to autologous serum (38.71%). Sera from patients with chronic urticaria released a significant amount of histamine compared with control subjects ($p<0.01$), the percentage of histamine released varied from 3.1% to 79.5% with a mean of 16.44% and a standard deviation (SD) of 14.26%, and sera of 27 among 62 chronic urticaria patients (43.55%) elicited histamine release more than 15%. The percentage of histamine release was also elevated in ASST+ chronic urticaria patients compared to ASST- chronic urticaria patients ($p<0.01$). We conclude that histamine-releasing activity, which was demonstrated in the sera of some patients with chronic urticaria, is important in the pathogenesis of chronic urticaria by stimulating or facilitating degranulation of cutaneous mast cells.

035

Ability to induce keratinocyte apoptosis determines pathogenicity of pemphigus IgGs

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Pemphigus vulgaris (PV) is associated with IgG antibodies against keratinocytes (KCs). Since autoantibody titers do not always correlate with disease severity, it remains to find out what determines pathogenicity of PVIgG. Apoptosis has been shown to precede acantholysis in PV patients' skin, PV patients' sera to cause KC apoptosis, and PVIgG to upregulate proapoptotic genes in KCs. We asked if the apoptotic and acantholytic activities of PVIgG correlate. PVIgGs were isolated by FPLC protein G affinity chromatography from sera of patients with different severity of PV but similar autoantibody titers. Apoptotic activity of PVIgGs was characterized by caspase-3 (Cs-3) assay, and acantholytic activity by extent of intraepidermal splitting in neonatal mice and cell-cell dyshesion in KC monolayers treated with test PVIgGs vs normal IgG. Time-course study revealed maximal Cs-3 activity in KC monolayers 12-24 h after addition of PVIgGs. Antibody from a patient with extensive mucocutaneous lesions exhibited highest activity. Similar correlation was observed in quantitative assays of acantholysis. At 24 h, the "apoptotic" and "nonapoptotic" PVIgGs produced 52 ± 7 and $20\pm2\%$ of acantholysis in vitro, and 76 ± 14 and $35\pm9\%$ in vivo, respectively ($p<0.05$). Effects of PVIgG vs normal IgG on gene expression along the apoptosis pathway were analyzed by RT-PCR and western blot. In the Fas signaling pathway, most pronounced changes occurred with Fas receptor (FasR), Fas ligand (FasL), FLIP long (FLIPl), Cs-8 and Cs-3. PVIgG upregulated FasL and Cs-3 and downregulated FLIPl, whereas normal IgG decreased Cs-8. The peak of PVIgG-induced changes occurred at 24 h, when both FasL and Cs-3 increased >200%. FasR decreased by ~40%, which might be a result of overstimulation of KCs with FasL. Thus, the pathogenicity of PVIgG reflects, at least in part, their ability to activate Fas pathway in KCs, whereas the effect of normal IgG suggests that IVIg may improve PV by blocking Fas signaling. Further studies of KC death receptor signaling in PV may clarify pathophysiology and suggest novel treatments of this disease.

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Skin inflammation is enhanced by sodium dodecyl sulphate-treatment in transgenic mice expressing type I interleukin-1 receptor under the control of keratin 14 promoter

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The role of IL-1-inducible keratinocyte cytokines in irritant contact dermatitis (ICD) is currently unknown. Using previously characterized transgenic mice expressing type I interleukin-1 receptor on basal keratinocytes (K14/IL-1RI transgenic mice), we carried out experiments with two strong irritants: sodium dodecyl sulphate (SDS) and phorbol 12-myristate 13-acetate (PMA). We measured ear swelling and cytokine production as an index of ICD. PMA and SDS increased ear swelling in both K14/IL-1RI transgenic mice and non-transgenic littermates; however, ear swelling in K14/IL-1RI transgenic mice was 50% and 100% greater than in non-transgenic littermates after 3 days or 7 days of consecutive application, respectively. The degree of neutrophil infiltration and epidermal hyperproliferation observed in PMA and SDS treated K14/IL-1RI transgenic mice was also much greater than in non-transgenic littermates. Gene expression of several inflammatory cytokines and chemokines in mouse ear was quantitated by real-time PCR. SDS- and PMA-treatment markedly induced several cytokine and chemokine genes tested, such as macrophage inflammatory protein (MIP)-2, KC, monocyte chemotactic protein (MCP)-1/CCL2, IL-1α, TNF-α, but not RANTES/CCL5, TARC/CCL17 or eotaxin/CCL11. Moreover, gene expression of MIP-2, MCP-1, IL-1α and TNF-α induced by SDS-treatment were significantly enhanced in K14/IL-1RI transgenic mice compared with those in non-transgenic littermates. Induced expression of MIP-2 and KC, which are ligands of chemokine receptors CXCR1 and CXCR2, was consistent with large number of neutrophils in inflammatory ICD sites of K14/IL-1RI transgenic mice. These results suggest that simple irritant SDS can cause skin inflammation through the IL-1-mediated, keratinocyte cytokine specific mechanism in fashion similar to PMA.

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Haplotype associations of the MHC with psoriasis vulgaris in Chinese Hans

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The objective of this study was to demonstrate the differential haplotype associations of the major histocompatibility complex (MHC) in psoriasis vulgaris (PV) patients in Chinese population stratified by the type of onset and sex. One hundred and thirty-eight PV patients and 149 normal controls were genotyped for HLA- A, B, C, DQA1, DQB1, and DRB1 using the polymerase chain reaction with sequence specific primers (PCR-SSP). The results showed: (1) HLA-A*26 (26.09% vs. 12.08%, $Pc<10^{-5}$), B*27 (17.03% vs. 1.01%, $Pc<10^{-7}$), Cw*0602 (15.58% vs. 5.03 %, $Pc<10^{-2}$), DQA1*0104 (19.93% vs. 9.40%, $Pc<10^{-3}$), DQA1*0201 (22.40% vs. 10.74%, $Pc<10^{-3}$), DQB1*0303 (18.12% vs. 9.73%, $Pc<10^{-7}$), and DRB1*0701/02 (26.09% vs. 9.73%, $Pc<10^{-7}$) were significantly increased in PV. HLA-Cw*0304 (5.07% vs. 14.43%, $Pc<10^{-3}$), DQA1*0501 (5.79% vs. 14.09%, Pc<0.05) were negatively associated with PV. (2) HLA-A*26-B*27 ($P<10^{-7}$), A*26-Cw*0602, ($P<10^{-4}$), B*27-Cw*0602 ($P<10^{-7}$), DRB1*0701/02-B*27 ($P<10^{-7}$), DRB1*0701/02-DQA1*0104 ($P<10^{-3}$), DRB1*0701/02-DQB1*0303 ($P<10^{-4}$), DQA1*0201-DQB1*0303 ($P<10^{-3}$), A*26-B*27-Cw*0602 ($P<10^{-3}$), A*26 -DRB1*0701/02 -DQA1*0201-DQB1*0303 (P<0.01) were identified as risk halpotypes for PV patients. (3) HLA-A*26-B*27 ($P<10^{-4}$), DQA1*0201-DQB1*0303 ($P<10^{-4}$), DRB1*0701/02 -DQA1*0104 ($P<10^{-3}$), DRB1*0701/02-DQB1*0303 ($P<10^{-5}$) and A*26-DRB1*0701 -DQA1*0201 -DQB1*0303 (P<0.01) were only significantly associated with type I psoriasis compared with controls. (4) These associated haplotypes with PV were not affected by sex, except that the frequencies of DRB1*0701/02-DQB1*0303 ($P<10^{-6}$) which was higher in male than in female patients. To summary, this study demonstrated the differential association of HLA and identified some special risk haplotypes in Chinese PV patients compared with other ethnic or racial populations.

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Thalidomide inhibits the induction of tumor necrosis factor-α by ultraviolet B irradiation of human keratinocytes

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TNF-α is induced by UV and contributes to UV-induced apoptosis of keratinocytes (KCs). Apoptotic antigens likely trigger a photosensitive immune response in subacute cutaneous LE (SCLE). In support of this model, we recently showed that a promoter polymorphism causing TNF-α overproduction is associated with SCLE (Werth et al. JID, 2000). Because Thalidomide is beneficial in diseases where TNF-α plays a pathogenic role, the purpose of the current study was to determine the effect of Thalidomide on ultraviolet B (UVB)–induced TNF-α secretion from cultured primary human KCs. Recent studies in mice have shown inhibition of UVB-induced TNF-α production in KCs in mice (Lu and Gaspari, Photoderm, 2003). Primary human KCs were cultured in serum-free medium, then pre-treated for 2 h with 0, 10, 25, 50, 200, or 800 μg of Thalidomide/ml. TNF-α secretion was then induced by 30 mJ UVB/cm2. TNF-α protein in conditioned medium was quantified by ELISA at 6h and 16h. KCs were harvested, and real-time PCR was used to quantify TNF-α mRNA, normalized to levels of cyclophilin mRNA. Consistent with prior work, TNF-α gene expression 16 h after UVB irradiation had increased 13.1 fold (range =12.0–14.4) over sham-irradiated controls. Thalidomide treatment of UV-treated KCs reduced the induction of TNF-α mRNA by two-thirds at all doses of 10 μg/ml and above. During a time course experiment, UVB-irradiated KCs at 6 hours showed a 15.6 (13.6–17.8) fold increase in TNF-α mRNA relative to no-drug, no-UV controls, and Thalidomide inhibited this mRNA level to 0.124 (0.109–0.141) of the no-drug, no-UV controls, i.e., far lower than control.Thalidomide also inhibited UV induced secretion of TNF-α protein in a dose dependent fashion. By 16 hours, a concentration of 50μg of Thalidomide/ml inhibited TNF-α secretion by 93%+/–4%(p=0.002), compared to cells treated with UV without drug. Overall, our results indicate that Thalidomide inhibits UV-induced TNF-α production from human KCs, an action that may explain the drug's therapeutic effects in photosensitive diseases.

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Comparative analysis of methods for detection of anti-laminin 5 autoantibodies in patients with anti-epiligrin cicatricial pemphigoid (AECP)

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AECP is a subepidermal blistering disease characterized by circulating anti-basement membrane autoantibodies to laminin 5. Recent studies showed that AECP is associated with an increased relative risk for fatal solid cancers. To study this and other potential associations further, practical yet sensitive methods to establish the diagnosis of AECP are needed. To evaluate the relative sensitivity of immunoblotting and immunoprecipitation techniques for the detection of anti-laminin 5 antibodies, comparative studies using reference laminin 5 antisera as well as sera from patients with AECP, other immunobullous diseases, and normal volunteers were performed. Equivalent amounts of protein from five different substrates (specifically, human keratinocyte [HK] extracts, HK extracellular matrix [ECM], A-431 cell extracts, HaCat cell extracts, and extracts of epidermal sheets [ES]) were immunoblotted using serial dilutions of reference laminin 5 antisera. HK ECM was the most sensitive substrate for detection of antibodies to laminin 5; extracts of HKs, A-431, and HaCat cells represented alternative test substrates (though the latter required higher amounts of protein input). ES extracts were a poor source of laminin 5. Sera from patients with AECP showed reactivity to laminin 5 in HK ECM at end titers exceeding those identified in indirect immunofluorescence microscopy studies of 1 M NaCl split skin. Studies of biosynthetically radiolabeled HK extracts found that a 10,000-fold dilution of reference laminin 5 antisera retained the ability to immunoprecipitate laminin 5. Maximal dilutions of sera from AECP patients retaining the ability to immunoprecipitate laminin 5 ranged from 500 to 5000. While immunoprecipitation was the most sensitive technique for detection of anti-laminin 5 antibodies, immunoblotting of HK ECM and HK extracts represents a practical alternative.

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Di-PPG-2-Myreth-10 Adipate reduces irritation and inflammation caused by cosmetic products

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RL Goldemberg has stated that nonionic surfactants - particularly ethoxylated esters - act as counter irritants in amphoteric/anionic shampoo systems; several studies in our laboratories have demonstrated this desirable property (ethoxylated modified glycerides, and PEG-150 Pentaerythrityl Tetrastearate). In the search for new molecules, we synthesized an alkoxylated myristyl alcohol derivative Di-PPG-2-Myreth-10 Adipate (DMA). DMA is both ethoxylated and propoxylated, which accounts for its multifunctionality. This emollient di-ester has multifunctional properties including broad solvent compatibility, a wide solubility range, and irritation mitigation. To study the irritation and inflammation mitigation properties of DMA 3 different surfactant based systems were investigated using 2 *in vitro* assays and 1 clinical trial. The addition of 5% DMA to 10% SLS significantly decreased the SLS induced cell death by 30% in the EpiOcular™ Model. Very mild surfactant systems cause intercellular permeability, the addition of 5% DMA significantly decreased the irritation potential in the TransEpithelial Permeability Model. Finally, female volunteers were patched using Occlusive Hill Top® chambers containing 1% SLS plus 0, 1, 5, 10% DMA, 100% DMA or dd water for 24H then examined using a chromameter (Minolta CR 200) and scanning laser doppler imager (Moor LDI). 100% DMA is less irritating than dd water. In 75% of the subjects DMA, at all concentrations, decreases the irritation of 1% SLS to that of dd water. From this data we can conclude DMA to be a potent mitigator of surfactant-induced irritation and inflammation. Future experimentation will try to elucidate the underlying mechanisms, which account for DMA's ability to decrease the irritation and inflammation potential of surfactant based cosmetic products.

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A novel murine model of ocular cicatricial pemphigoid (OCP) mediated by anti-laminin 5 IgG

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OCP is an autoimmune subepithelial blistering disease that often causes chronic ocular injury and/or blindness. Like other forms of CP, OCP is characterized by in situ deposits of IgG and/or complement in ocular epithelial basement membranes (BMs). Patients with one form of CP that features ocular involvement have IgG anti-BM autoantibodies directed against laminin 5 (L5); prior studies have shown that such autoantibodies are pathogenic in vivo. To devise an animal model of OCP, high titer rabbit anti-L5 antisera was produced for use in passive transfer experiments. This antisera bound murine epidermal BM (titer=20,480), immunoblotted all subunits of isolated murine L5, and immunoprecipitated L5 from biosynthetically radiolabeled murine keratinocytes. IgG in L5 antisera (as well as normal rabbit sera, control) was purified on protein G columns, concentrated by ultrafiltration, and injected into subcutaneous facial tissue 1 cm below the lower eyelid of adult BALB/c mice (n=9) (thus avoiding frank physical ocular injury as occurs in OCP models using wounding or caustics). Diffusion of L5 IgG (2.5 to 5 mg, single doses) into ocular tissues prompted erosion of corneal epithelia and eye closure within 48 hours; immunofluorescence and light microscopy studies identified in situ deposits of rabbit IgG and murine C3 in ocular epithelial BMs as well as noninflammatory subepithelial blisters within 24 and 48 hours, respectively. Mice treated with 5 mg of anti-L5 IgG weekly x 4 (n=5) or daily x 7 (n=4) regularly showed these same alterations as well as periocular hair loss, an apparent reduction in ocular diameter, and reduced numbers of conjunctival goblet cells. Control mice (n=9) receiving identical amounts of nonimmune purified rabbit IgG (single and repeated injection protocols) did not develop clinical, histologic, or immunopathologic alterations. These studies established a novel murine model of OCP that can be used to study how antibody-mediated ocular injury results in conjunctival blisters, unstable tear films, mucosal fibrosis, and compromised vision.

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Development of Rag2-/-/hBPAG2 transgenic (Tg) mice for the study of *in vivo* unbiased immune responses to human bullous pemphigoid antigen 2 (hBPAG2) in skin

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Previously, C57BL/6 Tg mice expressing hBPAG2 under the control of a K14 promoter were produced and characterized. Grafts of Tg skin to syngeneic Wt mice elicited hBPAG2-specific IgG of high and durable titer whose kinetics of production and localization in skin corresponded with blister formation within, and loss of, Tg grafts. To develop an adoptive transfer animal model where in vivo unbiased immune responses to hBPAG2 in skin can be characterized, Tg mice were crossed with syngeneic recombinase-activating gene-2-null (Rag2-/-) mice and bred to double homozygosity. Intravenous injection of large numbers ($\geq 10^8$) of lymph node (LN) cells and splenocytes from naive Wt mice resulted in only transient levels of low titer (e.g., 40) IgG specific for hBPAG2. Conversely, adoptive transfer of 1-5x10^7 LN cells and/or splenocytes from Wt mice previously grafted with hBPAG2 skin (i.e. immune lymphocytes) resulted in high titer (≥5120) and durable (⟩210 days) hBPAG2-specific IgG in Rag2-/-/hBPAG2 Tg mice. Adoptive transfer of CFSE-labeled immune lymphocytes showed that T and B cells proliferated in both Rag2-/-/hBPAG2 Tg and control Rag2-/- mice while hBPAG2-specific IgG only appeared in the former. Moreover, epidermal BM deposits of murine IgG were seen in Rag2-/-/hBPAG2 Tg, but not in Rag2-/- recipients. These findings showed that expression and presentation of hBPAG2 in recipients was required for durable production of hBPAG2-specific IgG. Interestingly, adoptive transfer of immune lymphocytes depleted of CD11c+ cells to Rag2-/-/hBPAG2 Tg mice resulted in high titer and durable hBPAG2-specific IgG, indicating that donor Wt dendritic cells were not required to maintain specific antibody production in Rag2-/-/hBPAG2 Tg mice. These studies have yielded an adoptive transfer animal model in which a transgene-encoded neoantigen in skin (i.e., hBPAG2) is processed and presented by host APCs to elicit and maintain model humoral immune responses that can be modulated and/or blocked in vivo.

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The regulation of toll-like receptor 2 expression in immortalized keratinocyte cell culture

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Toll-like receptors are a family of pattern recognition receptors that constitute the first line of defense against many pathogens and play a crucial role in the function of the innate immune system. Although toll-like receptor 2 (TLR-2) expression has been demonstrated in a number of myeloid cells, much less is known about the expression and function of these receptors on nonleukocytes. This study was conducted to investigate the expression of TLR-2 on keratinocytes and its regulation by *Propionibacterium acne* and cytokines, as well as the effect of drugs and UVB.

hTERT keratinocytes, obtained by stable transfection of primary cell culture with human telomerase reverse transcriptase, were treated with various concentrations of LPS, *P.acne* cell wall component, INF-γ, TNF-α and IL-1α. Hydrocortisone was used at 1uM, 10uM and 100uM and cells were exposed to UVB (310nm). The expression of TLR-2 was determined by immunofluorescence using quantitative CytoFluor reader at 48 hours.

The results showed that IL-1α, INF-γ, TNF-α and the combination of INF-γ and TNF-α upregulate the expression of TLR-2 in hTERT cells. TLR-2 levels were increased by 11, 13, 19 and 23% respectively, each producing a linear dose-response curve. UVB also appears to modulate TLR-2 activity. However, *P.acne* and LPS did not show significant effect on TLR-2 expression in keratinocytes. In addition, hydrocortisone was found to elevate the levels of TLR-2 up to 40% at the highest concentration tested. It has been shown that *P. acne* can directly induce the expression of pro-inflammatory cytokines such as IFN-γ and TNF-α from monocytes, thereby contributing to the inflammatory nature of acne. In this study, we demonstrate that human keratinocytes are capable of constitutively expressing TLR-2 that was augmented by exposure to various cytokines, including IFN-γ and TNF-α, as well as IL-1α. The expression of TLR-2 in keratinocytes indicates that activation of TLR-2 can contribute to inflammation at the site of disease activity and clarify the molecular mechanism by which *P. acne* induces inflammation.

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CD40/CD40L blockade prevents experimental immune responses to human bullous pemphigoid antigen 2 in epidermal basement membrane *in vivo*

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CD40/CD40 ligand (CD40L) interaction is required for the development of T cell-dependent antibody responses in vivo and thus is an attractive target for therapeutic interventions in many autoimmune disorders. Previously we developed and characterized C57BL/6 Tg mice expressing hBPAG2 in skin under the control of a K14 promoter. Tg skin placed on syngeneic Wt mice consistently elicited hBPAG2-specific IgG of high and durable titer as well as graft loss. Graft loss in this model was dependent upon CD4+ T cells and correlated with the production and tissue deposition of hBPAG2 IgG. To examine the relative contribution of the CD40/CD40L pathway to immune responses in our murine model, grafts of Tg skin were placed on CD40L -/- as well as hamster anti-CD40L monoclonal antibody (MR1)-treated mice to block hBPAG2-specific IgG production in vivo. In contrast to matching controls, CD40L -/- mice grafted with Tg skin did not develop hBPAG2-specific IgG or graft loss. Similarly, Wt mice grafted with Tg skin and treated with MR1 (days 0, 2, 4, 7, and 14) did not develop hBPAG2-specific IgG or graft loss (in contrast to grafted Wt mice treated with equivalent doses of control IgG). Successful inhibition of hBPAG2-specific IgG production and Tg graft loss following blockade of the CD40-CD40L pathway in this model provides opportunities to study induced operational tolerance and generate regulatory cells. These findings have direct relevance to blocking unwanted immune responses in patients with pemphigoid as well as patients undergoing gene replacement therapy for GABEB, a form of junctional epidermolyis bullosa characterized by null mutations in the gene encoding hBPAG2.

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Gene expression profiling of skin and peripheral blood in vitiligo

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The cellular and molecular disease mechanisms involved in vitiligo, which affects 2% of the world population, are yet to be elucidated. Microarray analysis and new emerging bioinformatics tools have enabled genome wide screening to enhance our understanding of complex disease. In previous work, we compared skin (lesional and non-lesional) samples from 6 vitiligo patients using DNA chips and found 63 genes that were differentially expressed. To study systemic alterations in disease we then collected peripheral blood from 8 patients affected with vitiligo and matched normals (age, ethnicity, gender). Affymetrix Hu95Aver2 chips were used to search for significant alterations in peripheral blood gene expression profiles of patients. Image data was first obtained and analyzed using MAS v5.0, and metrics files were subsequently exported and analyzed using GeneSpring ™ v6.0 using a nonparametric test with p cutoff of 0.05. Three hundred and fifty-five genes were found to be differentially regulated comparing vitiligo vs normal blood samples. Of these, 211 were up-regulated and 144 were down-regulated in disease. We observe the dysregulation of genes involved in oxidative stress (DUSP1, PIP3-E, GSS), stress response (MAPK14, MAP3K4, CGR19), apoptosis (DED, CASP10, TRAF5. DAP3) and immune response (RI58, PF4, TMSB4X, PVRL1, HLA-DRA, IL6ST, IFIT4, Ccl2, MRPL28, GATA3, KLRD1, TACTILE, XP1, EBI2, KLRC3, KLRC1, ANXA1, KLRB1, CHS1, MGEA6). We further observe differentially expressed genes functionally relevant in signal transduction, enzyme activity, DNA repair, transcription, transport, protein modification, RNA binding, and cell cycle, growth and maintenance. Comparison of differentially expressed genes in the skin (lesional and non-lesional) and peripheral blood (vitiligo blood and matched normal blood) reveals an overlap of 6 genes (MLC1, TGFB1, SARS, EIF3S6, MAP3K4, SH2BP1). These findings are being confirmed by real time RT-PCR. Supplementary samples are being collected to increase the sample size.

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Disturbed enzymatic antioxidant defense and increased oxidative damage repair in lichen sclerosus

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Lichen sclerosus (LS) is a chronic inflammatory skin disease of unknown aetiology. The mechanisms which lead to the immunological changes in all levels of the skin and the alterations of the dermal extracellular matrix are poorly understood. We have recently demonstrated an involvement of oxidative stress in the pathogenesis of LS. In this study, we investigated antioxidant defense mechanisms and the expression of the oxidative damage repair enzyme methionine sulfoxide reductase A (MSRA) in LS. Skin biopsies from 16 patients with untreated, histologically confirmed vulval LS were examined immunohistochemically. Antioxidant enzyme expression of catalase (Cat), copper-zinc superoxide dismutase (CuZnSOD) and manganese SOD (MnSOD) was investigated as well as the distribution of the oxidative damage repair enzyme methionine sulfoxide reductase A (MSRA). Normal vulval tissue from 16 subjects served as control. In lichen sclerosus tissue the enzymatic antioxidant defense was disturbed as demonstrated by a significantly reduced expression of MnSOD within the stratum corneum and epidermis, and an increased expression of Cat in epidermal and dermal layers. Furthermore, MSRA was shown to be increased in dermal layers of LS lesions suggesting the implication of a recently described oxidative protein damage repair mechanism. This is the first study investigating the antioxidant defense and oxidative damage repair capacities in LS. Further studies are required to elucidate the role of the observed modulation in the enzymatic antioxidant system in order to provide new insight into the possibilities of antioxidant therapeutic strategies.

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Th-1 cytokines in alopecia areata pathogenesis: IFN-γ is essentially involved while IL-2 plays a minor role

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Alopecia areata (AA) was regarded as a Th-1 mediated autoimmune disease of the hair follicle, because the Th-1 cytokines IFN-γ and IL-2 are expressed in AA-skin. Recently we have shown, that also the Th-2 cytokine IL-10 is expressed in skin infiltrating leucocytes in AA of C3H/HeJ mice and that IL-10-/- mice are resistant to the development of AA, pointing towards an involvement of Th-2 cytokines in AA pathogenesis. To prove the functional relevance of Th-1 cytokines in AA, we induced AA in IFN-γ-/- mice and in IL-2+/- mice by grafting lesional AA skin from AA-affected mice. After experimental induction by skin-grafting 90% of normal C3H/HeJ mice (control) developed AA, while none of the IFN-γ-/- mice and 47% of IL-2+/- mice developed AA. Immunhistochemistry showed dense peri- and intrafollicular infiltrates of CD4+ and CD8+ cells and an aberrant expression of MHC-I on hair follicle epithelium in control mice with AA, while infiltrates and MHC-I-expression were absent in IFN-γ-/- and IL-2+/- mice without AA. FACS-analysis of skin-draining lymph node cells revealed a significant reduction of T-cell activation markers and co-stimulatory molecules in IFN-γ -/- mice (CD25, CD69, CD28, CD86) and IL-2+/- mice (CD25, CD28, CD80) and a reduced expression of pro-inflammatory cytokines in IFN-γ-/- mice (IL-6, TNF-α) and IL-2+/- mice (IL-4, IL-5, IL-6, IFN-γ, TNF-α). Our data show that IFN-γ-/- mice are resistant to the development of AA, pointing towards an essential role of IFN-γ in AA pathogenesis. FACS-analysis and immunohistochemistry demonstrate that IFN-γ is irreplaceable for activation of autoreactive T-cells in the lymph nodes. A pathogenetic role for IL-2 in AA could also be demonstrated, but the expressiveness of our data is limited because only heterozygous IL-2+/- mice were available for these experiments. FACS-analysis and immunohistochemistry point towards an involvement of IL-2 in the activation of autoreactive T-cells in the lymph nodes.

048

Macrophage migration inhibitory factor is important for eosinophil migration *in vivo*

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Eosinophils are thought to mediate inflammatory and cytotoxic events associated with allergic disorders, including atopic dermatitis, urticaria and bronchial asthma. To investigate the effects of MIF, a pleuripotent cytokine on the allergic disorders, we engineered transgenic (Tg) mice that overexpress MIF. We first examined its effects on antigen-induced eosinophilia in vivo. MIF Tg mice were sensitized subcutaneously with ragweed pollen and were challenged intraperitoneally with the allergen 24 hours later. The eosinophilia induced by ragweed pollen extract was quantitatively evaluated. Eosinophilia in response to ragweed pollen extract exacerbated in MIF Tg mice compared with that of wild-type mice. The MIF and eotaxin contents of the peritoneal fluid were increased in the MIF Tg mice compared with wild-type mice, respectively. Moreover, we found that the eosinophilia induced by ragweed pollen extract was suppressed by the treatment with neutralizing anti-MIF antibody i.p. prior to the challenge. Furthermore, eosinophilia induced by ragweed pollen extract were found to be significantly reduced by orally treated cetirizine of 20 mg/kg, a putative H1-receptor antagonist. MIF and eotaxin were also significantly decreased at 24 hours in the peritoneal fluid by cetirizine treatment. Based on these results, it is suggested that MIF is profoundly involved in the eosinophil accumulation, and blockade of MIF may become a promising therapeutic treatment for allergic diseases.

049

Humoral immunity to extracellular matrix protein 1 in lichen sclerosus

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Lichen sclerosus is a chronic inflammatory dermatosis of unknown etiology. Evidence for an autoimmune basis to the disorder is emerging with circulating autoantibodies to the glycoprotein extracellular matrix protein 1 (ECM1) recently demonstrated in the sera of about 75% of affected individuals. In this study, we determined the site(s) of autoantibody targeting of ECM1 in lichen sclerosus sera, established the immunoglobulin sub-type profile of the anti-ECM1 antibodies, and looked for clinicopathological correlation. Immunoblotting was performed using a series of bacterial recombinant proteins spanning different domains of ECM1 and 90 lichen sclerosus sera. Blotting revealed multiple antigenic reactive sites within both the amino-terminus (50/90, 55.6%) and the protein loop cysteine-rich domain (54/90, 60%), although few sera reacted to the distal carboxyl-terminus (7/90, 7.8%). Immunoabsorption studies using affinity-purified lichen sclerosus sera showed no antibody cross-reactivity with other ECM1 epitopes. IgG subclass analysis revealed that the anti-ECM1 autoantibodies were mainly IgG2 (48/54, 88.9%), either IgG2 alone (28/54, 51.9%) or in combination with one or more other IgG subclasses. Clinical appraisal of 26 subjects (including 3 with extragenital disease) showed no relationship between extent and duration of disease or skin histology findings and any specific ECM1 epitope nor any particular type of IgG response, although in those with lichen sclerosus of 5-10 years' duration, a solitary IgG2 response predominated. Overall, these findings expand insight into humoral autoimmune responses and possible disease mechanisms in lichen sclerosus.

050

Flexible docking of self-epitopes to HLA class II DR and DQ alleles in pemphigus vulgaris

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The strongest genetic influence on PV susceptibility maps to the MHC II region. Over 95% of PV patients serotype as either HLADR4 and/or HLADR6, and the strongest associations are with the DRB1*0402 and DQB1*0503 allelic subtypes. In an attempt to further elucidate the functional correlation between MHC class II alleles and PV, we constructed atomic models of DRB1 subtypes (*04011, *0402, *0404, *0406, *1401, *1404, *1405) and one DQB1 subtype (*0503). In subsequent analysis, eight fifteen-residue Dsg3 peptides (Dsg3 341-355, Dsg3 809-823, Dsg3 962-976, Dsg3 206-220, Dsg3 190-204, Dsg3 251-265, Dsg3 762-786 and Dsg3 380-396) capable of eliciting T cell responses in PV patients were modeled into the binding groove of each HLA model using a novel MHC-peptide docking protocol. Our modeling procedure first applies a sliding window input of size 9 to each peptide to generate all combinations of core residues to be modeled into the binding groove of each allele. Docking of peptides was subsequently performed in four steps as follows: (i) pseudo-Brownian rigid body docking of core peptide fragments at the ends of the binding groove, (ii) loop closure by satisfaction of spatial constraints, (iii) refinement of the backbone and side-chains of peptide core residues as well as atomic clash regions at receptor; and finally, (iv) extension of flanking residues. These studies provide further evidence that the DRB1*0402 allele is directly involved in the selection of specific self-peptides in PV. Moreover, these data indicate that a non-overlapping set of self-epitopes are associated with the DRB1*0402 vs. DQB1*0503 linked responses. Our modeling results are consistent with previous functional data obtained from in vitro competitive binding assays and T cell proliferation studies in PV patients and provide structural insights into the molecular basis of MHC-linked control of the autoimmune response in PV.

051

CX3CR1 single nucleotide polymorphisms are associated with type 1 psoriasis

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The purpose of this study was to investigate the association of polymorphisms of the fractalkine receptor gene (CX3CR1) with Type 1 psoriasis. Five single nucleotide polymorphisms (SNPs) (rs3732378 G/A, rs3732379 C/T, rs2669843 G/A, Hcv468342 G/C and Hcv11578468 T/C) were genotyped in 669 psoriasis cases and 184 unrelated controls. A fluorescence based primer extension method (SNaPshot, PE-ABI) (Applied Biosystems, Warrington, UK) was used with products electrophoresed on an ABI PRISM 3100 Genetic Analyser. Case and control cohorts were tested for population stratification. Single-marker associations between SNPs and the disease phenotype were analysed using the statistical package Stata 7 (StataCorp. 2001). The frequency of the G allele for the (non synonymous Met > Thr) SNP marker rs3732378 was increased in the case cohort (85%) compared with the controls (80%) ($p = 0.04$). In addition, using the Expectation Maximization (EM) algorithm as implemented in SNPHAP statistical software (Clayton, D. http://www-gene.cimr.cam.ac.uk/clayton/software/ 2002), estimated haplotype frequencies were generated, and these frequencies were compared between the case and control groups. A 2-marker haplotype, rs3732378 G and Hcv11578468 C, was over represented in the case cohort (50%) compared with the controls (43%)($p = 0.03$). In conclusion, these results suggest a possible involvement of the CX3CR1 gene in the pathogenesis of psoriasis.

052

Topical bexarotene 1% gel is anti-inflammatory in the mouse ear model of inflammation

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The mouse ear model of irritation or allergic response has been used to profile the potential anti-inflammatory activity of topical drug candidates. A standard concentration of the phorbol ester, 12-O-tetradecanoyl phorbol acetate (TPA) is applied to the ears to induce a standardized inflammation via the cascade of eicosinoids and prostaglandins derived from arachidonic acid. Objective measures of the degree of inflammation can be made with measures of ear thickness (edema), weight from a standardized biopsy of the ear, and the leukocyte infiltration into the tissue. The RXR-selective retinoid, bexarotene, was evaluated for its anti-inflammatory potential in the mouse ear model to identify potential mechanisms associated with its clinical activity. Male CD-1 mice were used in a standard test protocol that included the high potency steroid, clobetasol 0.05%, as a positive control and the acetone vehicle for TPA as a negative control. Reductions in TPA-induced inflammation with bexarotene 0.1-1.0% were dose-related. Reductions with bexarotene 1% were 66% for the TPA-induced ear thickness and 50% for biopsy weight. This result was comparable to clobestasol 0.05% (54% for ear thickness and 45% for biopsy weight). The anti-inflammatory action of bexarotene is likely to be mediated through modulation of gene expression via RXR activation.

053

An active animal model of skin basement membrane antigens-induced autoimmune alopecia resembling human alopecia areata is mediated by autoreactive T cells

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In order to develop a currently unavailable animal model of active autoimmune hair loss disease to study the pathogenesis of human alopecia areata (AA), mouse skin basement membrane zone (BMZ) proteins, components of hair follicle, were used to immunize mice for inducing hair loss. Immunization of BMZ antigens (four times, three weeks apart) resulted in progressive hair loss in 78% of C57BL/6 mice and 11% of SJL/j and BALB/c mice, with feature of exclamation point hair but without blister formation. Histopathology documented the presence of peri- and intra-follicular mononuclear cell infiltrates in mice with hair loss, but not in normally haired mice. Adoptive transfer of BMZ antigens autoreactive spleen cells (30 millions/mouse) from skin BMZ antigens-immunized mice to naïve syngeneic mice intra-peritoneally resulted in hair loss in all recipient mice in four weeks, whereas none of mice received spleen cells from mice immunized with control protein mouse serum albumin developed hair loss. The mice received spleen cells from BMZ antigens-immunized mice, but not the mice received spleen cells from albumin-immunized mice, demonstrated both humoral and cellular autoimmunity to the BMZ antigens: possess circulating IgG autoantibodies to the BMZ antigens (by ELISA) and exhibit positive autoreactive T cell responses to the BMZ antigens (by T cell proliferation assays). Moreover immunopathology detected a CD4+ T cell-dominated perifollicular infiltrate in the hair loss skin of mice receiving BMZ antigens-immunized spleen cells, but not in the normally haired skin of mice receiving albumin-immunized spleen cells. Our data supports a novel mouse model of active, experimentally induced, autoimmune hair loss disease resembling human AA clinically, histopathologically, and immunopathologically. Although autoimmune response to skin BMZ proteins has not been demonstrated in human patients with AA, this model may be useful in shedding light to the pathomechanism of autoimmune alopecia.

054

A case of IgA pemphigus with dual autoantigen-reactivity against desmoglein 1 and desmocollin 1

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The IgA pemphigus is an uncommon vesiculo-pustular autoimmune bullous disease with IgA anti-keratinocyte cell surface antibody reactivity. Two subtypes can be discerned; in the subcorneal pustular dermatosis type desmocollin 1 (Dsc 1) was identified as a target autoantigen, while in few cases of the intraepidermal neutrophilic type desmoglein 1 (Dsg 1) reactivity was observed. We report a 48 year-old Caucasian male with generalized pustular blistering lesions. Histopathology revealed subcorneal pustules. Direct immunofluorescence (IF) exclusively showed in vivo bound IgA anti-keratinocyte cell surface antibodies, while circulating autoantibodies were undetectable by indirect IF and by immunoblotting. Enzyme-linked immunosorbent assays using baculovirus-expressed recombinant Dsg 1 revealed anti-Dsg 1 IgA but not IgG reactivity. No IgA anti-Dsg 3 reactivity was present. Sequential absorption with immobilized Dsg 1, but not with Dsg 3 completely abrogated IgA anti-Dsg 1 reactivity. Moreover, Dsc 1 was revealed as a target of IgA autoantibodies by indirect IF utilizing Dsc 1 transfected COS-7 cells.

In summary, these data identify desmoglein 1 and desmocollin 1 as autoantigens in a patient with IgA pemphigus. To our knowledge this is the first IgA pemphigus case with a dual autoantigen-reactivity.

055

Antigen-specific ELISA for detection of circulating autoantibodies to extracellular matrix protein 1 in lichen sclerosus

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Lichen sclerosus is a common mucocutaneous scarring disease of unknown etiology. However, accumulating evidence suggests an autoimmune component. Recently, circulating autoantibodies to the glycoprotein, extracellular matrix protein 1 (ECM1), have been reported in the sera in the majority of subjects with lichen sclerosus. However, very little is known about the potential clinical relevance of these anti-ECM1 antibodies. In this study, we examined ECM1 epitopes in lichen sclerosus sera to develop an ELISA for the serologic diagnosis of the disease, and correlated the ELISA titer with clinicopathological parameters. Using a series of bacterial recombinant proteins, most lichen sclerosus sera (28/39, 72%) recognized the distal second tandem repeat domain and carboxyl-terminus of ECM1. By antigen-specific ELISA, we analyzed serum autoantibody reactivity against the most immunodominant ECM1 epitope in 413 individuals (95 with lichen sclerosus, 161 normal controls, and 157 with other autoimmune basement membrane or sclerosing diseases). The results showed that the ECM1-ELISA is highly sensitive as 76 of 95 patients with lichen sclerosus (83%) exhibited IgG reactivity, and also highly specific (94%) in discriminating lichen sclerosus from other disease/control sera. Higher ELISA titers correlated with more longstanding and refractory disease, as well as cases complicated by squamous cell carcinoma (n=3). Our findings suggest that the ECM1-ELISA is a valuable tool for the accurate detection and quantification of anti-ECM1 autoantibodies, which may be relevant to the clinical status of patients with lichen sclerosus.

056

CGRP induces nitric oxide in human keratinocytes through modification of intracellular nitrosothiols

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We previously showed that calcitonin gene-related peptide (CGRP) induced intracellular nitric oxide (NO) in immortalized human keratinocytes. This NO production was biphasic, with an initial peak within minutes, and an additional upregulation from 24 to 48 hrs. Late NO could be attributed to the observed CGRP induction of iNOS. However, early (within 15 min) NO could not be attributed to induction of iNOS. We therefore investigated a possible source of early NO. S-nitrosothiols have been considered to be a NO reservoir, and can be important for intercellular signaling and vasodilation as well as in intracellular signaling mechanisms. Our hypothesis was that the early burst of CGRP-induced NO was due to release of NO from nitrosothiols. Human telomerase immortalized keratinocytes were treated with CGRP (0.1nM to 100nM), for time periods up to 48 hrs. This treatment decreased levels of nitrosothiols in a dose related manner during the time frame corresponding to non-iNOS associated NO. Nitrosothiols were decreased significantly by CGRP within 15 min. The levels were remained lower than control for at least one hour but returned to control by 3 hours. UVB did not have this effect. Neither NO production during this time nor nitrosothiol depletion was affected by an iNOS inhibitor. Nitrotyrosine levels were not affected at any time point examined up to 24 hrs by CGRP at the concentrations used. This data suggests that CGRP may exert subtle but meaningful effects on keratinocyte intracellular signaling or cytokine expression by means of NO release from pre-stored nitrosothiols.

057

Lysophosphatidic acid induces chemotaxis, oxygen radical production, CD11b up-regulation, Ca2+-mobilization and actin reorganization in human eosinophils via G proteins

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Eosinophils play a major role in the pathopysiology of atopic diseases. Lysophosphatidic acid (LPA) is a bioactive lipid mediator, which is generated by secretory type II phospholipase A2 and is thought to play a major role in the pathogenesis of atopic diseases. Here, the biological activity of LPA on human eosinophils was characterized. We showed by reverse transcription and polymerase chain reaction that human eosinophils express the mRNA of the LPA receptors EDG-2 and EDG-7. Experiments revealed that LPA has chemotactic activity towards eosinophils, stimulates the production of reactive oxygen metabolites, and induces up-regulation of the integrin CD11b. Signal pathway measurements indicated Ca2+-mobilization from intracellular stores and transient actin polymerization upon stimulation with LPA. Cell responses elicited by LPA were inhibited by pertussis toxin indicating that in eosinophils the LPA receptor(s), presumable EDG-2 or/and EDG-7, couple to Gi/o proteins. Moreover, LPA-induced activation of esosinophils could be completely blocked with the EDG-2/EDG-7 antagonist diacylglycerol pyrophophate. These results indicate that LPA is a strong chemotaxin and activator of eosinophils. These findings point to a novel role of LPA in the pathogenesis of diseases with eosinophilic inflammation such as atopic diseases as chemotaxin as well as activator of proinflammatory effector functions.

058

Inosine stimulates chemotaxis, Ca2+-transients and actin polymerization in immature human dendritic cells via a pertussis toxin-sensitive mechanism independent of adenosine receptors

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Inosine is an endogenous purine nucleoside, which is formed by adenosine deaminidase during adenosine breakdown and is released into the extracellular space from the sympathetic nervous system or injured cells. Here, we studied the biological activity of inosine on human dendritic cells (DC), which are specialized antigen presenting cells characterized by their ability to migrate from the blood to peripheral tissues, and then to secondary lymphoid organs where they initiate adaptive immune responses. In immature DC, inosine concentration-dependently stimulated Ca2+ transients, actin polymerization and chemotaxis. Experiments with adenosine receptor antagonists and pertussis toxin as well as desensitization studies suggested that the activity of inosine was mediated by a G protein-coupled receptor pathway independent of adenosine receptors. DC induced to mature by lipopolysaccharide lost their ability to respond towards inosine with these activities. Moreover, inosine did neither influence membrane expression of CD54, CD80, CD83, CD86, HLA-DR and MHC class I molecules nor modulated secretion of interleukin (IL)-12, IL-10 and tumor necrosis factor alpha in immature and lipopolysaccharide-matured DC. In aggregate, our study indicates that inosine may be involved in the trafficking control system of immature DC, and mediates its chemotactic activity by a pertussis toxin-sensitive mechanism independent of adenosine receptors.

059

Alopecia areata induced in young C3H/HeJ mice by interferon-gamma

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Hair follicle epithelium is an immune privileged site, with minimal expression of MHC class I and II. Paus proposed that alopecia areata results from loss of immune privilege with induction of MHC class I and II, followed by loss of tolerance to autoantigens. Interferon-gamma (INF-g) is known to induce MHC class I and II on hair follicle epithelium. C3H/HeJ mice develop spontaneous alopecia areata with age (20% at 12 months). It was hypothesized that injection of INF-g would induce loss of tolerance and alopecia areata in genetically susceptible young C3H/HeJ mice. C3H/HeJ mice (4 mice/group; 8 wks old) were injected intravenously with INF-g 2 X 10e4 U or saline for 3 consecutive days, and at days 7 and 14. Generalized alopecia was observed in all 4 mice injected with INF-g, and in none of the mice injected with saline. Histology demonstrated peri-follicular mononuclear cell infiltrate with dystrophic hair follicles. Regrowth of white hairs was noted 2 wks after the last injection of INF-g. The histology, and regrowth of white hairs supports alopecia areata, rather than telogen effluvium as the cause of hair loss. This data supports the hypothesis that alopecia areata results from loss of immune privilege, and provides a new model for induction of the disease.

060

Correlation of anti-desmoglein-1 epitope profiles with disease status in endemic pemphigus foliaceus

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Fogo Selvagem (FS) is an endemic form of pemphigus foliaceus caused by pathogenic autoantibodies against desmoglein-1 (Dsg1). Autoantibody titers in the sera of patients roughly correlate with disease severity. Using eight chimeric recombinant proteins that contained different ectodomains (EC1, EC1-2, EC1-3, EC1-4, EC2-5, EC3-5, EC4-5, and EC5) of Dsg1, we have previously found that patients with active disease all have anti-Dsg1 antibodies specific for the EC1 and/or EC2 domains, whereas patients in clinical remission have anti-Dsg1 specific for EC5 only. Two major epitope profiles of anti-Dsg1 antibodies were revealed in patients with active disease. About 50% of sera showed reactions predominately with the chimerias containing either the EC1 or EC2 domain of Dsg1 (profile 1). The other half of the sera recognized all eight chimeras (profile 2), indicating reactivity with the EC1 and EC5 domains. The aim of this study was to analyze the relationship between the anti-Dsg1 epitope profiles, the antibody immunofluorecent titers and the disease status. We found that sera with higher antibody titers were prone to have epitope profile 1, whereas sera with low antibody titers predominantly showed epitope profile 2. Of the 15 sera with antibody titers > 1:160, 12 sera (80%) displayed epitope profile 1. On the contrary, 10 of 11 sera (91%) with negative or antibody titers < 1: 80 presented epitope profile 2. This trend was statistically significant when analyzed by McNemar test. Interestingly, sera showing epitope profile 1 were all collected from patients with severe disease. Sera showing epitope profile 2 were obtained from patients with mild disease who were at: 1) early stage of FS, 2) FS entering remission, or 3) early relapse of FS. This study demonstrates that determination of anti-Dsg1 epitope profiles is a novel tool to assess disease activity in FS. Profile 1 is a feature of patients with extensive disease whereas profile 2 reflects disease with minimal skin involvement.

061

Minocycline inhibits antigen processing for presentation to human T lymphocytes: possible anti-inflammatory mechanism

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Minocycline (MINO) has long been used for acne as an anti-inflammatory agent. Recent clinical trials suggest it has anti-inflammatory action in rheumatoid arthritis and multiple sclerosis. MINO has 4 ionizable groups with pKa1=2.8; pKa2=5.0; pKa3=7.8; and pKa4=9.3 and an isoelectric point of 6.4 which should buffer a pH4.7 lysosome. Chloroquine inhibits antigen processing, and T-cell responses by interfering with acidification of lysosomes in antigen presenting cells. The ability of MINO to inhibit processing of tetanus toxoid (TT) for presentation to human T-cells was tested. Peripheral blood monocytes were incubated with TT for 1hr then fixed with 0.12% paraformaldehyde and added to an autologous TT responsive T-cell line. T-cell proliferation was determined by 3H-thymidine uptake. MINO (0.1 - 0.4mM) or chloroquine (0.4mM) were added to test groups for 4hr incubation prior to addition of TT, and fixation. MINO gave significant inhibition (65-100%) of T-cell response to TT and was epi-potent to chloroquine. Inhibition was not observed when TT was added for 1hr prior to the MINO, indicating that presentation of processed antigen was not inhibited. MINO and chloroquine have a high tissue distribution in lysosomes, and chloroquine is known to be inhibitory in humans. Both drugs are administered at similar doses, suggesting that the in vitro inhibition by MINO has relevance to clinical use.

062

Passive transfer of epidermolysis bullosa acquisita antibodies induces blisters in human skin grafted onto nude mice

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Epidermolysis bullosa acquisita (EBA) is an antibody-mediated autoimmune blistering disease in which patients develop subepidermal blisters and antibodies directed against type VII (anchoring fibril) collagen. The dysfunction of anchoring fibrils caused by these autoantibodies to type VII collagen may play an important role in the pathogenesis of EBA. However, direct evidence for a pathogenic role of EBA autoantibodies has not been demonstrated. To develop an animal model that would duplicate the findings in the skin of patients more closely, full-thickness normal human skin was grafted onto nude mice. Purified IgG fractions prepared from two EBA patients and two normal control sera were injected into the dermis of the human grafts (total dose of 10 mg of IgG per graft) every 24 hours for one week. Human skin grafts injected with either fraction of purified EBA IgG autoantibodies developed widespread erosions, crusted lesions and blisters. By clinical, histological, and immunological parameters, the induced lesions were reminiscent of human EBA. Histological examination of tissue sections from lesion sites demonstrated epidermal-dermal separation in the sub-lamina densa zone. Direct immunofluorescence of biopsies obtained from human skin grafts injected with purified EBA IgG antibodies showed IgG deposits at the basement membrane zone (BMZ). Indirect immunofluorescence of sera from mice injected with EBA IgG antibodies showed high titers of circulating antibodies that labeled the BMZ of human skin even 3 weeks after the termination of injections. Similar concentrations of control normal IgG did not induce any pathology when injected into human skin grafts. We conclude that antibodies to type VII collagen play an important role in the pathogenesis of EBA. This experimental human skin graft mouse model may provide a useful tool for dissecting the molecular and immunological mechanisms of subepidermal blister formation in EBA and for developing novel strategies for treatment of EBA.

063

A novel flow cytometry-based assay for direct enumeration and characterization of human autoantigen-specific T cells in pemphigus vulgaris

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Pemphigus vulgaris (PV) is a blistering skin disorder mediated by autoantibodies targeting the epidermal adhesion molecule desmoglein 3 (Dsg3). Dsg3-specific T cells, responsible for coordination of the humoral immune response, have been identified in the peripheral circulation of PV patients and it has been suggested that a shift from Th1- to Th2-predominant activity is associated with active disease (Veldman et al. JI170:635,2003). Accurate data characterizing these cells is not yet available, however, due to limitations in technologies for the analysis of antigen-specific T cells. We have developed a novel technique combining cell surface matrix technology with flow cytometry to enumerate and characterize autoreactive T cells. Our method enables simultaneous detection of multiple cytokines secreted by single cells and enumeration of rare cells present at a frequency as low as 1 in 10^6 cells, compared with a resolution of 1 in 10^4 cells for intracellular cytokine staining. Using this method, peripheral blood mononucleocytes sampled from PV patients stratified by active (n=10) or remittive disease (n=4) and healthy HLA-matched controls (n=4) were tested for activation by recombinant extracellular Dsg3. We evaluated IFN-γ-producing CD4+ cells (Th1), IL-10 or IL-4-producing CD4+ cells (Th2), and the non-standard populations of CD4+ IFN-γ and IL-4 or IL-10-producing cells. An autoantigen-specific CD4+ IL-10 response and a CD4+ IL-4 response were each detected in 1 patient with active disease, while CD4+ IFN-γ responses were detected in 2 patients with remittive disease. One patient with remittive disease was shown to convert from Th1 to Th2 response with the initiation of disease activity. IFN-γ secretion by CD4- cells was detected in 4 patients. These results are consistent with the prevailing ideology that Th1 cells exert a restraining role on Th2 lymphocytes that may be overpowered by a rampant Th2 response during the course of disease development. The CD4- T cell response is provocative and warrants further investigation.

064

A passive transfer model of epidermolysis bullosa acquisita using antibodies generated against the noncollagenous (NC1) domain of human type VII collagen on human skin grafted onto mice

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Epidermolysis bullosa acquisita (EBA) is an autoimmune subepidermal blistering disease associated with autoantibodies to type VII collagen, a major component of anchoring fibrils. EBA autoantibodies recognize four major immunodominant epitopes localized within the amino-terminal noncollagenous (NC1) domain of type VII collagen. We previously showed that passive transfer of a high titer rabbit polyclonal antibody against the NC1 domain of type VII collagen into SKH 1 hairless mice induced skin blisters. To develop a more relevant animal model that would better mimic the EBA disease process, full-thickness human skin was grafted onto either immunodeficient nude mice or immunocompetent SKH 1 hairless mice. IgG purified from a rabbit immunized with NC1 was injected intradermally into the skin grafts (total dose of 10 mg of IgG per graft) every 24 hours for up to 5 days. Human skin grafted on either immunodeficient or immunocompetent mice injected with anti-NC1 IgG developed widespread erosions and crusted lesions. By clinical, histological, and immunological criteria, the induced lesions were reminiscent of human EBA. Tissue sections from lesion sites demonstrated epidermal-dermal separation in the sub-lamina densa zone. Injected antibodies bound to the basement membrane zone (BMZ) of human skin grafts in vivo by direct immunofluorescence. Serum samples from mice receiving anti-NC1 IgG antibodies had high titers of circulating antibodies that labeled the BMZ of human skin. Human skin grafts injected with similar concentrations of control IgG purified from normal rabbits showed no pathological changes. We conclude that antibodies to the NC1 domain of type VII collagen play an important role in the pathogenesis of EBA. This experimental mouse model can be used to identify pathogenically relevant epitopes on NC1 and may provide a useful tool for dissecting the molecular and immunological mechanisms of subepidermal blister formation in EBA.

065

Molecular characterization of OAS1 overexpression in cutaneous LE suggests a novel apoptotic pathway relevant in disease

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The molecular basis for disease initiation and mechanisms of progression in systemic and cutaneous LE are not well understood. Experimentally there is a generalized immune dysregulation that is at least in part genetically based, resulting in a broad spectrum of autoantibody specificities associated with a combination of humoral and cellular abnormalities that ultimately lead to tissue destruction. There is mounting evidence that abnormal apoptotic pathways contribute to the immune dysregulation in LE. Apoptosis is crucial for maintaining cellular homeostasis and occurs as a part of normal keratinocyte differentiation. However, increased apoptosis is observed in keratinocytes present in the basal and suprabasal layers of cutaneous LE lesions. Release of apoptotic debris locally within tissues or into the circulation could then result in heightened autoantigen display and support the generation of and/or preservation of autoimmune lymphocytes. Moreover, there is evidence for resistance to apoptosis in LE peripheral lymphocytes with autoreactive potential. However, the exact mediators of altered apoptosis in cutaneous LE remain to be elucidated. In microarray analysis of global gene expression in cutaneous LE, we observed the up-regulation of a number of apoptotic genes - including the interferon-inducible pro-apoptotic OAS1 gene encoding the small (p42) 2′-5′-oligoadenylate synthetase - in the skin and peripheral blood of patients. This observation is of potential significance to disease as recent work suggests a link between small (OAS1) 2′-5′-oligoadenylate synthetase gene expression and isozyme activity with apoptosis in vitro. Molecular analysis reveals the 9-2 isoform of the OAS1 gene is overexpressed in cutaneous LE skin and blood. To our knowledge, our data provide the first experimental evidence demonstrating an increased expression of the small 2′-5′-oligoadenylate synthetase gene OAS1 in human autoimmune disease, suggesting novel apoptotic pathways of relevance in cutaneous LE.

066

Divergent dose-dependent responses to arachidonic acid in normal human keratinocytes

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Arachidonic acid (AA), a C20:4 Δ5,8,11,14-eicosatetraenoic acid, is a precursor fatty acid in the synthesis of many pro-inflammatory lipid mediators. In recent years, oxidative stress and inflammation have been recognized as inseparable phenomena. Similarly, inflammation and malignancy seem to be related biological responses. Chronic inflammation causes loss of epithelial polarity, altered deposition of extracellular matrix proteins, enhanced cellular migration and dedifferentiation of epithelial cells. We have therefore investigated the pro-inflammatory mediator, AA, and its effect on keratinocyte viability, stress-activated signaling and cell morphology. To assess cell viability, fluorescent markers, calcein-AM and ethidium homodimer-1, identifying live and dead cells, respectively, were used. Keratinocytes were exposed to AA concentrations ranging from 1 to 100 μM. Live/dead read-outs obtained by flow cytometry were highly reproducible. 100% of cells exposed to 100 μM AA and 70% of cells exposed to 30 μM AA died within 24 hours post exposure. In contrast, 0% of cells exposed to 10 μM AA or less died within 24 hours, even if they were re-exposed hourly to AA. Instead, these cells exhibited impressive morphologic alteration with lamellipodia and filopodia formation. Adenoviral overexpression of catalase but not superoxide dismutase completely inhibited these morphology changes. Moreover, catalase-overexpressing keratinocytes were more prone to AA-induced cell death than βGal-infected controls. These findings implicate divergent dose-dependent oxidative/survival vs. pro-oncogenic roles for AA, where lower concentrations of AA facilitate migratory responses and cell survival in cultured human keratinocytes.

067

Pemphigus mouse model as a tool to evaluate various immunosuppressive therapies

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Pemphigus vulgaris (PV) is an autoimmune bullous skin disease caused by IgG autoantibodies against a cadherin type adhesion molecule, desmoglein 3 (Dsg3). We have generated an active disease mouse model for PV by adoptive transfer of lymphocytes from immunized Dsg3-/- mice to Rag2-/- mice. The mice stably produce anti-Dsg3 IgG without general immune activation and show weight loss due to inhibition of food intake by mucosal erosions. In this study, we used this model to evaluate effects of two kinds of corticosteroids and five immunosuppressive agents on the pathogenic antibody production and development of the PV phenotype. Treatment was initiated at the time of the adoptive transfer and continued for 4 weeks and the mice were evaluated for 8 weeks in general. Five PV model mice were treated along with five untreated control mice. We used dexthamethasone (DEX), methylprednisolone (m-PSL), azathioprine (AZP), cyclophosphamide (CPA), cyclosporine A (CYA), tacrolimus hydrate (FK506), and mycophenolate mofetil (MMF). Compared to untreated control mice at week 2, the titers of anti-Dsg3 IgG and the degree of weight loss were, respectively, 0.83 and 0.88 in mice treated with DEX (i.p. 10 mg/kg daily), 0.70 and 0.88 in mice treated with m-PSL (i.p. 100 mg/kg daily), 0.33 and 0.75 in mice treated with AZP (p.o. 30 mg/kg daily), 0.03 and 0.03 in mice treated with CPA (i.p. 40 mg/kg three-times a week), 0.20 and 0.27 in mice treated with CYA (i.p. 40 mg/kg daily), 0.68 and 1.42 in mice treated with FK506 (i.p. 5mg/kg daily), 1.22 and 0.81 in mice treated with MMF (p.o. 80 mg/kg daily). Thus, we could grade various immunosuppressive therapies on their effects to pemphigus model mice. Throughout the course of this study, CPA showed the most significant therapeutic effect on the pathogenic anti-Dsg3 IgG production as well as the appearance of the PV phenotype. Although there is difference between mice and human, this model provides a unique tool to evaluate and compare in vivo effects of various therapeutic strategies against organ-specific autoimmune diseases.

068

Non-pathogenic anti-Dsg3 monoclonal IgG antibodies become pathogenic and induce pemphigus vulgaris phenotype in combination

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Pemphigus vulgaris (PV) is an autoimmune blistering disease caused by anti-desmoglein3 (Dsg3) IgG antibodies. We have obtained 8 AK and 10 NAK anti-Dsg3 IgG monoclonal antibodies (mAbs) from PV model mice receiving immunized and naive splenocytes from Dsg3-/- mice, respectively. In this study we evaluated the blister-inducing activity of NAK mAbs in monoclonal vs. polyclonal condition in two different assays; *in vitro* keratinocyte dissociation assay and ascites formation assay. In dissociation assay normal mouse primary keratinocytes were incubated with NAK mAbs and obtained a dissociation index by comparing numbers of cell particles after mechanical stress by pipetting. In ascites formation assay, hybridoma cells were inoculated in peritoneum of Rag2-/- mice and development of the PV phenotype was evaluated. The dissociation assay showed that all the NAK mAbs had low activities. None of the NAK mAbs induced the PV phenotype when single clones were inoculated in mice. In contrast, when several clones recognizing different Dsg3 epitopes were mixed the dissociation assay showed high activities equivalent to that of AK23, which was previously shown to be pathogenic. Furthermore, when several clones of NAK mAbs were combined and inoculated in mice, the mice developed the PV phenotype, including oral erosions with suprabasilar acantholysis in histology. These findings indicated that non-pathogenic anti-Dsg3 mAbs show the pathogenic activity in inducing the loss of cell adhesion of keratinocytes with resultant PV blister formation when combined. Considering patients' sera are polyclonal, these mAbs provide a valuable tool to mimic the human disease and dissect molecular mechanism of blister formation in PV.

069

Desmogleins expression in regulating pemphigus activity

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Pemphigus is an autoimmune disease caused by anti-desmogleins autoantibodies, toward the components of desmosome, cell adhesion structure between keratinocytes. Systemic administration of glucocorticoid has been used effectively for the treatment of pemphigus patients. Glucocorticoid can reduce serum concentration of anti-desmogleins antibodies through its immunosuppressive effect in a long term. Although its effect usually appears within a few days of administration, clinically, the mechanism of its effect in a short period remains to be elucidated. We compared mRNA and protein tissue expression of desmoglein 1 and desmoglein 3 by means of in situ hybridization and immunostain, in three pemphigus patients before and one or two weeks after the treatment. Also, the change of the amount of desmogleins mRNA in serum were analyzed with semiquantitative RT-PCR in nine pemphigus patients. The mRNA and protein tissue expression of desmoglein 1 and 3 decreased markedly in two patients in whom glucocorticoid treatment was effective. In another patient who did not respond to glucocorticoid treatment the desmogleins expression increased, instead. The RT-PCR analysis also revealed that the serum concentration of desmogleins mRNA decreased in whom glucocorticoid treatment was effective. In all patients serum concentration of anti-desmogleins antibodies, measured in ELISA, were not changed significantly within one or two weeks after the treatment. We speculated that glucocorticoid showed its immediate effect by suppressing desmogleins expression transcriptionally, then reducing the immunecomplex formation in situ and halting the acantholytic change of keratinocytes. Searching the regulator of desmoglein expression other than glucocorticoid may lead a new class of pemphigus treatment.

070

Low-producing polymorphisms of mannose binding lectin but not the C1qA SNP are associated with DM

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Mannose binding lectin (MBL) and C1q are structurally related molecules that bind apoptotic blebs and are thought to play a role in the non-inflammatory clearance of autoantigens on apoptotic cells. Deficiencies of these two molecules may contribute to photosensitive cutaneous autoimmune diseases: dermatomyositis (DM), but not subacute cutaneous lupus erythematosus (SCLE), is associated with low-producting MBL single nucleotide polymorphisms (SNPs) (Werth et al, 2002), and SCLE has been associated with C1qA, a low-producing C1q SNP (Racila et al, 2003). We now sought to compare in a single study the frequencies of low-producing MBL and C1q SNPs in four groups: adult DM (n = 73), SCLE (n = 53), discoid LE (DLE, n = 52), and healthy controls (n = 75). DNA was isolated from buffy coats, and allele-specific polymerase chain reactions (PCR) were used to genotype the MBL (Asp^{54}, Glu^{57}, and the LX promoter polymorphism at −221) and C1qG/A ($Gly70_{GGG}Gly\ 70_{GGA}$) polymorphisms in each patient and control. We found that the incidence of lowest-producing combinations of MBL polymorphisms (group 3) was increased in DM, with 27% of DM patients having lowest-producing variants vs. 12% of SCLE, 17% of DLE, and 9% of controls ($p \leq 0.025$ by χ^2 test, 4x2 table). We found the incidence of the low-producing C1qA SNP genotype was 36% in SCLE, 22% in DM, 21% in DLE, and 35% in controls (NS). To compare the contributions of individual low-producing SNPs, we found significant differences between diseases in the numbers of patients with low-producing MBL SNPs but wild-type C1q (47% of DM, 24% of SCLE, 25% of controls) vs. low-producing C1q SNPs but wild-type MBL (10% DM, 19% of SCLE, 17% controls; p=0.034). Thus, low-producing MBL SNPs, but not the low-producing C1qA polymorphism is associated with DM.

071

Detection of serum IgE auto-antibodies against epidermal proteins in severe forms of atopic dermatitis

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Many symptoms of atopic diseases result from events initiated by cross-linking of cell-bound IgE specific for exogenous allergens. Previous studies showed that sera of patients with severe forms of atopic dermatitis might also contain IgE specific for self-proteins, supporting the hypothesis of autoreactivity as pathogenic factor in atopy. Because these studies were mainly performed with extracts of cell lines and peripheral blood-derived mononuclear cells, it remained unknown whether sensitization to skin-derived self-proteins may occur in atopic dermatitis. Addressing this question, we analyzed the sera of 21 atopic dermatitis patients and 10 healthy donors for specific IgE antibodies against protein extracts of normal epidermis, dermis and the epithelial cell-line A431. No specific reactivity against these extracts was detectable when sera derived from healthy persons were tested in western-blots. In contrast, four patients with severe forms of atopic dermatitis (defined by high levels of total serum IgE and a high investigator global assessment score) exhibited IgE-reactivity against A431-derived proteins, thus confirming previous findings. Importantly, serum IgE of two of these four patients recognized also proteins in epidermal extracts. These latter proteins exhibited molecular weights different from those detected in A431 extracts indicative of distinct IgE specificities. Interestingly, all analyzed sera failed to react with dermal protein extracts. These results suggest that elicitation of auto-reactive IgE against epidermal self-proteins occurs in severe forms of atopic dermatitis, possibly contributing to the chronicity of the disease.

072

Fas ligand-mediated keratinocyte apoptosis in fixed drug eruption

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The pathogenesis of fixed drug eruption (FDE) is not fully understood. Histologically, like lichen planus, graft-versus host reaction, Stevens-Johnson syndroma, and toxic epidermal necrolysis, FDE is characterized by keratinocyte apoptosis. The aim of this study is to clarify the role of cytotoxic T cells and Fas/Fas ligand (L) pathway in keratinocyte apoptosis of FDE. Lesional punch biopsy specimens and normal skin samples of 32 patients with established FDE were evaluated. Apoptosis was assessed by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling(TUNEL). Immunohistochemistry for Fas, FasL, and caspase-3 as well as CD4 and CD8 was performed. In contrast to normal skin showing focal apoptosis confined to stratum corneum and/or granulosum, there was marked apoptosis of lesional keratinocytes. Evaluation of the subpopulation of T cells infiltrated into the epidermis demonstrated the dominant CD8+ T cells. Compared to normal skin and old lesions, FasL expression was higher in the early acute lesional keratinocytes and some dermal lymphocytes. These FasL positive cells were also positive for caspase-3. Our results strongly suggest that a potential mechanism of keratinocyte death in FDE is apoptosis, which may be mediated by CD8+ cytotoxic T cells and the Fas/FasL pathway.

073

V-gamma-5 dendritic epidermal T cells are required for optimal regulation of cutaneous inflammation and protection of the epidermal barrier

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We have previously demonstrated that mice deficient in all γδ T cells demonstrate augmented dermatitis responses, as well as a defective transepidermal water barrier (TEWB). To investigate the role of Vγ5+ dendritic epidermal T cells (DETC) in the regulation of cutaneous inflammation and maintenance of the TEWB, we backcrossed (10+ generations) the T cell receptor (TCR)δ-/- and Vγ5-/- mutations each onto the FVB background. While the TCRδ-/- mice show no intraepidermal γδ(+) T cells, the Vγ5-/- mice demonstrate normal numbers of Vγ5(-) γδ(+) DETC. Ear swelling responses to irritant (tetradecanoylphorbol acetate; TPA) and allergen (dinitrofluorobenzene; DNFB) challenges were assessed, and skin surface hydration (SSH) was determined as instantaneous ($t = 0$ sec) skin surface electrical capacitance (SSEC; NOVA Dermaphase Meter 2003) while transepidermal water loss (TEWL) was measured as the change in SSEC over time (1 to 10 sec). At 24hrs after exposure to a 40 nmol TPA irritant challenge, both TCRδ-/- and Vγ5-/- mice showed increased ear swelling (19.4±1.2 mm^{-2} and 16.6±1.2 vs 10.3±0.9 in controls, $p<0.00001$), as well as increased SSH (138.6±7.7 picoFarads and 124.6±2.4 vs 112.2±3.4 in controls; $p<0.004$ for both). TEWL was significantly greater in the TCRδ-/- (3.64±0.52, $p<0.005$) but not the Vγ5-/- mice (2.14±0.23), relative to controls (1.86±0.30). Following challenge with 0.2% DNFB to ear skin, one week after sensitization with 0.5% DNFB to abdominal skin, both TCRδ-/- (25.7±2.4, $p<0.0001$) and Vγ5-/- mice (17.2±3.0, $p=0.01$) showed increased ear swelling relative to controls (10.2±0.9). Hence, a major component of the capacity of γδ T cells to regulate cutaneous inflammation and maintain epidermal integrity is mediated by Vγ5(+) DETC; however, other non-Vγ5 γδ(+) DETC may also have functional activity.

074

Estradiol and the C1QA G288>A (AF 135157) exonic single nucleotide polymorphism control SC35 splicing factor activity in the processing of C1QA pre-mRNA

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Complete congenital deficiency of C1q, the first component of the classical pathway of complement, is the strongest single genetic risk factor for the development of systemic lupus erythematosus (SLE) yet identified. Greater than 90% of individuals so affected develop SLE. In addition to its regulatory roles in immune complex diseases such as SLE (immune complex solubilization, modulation of B cell and T cell function), C1q is being increasingly recognized to play a multifunctional role in the innate immune response. Functional human C1q consists of 6 subunits of a heterotrimer formed by the association of three related but distinct gene products: C1qA, C1qB, and C1qC. Using a candidate gene approach, we recently observed that a new SNP in the second exon of the C1QA gene (C1QA G288>A [AF 135157])(C1QA G288>A) is strongly associated with photosensitive subacute cutaneous LE (SCLE) skin lesions and relatively low serum C1q levels (Lupus 12:124-132, 2003). A web-based exonic splicing enhancer (ESE) identification algorithm (ESE Finder) predicted that the C1QA G288>A SNP disrupts an ESE that serves as a binding site for the estradiol-regulated SC35 splicing factor (syn. SFRS2). Using 5'/3' RACE PCR and Southern blotting, we now have evidence that C1qA pre-mRNA does in fact undergo alternative splicing. In homozygous C1QA G288>A individuals, the resulting spliced mRNA appears to encode a truncated form of C1qA that is missing the first exon. We speculate that this truncated variant of the C1qA polypeptide is less efficient in associating with C1qB and C1qC polypeptides to form fully functional C1q molecules. Relatively deficient C1q levels in homozygous C1QA G288>A individuals could interfere with C1q's immunoregulatory functions including participation in the physiologic clearance of apoptotic cells thereby potentiating the development female-dominant autoimmune diseases such as SLE and SCLE.

075

Role of TNF-α, IL-1, and IL-6 in experimental autoimmune bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune inflammatory disease characterized by subepidermal blisters and autoantibodies against two hemidesmosomal proteins - BP230 and BP180. Inflammatory cells, including eosinophils, neutrophils, lymphocytes, and monocytes/macrophages are identified in the upper dermis and bullous cavity. Various inflammatory mediators that can recruit and activate inflammatory and residential cells are present in lesional skin and/or blister fluids of BP patients. We previously showed that passive transfer of anti-BP180 IgG into neonatal mice induces a BP-like disease in the animals. Subepidermal blistering in experimental BP depends on complement activation, mast cell degranulation, and neutrophil infiltration. TNF-a released by activated mast cells plays a critical role in neutrophil recruitment and subsequent blistering. In this study we investigated the role of IL-1 and IL-6 and their functional relationship with TNF system using the BP mouse model. Mice deficient in TNF-α were resistant to experimental BP with minimal neutrophil infiltration. Levels of IL-1 and IL-6 in the skin of TNF-α-deficient mice at the pathogenic IgG-injection site were significantly reduced compared to wild-type (WT) control. TNF-α-deficient mice when reconstituted locally with recombinant IL-1 or IL-6 became susceptible to experimental BP. Pretreatment of WT mice with IL-1 or IL-6 neutralizing antibody blocked the pathogenic activity of anti-BP180 IgG. Furthermore, mice deficient in IL-1 receptor or IL-6 were resistant to experimental BP. These results suggest that TNF-αupregulates levels of IL-1 and IL-6, which in turn leads to neutrophil recruitment causing subepidermal blistering triggered by anti-BP180 IgG.

076

Cutaneous gene expression by DNA microarray in murine sclerodermatous graft versus host disease, a model for human scleroderma

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Chronic graft versus host disease (GVHD) is a major complication of allogeneic bone marrow transplantation that can have autoimmune features resembling scleroderma (sclerodermatous GVHD). The molecular mechanisms governing skin fibrosis in Scl GVHD are not known. We used DNA microarrays representing > 14,000 mouse genes to characterize global gene expression in skin during early stages of GVHD from irradiated BALB/c (H-2d) mice transplanted with B10.D2 (H-2d) bone marrow and spleen cells. These mice develop skin thickening, while control mice transplanted with BALB/c (H-2d) bone marrow and spleen cells do not develop skin thickening. We found consistent differences in gene expression between mice with Scl GVHD and controls. Cytokine mRNA for both Th1 (IFN gama) and Th2 (IL-6, IL-13, IL-4) processes and chemokine mRNA (MCP-1, RANTES) were elevated. TGF beta 1, CTGF and PDGF mRNA were elevated, similar to results in human scleroderma. This validates the murine model and identifies potential markers for the disease. Such marker will help to understand sclerodermatous pathogenesis and to devise more effective strategies for intervention in early scleroderma and GVHD.

077

Innate immunity in the development of murine sclerodermatous GVHD, a model for scleroderma

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Molecular mimicry due to bacterial/viral infection has been proposed as a possible explanation of autoimmune diseases such as scleroderma, a Th2 predominant immune process. In addition to providing cross-reactive proteins, viruses and bacteria also provide components that can interact with innate immunity through toll-like receptors (TLRs). We are modeling human scleroderma with murine sclerodermatous graft versus host disease (Scl GVHD), in which transplantation of bone marrow and spleen cells of B10.D2 (H2d) mice into lethally irradiated BALB/c (H-2d) mice across minor histocompatability loci (MiHC) produces skin thickening and lung fibrosis rather than classic cytotoxic GVHD. In scleroderma and Scl GVHD, monocytes/macrophages are prominant in cutaneous inflammatory infiltrates. Examining the MiHC reaction in vivo in Scl GVHD, and in vitro, in mixed leukocyte reactions (MLR) of spleen cells using donor T cells with host antigen presenting cells (APCs), we have identified activated CD4 T cells as the major responding cell. When TLR agonists CpG oligonucleotide and lipopolysaccharides are added to MLR we see an increase in T cell proliferation when either dendritic cells (DCs) or macrophage are used as APCs. CD4 T cells stimulated by splenic DCs show 2-fold increase in the number of T cells producing Th1-like cytokine IFN-gamma, while there is a 9-fold increase in the number of T cells producing Th2-like cytokines IL-4 and IL-10 when incubated with splenic macrophages. These data suggest that MiHC-reactive T cells appear to be differentially responsive to TLR agonists depending on the dominant APC present, and that the interactions between macrophages and TLR agonists may be essential for initiating the events leading to Scl GVHD.

078

IgG4 anti-basement membrane zone (BMZ) antibody is the first and last antibody in prodromal bullous pemphigoid (PBP)

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This study was performed to investigate the role of IgG4 subclass anti-BMZ Ab in PBP patients by using DIF and IIF. Skin specimens were obtained from 45 PBP patients, with sera being available from 26 patients. Among these 26 patients, serial serum specimens were available from 5 PBP patients. RESULTS: Using DIF, 36 patients had IgG and C3 staining at the BMZ, while 9 patients had only C3 staining. When skin samples from these 9 patients were evaluated for IgG subclass deposition by IIF, 4 demonstrated linear IgG4 staining along the BMZ. BMZ Ab were found in 10 PBP patients, while it was negative in the remaining16 PBP patients. By using IIF for IgG subclass BMZ Ab, 4 of these 16 PBP patients were found to have IgG4 BMZ Ab. When the serial serum samples from the 5 PBP patients were analyzed by IgG subclass IIF, antibody titers were found to be higher at the beginning of their disease and most of the IgG subclasses were present. However, as the condition of the PBP patients improved, serum BMZ Ab titers decreased slowly and IgG4 BMZ Ab was the last subclass to disappear. If the patient relapsed or had a worsening of their disease, IgG4 was the first IgG subclass BMZ Ab to be detected and detection of other IgG subclasses BMZ Ab would subsequently follow. CONCLUSION: IgG4 is the predominant BMZ Ab in PBP patients and persists throughout the course of the disease. IgG4 is the first IgG subclass to appear and the last to disappear. There is a switching of circulating BMZ Ab from IgG4 to other subclasses back and forth.

079

Crucial role for intraepidermal T cells expressing the collagen-binding integrin α1β1 in psoriasis

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Psoriasis is a chronic relapsing T cell-mediated skin disorder. Activated T cells migrating into the epidermis seem to be required for disease formation. Trafficking of leukocytes within tissue and thereby generating an inflammatory response is critically dependent on interactions with extracellular matrix (ECM) proteins such as collagens. We investigated the functional expression of a major collagen-binding receptor: α1β1 integrin (VLA-1) in psoriasis. VLA-1 was exclusively expressed by infiltrating epidermal but not dermal $CD8^+$ and $CD4^+$ T cells in active psoriasis lesions. To determine the *in vivo* relevance of VLA-1 expression on activated T cells in psoriasis we took advantage of a recently described xenotransplantation model with spontaneous development of psoriasis *(JEM, in press)*. Uninvolved human skin from patients with psoriasis was engrafted onto AGR mice, deficient in type I and II interferon receptors in addition to being $RAG^{-/-}$. Upon transplantation, mice were treated with either murine anti-human α1-integrin mAb or isotype control antibody. Skin grafts on mice receiving control antibody developed a fully-fledged psoriasis after 6 weeks as expected. Blockade of VLA-1 inhibited psoriasis formation in 6 out of 6 mice (n=3 patients). Anti-VLA-1 treatment resulted in a significant reduction of both acanthosis and papillomatosis index. A significant, more than two-fold expansion of epidermal and dermal T cells during disease formation was observed in transplanted grafts treated with control antibody. Expression of VLA-1 was restricted to the pool of expanded epidermal T cells in converted psoriatic skin. Expansion of intraepidermal VLA-1 expressing T cells was completely blocked after anti-VLA-1 treatment. Our findings suggest a crucial role for intraepidermal VLA-1 expressing T cells in psoriasis and might provide the basis for new strategies in psoriasis treatment focusing on T cell/ECM interactions.

080

Dissecting the role of NF-κB and TNFα on cutaneous inflammation in K5-PKCα overexpressing mice

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Protein kinase C (PKC) isoforms are major regulators of cutaneous homeostasis and mediate inflammation in response to 12-0-tetradecanoylphorbol-13-acetate (TPA). We have previously reported that transgenic mice overexpressing PKCα in the basal layer of epidermis exhibit severe intraepidermal neutrophilic inflammation and keratinocyte apoptosis when treated topically with TPA. Depletion of granulocytes in K5-PKCα mice by ip administration of mAb Ly-6G abolishes intraepidermal neutrophilic infiltrates but does not prevent epidermal apoptosis suggesting that these processes are independently regulated. Activation of PKCα increases the production of TNFα and the transcription of chemotactic factors (MIP-2, S100A8/A9), VEGF and GM-CSF in K5-PKCα keratinocytes. In response to PKCα activation, NF-κB translocates to the nucleus, and this is associated with IκB phosphorylation and degradation. Preventing IκB degradation in K5-PKCα keratinocytes using an adenoviral IκBα mutant prevents NF-κB nuclear translocation and reduces both the expression of inflammation associated genes and chemoattractant release. To determine if TNFα mediated NF-kB translocation and subsequent expression of pro-inflammatory factors, mice were treated systemically with a dimeric soluble form of p75 TNF receptor (Etanercept) and keratinocytes were cultured in the presence of TNFα neutralizing antibodies. The *in vivo* treatment did not prevent inflammation, and the *in vitro* treatment did not prevent TNFα nuclear translocation after TPA. These results were confirmed using TNFR null primary keratinocytes adenovirally transduced to overexpress PKCα and suggest that PKCα can activate NF-κB independently of secreted TNF. Together these results implicate PKCα as a regulator of a subset of cutaneous cytokines and chemokines responsible for intraepidermal inflammation independent of soluble TNFα. Understanding the function of TNFα in this model will await the outcome of current studies using K5-PKCα mice genetically ablated for TNF receptors.

081

Circulating IgA autoantibodies to epidermal transglutaminase 3 can not distinguish dermatitis herpetiformis from celiac disease

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The discovery of tissue transglutaminase (TGase) as the main endomysial autoantigen failed to explain why only a proportion of gluten sensitive patients show symptoms of dermatitis herpetiformis (DH). By comparing the antibody responses to TGase 2 and 3, a recent study suggested that target antigens in DH and celiac disease (CD) are distinct: TGase 2 is the target in CD and TGase 3 in DH. We here provide evidence that TGase 3 is also recognized by IgA antibodies in sera from patients with CD. We examined IgA reactivity of DH and CD sera against a human recombinant TGase 3 and a purified guinea pig TGase 2 by Elisa assay. Selected sera were also tested by Western blot using recombinant TGase 3 and purified TGase 2. The diagnosis of CD was confirmed by presence of anti-endomysial antibody and jejunal biopsy while DH was diagnosed by skin biopsy using both light and immunofluorescence microscopy. Sera were obtained from 69 patients with DH and 45 with CD, as well as 10 with linear IgA disease (LAD) and 10 with cicatrical pemphigoid (CP). Sera from 31 healthy individual and rabbit antibodies to TGase 2 and 3 were used as controls. By Elisa, IgA to TGase 3 was present in 39 of 45 (87%) CD and 62 of 69 (90%) DH sera. IgA to TGase 2 was present in 41 of 45 (91%) CD and 61 of 69 (88%) DH sera. One of 10 CP sera also contained IgA to both TGase 2 and 3. No sera from healthy individuals or LAD contained IgA to TGase 2 or 3. IgA reactivity to TGase 2 and 3 was also confirmed by Western blot assay using selected DH and CD sera.These data provide strong evidence that IgA autoimmune response to TGase 3 is not exclusively present in DH. CD sera also contain IgA to TGase 3. Accordingly, autoimmune response to TGase alone may not explain presence of two clinically distinct forms of gluten sensitive disease, i.e., DH and CD.

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Anti-interleukin-5 decreases dermal eosinophilia but increases epidermal hyperplasia in chronic proliferative dermatitis (*cpdm*) mice

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Chronic proliferative dermatitis (*cpdm*) mice develop chronic eosinophilic inflammation in tissues, most prominently in the skin. To evaluate the role of cutaneous eosinophilia, mutant mice were treated with anti-IL-5 and examined for changes in phenotype. Monoclonal rat anti-mouse IL-5 (TRFK5) was generated from hybridoma cells in serum free medium, concentrated, checked for endotoxin, and diluted in phosphate buffered saline (PBS). Lyophilized rat IgG was diluted in PBS and used as a control. Five pairs of gender-matched *cpdm/cpdm* littermates, 3-4 weeks of age, were injected intraperitonealy three times with 0.2 mL (0.3 mg) of anti-IL-5 or IgG control with 3 day intervals. Mice were euthanized 3 days after the last injection, and blood and skin collected. Absolute and differential leukocyte counts were determined. The number of eosinophils in the blood was decreased 9-fold in mice treated with anti-IL-5 versus controls. The number of eosinophils in the dermis of mice treated with anti-IL-5 was half that of the controls ($790 \pm 180 /mm^2$ versus $1860 \pm 120 /mm^2$). While the eosinophils decreased in mice treated with anti-IL-5, epidermal thickness was unexpectedly and significantly increased (46.8 ± 1.2 µm versus 40.4 ± 2.5 µm). The number of mast cells was not significantly affected. The concentrations of eotaxin (CCL11), IL-4, and IL-5 in skin homogenates were determined by ELISA. The concentrations of eotaxin, IL-4, and IL-5 were significantly increased in the skin of mice treated with anti-IL-5. These results indicate that treatment with IL-5 neutralizing antibodies is less effective in decreasing the accumulation of eosinophils in the skin than in the blood, and causes an unexpected increase of IL-4, IL-5, and eotaxin in the dermis. The increased thickness of the epidermis suggests that eosinophils may inhibit keratinocyte proliferation in the skin of *cpdm/cpdm* mice.

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Corticosteroids prevent acantholysis by strengthening keratinocyte cell-cell adhesion

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Pemphigus Vulgaris (PV) is a disease of skin fragility caused by autoantibodies to the desmoglein class of cell-cell adhesion molecules. To determine the mechanism of corticosteroid therapy in PV, we examined the effect of methylprednisolone on keratinocyte cell-cell adhesion strength. Primary keratinocytes (HEK) or a non-transformed keratinocyte (HaCaT) cell line were treated with methylprednisolone for 24 hours. Monolayers of keratinocytes were detached from the plates by dispase, and then subjected to mechanical disruption (adhesion assay). The ratio of released single cells to total cells was used as an indication of cell-cell adhesive strength. Corticosteroids were found to increase keratinocyte adhesive strength in both HEK and HaCaT cultures. PV IgG dramatically reduced keratinocytes adhesion strength. To determine if corticosteroids could block the effects of PV antibodies on keratinocytes adhesion, cultures were pretreated with methylprednisolone for 24 hours and then incubated with PV or normal IgG (control) for 24 hours. Pretreatment with methylprednisolone inhibited the PV IgG induced decrease in cell-cell adhesion. Our data show that pretreatment with corticosteroids prevents the acantholysis that occurs following incubation with PV antibodies, and also increases keratinocyte cell-cell adhesive strength. The findings that corticosteroids increase keratinocyte adhesive strength irrespective of PV antibody treatment, indicate that the enhancement in desmosomal adhesion is a generalized effect of corticosteroids and not specific to the pathophysiology of PV.

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Profiling *Staphylococcus aureus*-mediated pro-inflammatory cytokine response in keratinocytes

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Skin infections caused by S. aureus continue to pose a serious medical problem and are associated with numerous skin diseases. Keratinocytes, the predominant cell type found in the epidermis and an important part of the immune system, produce a great variety of cytokines. While cytokines are implicated in the development and persistence of inflammatory immune responses and S. aureus is known to induce cytokines, the full scope of cytokine responses to S. aureus infection in primary human keratinocytes is unknown. To elucidate the response of pro-inflammatory cytokines in the pathogenesis of S. aureus infection, we determined cytokine expression by real-time reverse transcription polymerase chain reaction (real-time RT-PCR), an approach that reveals the sequential order of expressed genes. Five pro-inflammatory cytokines were selected: tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), interleukin 1-alpha (IL-1α), IL-1β. These cytokines were tested for their response to S. aureus at a multiplicity of infection (MOI) 100:1 (bacteria:keratinocytes). RNA was extracted at a serial of time points including (t = 0, 2, 4, 6, 8 h) and reverse transcribed to prepare cDNA. Real-time PCR was performed for each time point and β-actin was used as an internal control and for standardization. Our results revealed that TNF-α increased rapidly (2h) and significantly (55x). In contrast, IL-1α, IL-1β increased slowly (6h) and modestly (3-6x). No induction of IFN-γ was observed. Soluble TNF-α was detected by enzyme linked immunoassays in the supernatants of S. aureus infected cells, confirming PCR results. The rapid and significant induction of TNF-α suggests that it plays a critical role in the S. aureus-mediated inflammatory response. TNF-α, a known inducer of other cytokines, is primed to initiate the induction of other inflammatory cytokines (IL-1). Furthermore, these results suggest that persistent TNF-α secretion as a result of S. aureus infection is likely to cause tissue damage and systemic spread of infection.

085

Withdrawn

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Redox targeted therapeutic approach for inflammatory skin diseases

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Recent data suggest that a subset of the triggers of inflammatory cascades resides in redox signaling pathways. While a body of literature linking these processes to dermatological diseases is emerging and our understanding of redox signaling and inflammation-mediated dermatological disease is increasing, a paucity of data remains about developing concerted drug discovery paradigms targeting redox pharmacology. As physical-chemical properties such as charge recognition and transfer are critical in modulating activity of redox-based targets, a counterpart to Structure Activity Relationships, PAR (Physical Chemical Activity Relationships) has been developed focusing on both structural and charge transfer parameters of biological activity. To test this hypothesis, a subset of compounds, diversified based on structure and redox attributes, were selected from a large library of compounds and their in vitro and cell-based anti-inflammatory and redox activities were determined. Compounds representing a distinct redox class demonstrated maximum activity across an assay panel. An expanded set of compounds were selected around the active redox class and further screened in secondary in vitro and in vivo assays of oxidative stress and inflammation. A robust anti-inflammatory activity was noticed with inhibition of ear edema. In addition, the lead compounds significantly reduced production of cytokines by PHA-activated human peripheral blood lymphocytes. The overall hit rate of this subset was ~30%. Herein, we suggest that PAR may be a useful tool to explore redox properties required for biological activity in inflammation-mediated dermatological diseases.

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Caspase activity in inflammatory skin disease

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Caspases have emerged as pivotal mediators in many aspects of tissue homeostasis by controlling cell numbers and eliminating abnormal cells. They, therefore, have been investigated as potential therapies, both for inhibiting apoptosis in cells with limited proliferation, as well as initiating apoptosis in tumor cells. The duality of scheduled death during epidermal terminal differentiation and premature death of keratinocytes in pathologic skin conditions make the study of caspase-mediated events in the skin particularly relevant. It has been proposed that the dyskeratotic keratinocytes seen in areas of lichenoid inflammation in certain inflammatory dermatoses are keratinocytes dying an apoptotic death. Additional investigators have described other findings of apoptosis in the basal and lower spinous layer of involved skin of lichenoid dermatoses such as lupus erythematosus (LE), graft-vs-host disease (GVHD), erythema multiforme (EM), and lichen planus (LP). These studies have used the morphologic features of the dying keratinocytes combined with the demonstration of nick-ended DNA to classify the death as apoptotic. The role of caspases or other proteolytic events leading to death in these disorders has not been delineated. In this study, we have measured caspase activity in involved and non-involved skin from patients with LE, EM, and LP. Using a panel of fluorimetrically-labeled caspase tetrapeptide substrates, we find that DEVD-ase (the preferred substrate of caspase-3) activity is increased 1000 fold in lesional compared to non-lesional skin in patients with EM and LE. Patients with LP did not have increased DEVD-ase activity compared to normal controls or to non-lesional skin. Further, substrates for caspases-1,-2,-6,-8, and -9 were not cleaved differentially in either the disease skin specimens or in non-involved skin. These studies show that despite similar histologic features, keratinocyte death in LE and EM differs from that of LP in the proteolytic pathway to cell death. These studies establish the rationale for use of specific caspase inhibitors as therapies for certain inflammatory skin diseases.

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The role of PAR-1 and PAR-2 in corneal epithelial inflammatory responses

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Proteinase-activated receptors (PARs) are a novel class of cell surface receptors that are activated by the cleavage of their N-terminal domain by serine proteases such as the PAR-1 activating thrombin and the PAR-2 activating trypsin. PAR activating proteases are widely distributed in a variety of tissues and are released locally after tissue injury and inflammation. Previous studies indicate that the activation of keratinocyte PARs can modulate cytokines production, cellular proliferation and differentiation. In this report we examined the role of PAR-1 and PAR-2 in mediating inflammatory responses of human corneal derived HCE-T epithelial cells which, like keratinocytes, are exposed to a wide variety of external insults and infectious agents. Our results indicate that the activation of both HCE-T PAR-1 and PAR-2 induces the production of IL-1 alpha and IL-1 beta, whereas only PAR-1 but not PAR-2 activation stimulates increased HCE-T sICAM-1 secretion. Next the effect of PAR activation on HCE-T MMP and TIMP expression was examined. These proteins play a key role in tissue inflammation, repair, and remodeling. Our results again demonstrated a differential effect between PAR-1 and PAR-2 activation on HCE-T MMP and TIMP production. Thrombin (PAR-1 activator) but not trypsin (PAR-2 activator) induced increased expression of MMP-1, MMP-2, TIMP-1 and TIMP-2. The results of this study indicate that like closely related keratinocytes, corneal epithelial PARs may serve as important modulators of tissue inflammation and repair in this important ocular barrier tissue. Modulation of these receptors may provide to be a useful therapeutic target for a variety of epithelial diseases.

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Negative feedback regulation of phosphatidylinositol 3-kinase pathway by overexpressed cyclooxygenase-2 in human epidermal cancer cells

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While enhanced expression of cyclooxygenase (COX)-2 has been observed in human skin epidermal cancer, the mechanisms underlying COX-2 expression have not been completely elucidated. Recently, a role of the phosphatidylinositol-3 (PI3) kinase pathway in COX-2 expression has attracted attention. We investigated COX-2 expression, PI3 kinase activity, and the phosphorylation level of Akt, a downstream effector of PI3 kinase, in human skin cancer cells HSC-5. Compared to the non-tumorigenic keratinocytes HaCaT, in HSC-5 cells, COX-2 protein expression and PI3 kinase activity were increased. The PI3 kinase inhibitor LY294002 reduced COX-2 expression in HSC-5 cells and contrary to our expectation, the phosphorylation of Akt was significantly decreased. The expression of Bcl-2, which is regulated by Akt, was reduced and apoptosis was induced in HSC-5 cells compared to HaCaT cells. COX-2 inhibitor NS398 up-regulated PI3 kinase activity and Akt phosphorylation. These results imply that constitutively over-expressed COX-2 down-regulates the PI3 kinase/Akt pathway through a negative feedback mechanism.

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Detection of human Papilloma virus DNA in Bowen disease, Bowenoid papulosis and squamous cell carcinoma

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It is well known that Human papilloma virus(HPV) induce benign warts. In the recently, however, HPV has been shown to be detected in malignant tumors of the skin. We tried to detect HPV DNA in Bowen disease(BD), Bowenoid papulosis(BP) and squamous cell carcinoma(SCC) of the skin. One of 21 cases of BD, 1 case showed?HPV 31. In 3 cases of BP, 1 case showed HPV 16 and another case 31. In 26 cases of SCC, 1 case showed HPV 16, 1 case HPV 34, and 4 cases HPV 31. In the present study, we showed that cervical type of HPV was detected in SCC. These results were different from the former ones that showed no detection of cervical type of HPV was detected in SCC.

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Inhibitory effect of topical applications of non-denatured soymilk on the formation and growth of UVB-induced skin tumors

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Treatment of female SKH-1 hairless mice with ultraviolet B light twice a week for 20 weeks resulted in a population of tumor-free mice with a high risk of developing skin tumors during the next several months in the absence of additional UVB treatment (high risk mice). Topical applications of non-denatured soymilk but not heat-denatured soymilk once a day 5 days a week to these high risk mice inhibited the formation and growth of skin tumors. Similar topical applications of Soybean Trypsin Inhibitor or Bowman-Birk Inhibitor also inhibited the formation and growth of skin tumors, but these agents were less active than non-denatured soymilk. Treatment of miniswine skin with non-denatured soymilk once a day for 5 days prior to UVB irradiation reduced or completely eliminated UVB-induced formation of thymine dimers and apoptotic cells in the epidermis. These data suggest that non-denatured soymilk could be applied to humans to prevent sunlight-induced skin damage and to reduce the risk of skin tumor formation and progression.

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Analysis of gene mutations in six cases of dermatofibrosarcoma protuberans

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Fusion of the collagen type I alpha 1 (COL1A1) gene with the platelet-derived growth factor B-chain (PDGFB) gene has been pointed out in dermatofibrosarcoma protuberans (DFSP). Various exons (exon 7, 8, 10, 11, 18, 22, 24, 25, 29, 32, 33/34, 37, 38, 39, 40, 43, 45, 46, 47) of the COL1A1 gene have been shown to be involved in the fusion with exon 2 of the PDGFB gene. We studied the breakpoints of the COL1A1 gene using the tumor specimens from six patients with DFSP. Reverse transcriptase - polymerase chain reaction (PCR) was performed using frozen or paraffin-embedded tissues. To amplify the COL1A1-PDGFB fusion transcript, PCR was carried out using 16 COL1A1 forward primers and a specific PDGFB reverse primer. Nucleotide sequence analysis was carried out using the PCR products to identify the breakpoints. Cases 1, 2, 3 and 4 were diagnosed as ordinary DFSP, case 5 as DFSP with fibrosarcomatous lesion (DFSP-FS), and case 6 as lung metastasis of DFSP-FS. The COL1A1-PDGFB fusion transcripts were detected from the tumor specimens. Sequence analysis revealed that the ends of exons 18, 42, 38, 44, 29 and 38 in the COL1A1 gene were fused with the start of exon 2 in the PDGFB gene in case 1, 2, 3, 4, 5 and 6, respectively. This study identified two novel COL1A1 breakpoints, namely, exon 42 and 44 of the COL1A1 gene. Detection of the aberrant fusion transcript seems to be useful at differential diagnosis both in primary and metastatic lesions.

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Reciprocal regulation of DEC1 and HIF1 in response to UV irradiation in cultured human keratinocytes

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UV radiation represents the most significant risk to human skin, causing skin cancer. AP1 and NFκB among others transcription factors play critical roles in UV signal transduction pathways, knowingly from EGFR, to PI3 kinase and MAP kinase, leading to expression of various genes involved in skin cancer. Recently, another transcription factor, hypoxia inducible factor or HIF, involved in cellular response to low oxygen tension or hypoxia, attracts unprecedented attention. Our preliminary study showed that UV irradiation also induces expression of HIF1α, one of the members of HIF complex. HIF1α expression is reportedly to be modulated by EGFR/PI3K/AKT pathway in human prostate cancer cells. However, the question whether this very pathway mediates UV-induced expression of HIF1α remains to be addressed. In addition to HIF, other two transcription factors DEC1 and DEC2, recently known to interact with HIF, have been shown to be involved in cell proliferation and survival. Our recent findings suggest that over-expression of DEC1 provides cells with an unusual survival mechanisms under hypoxia condition. While data from others indicated that HIF1α up-regulates DEC1, our preliminary studies showed that UV-induced HIF1 expression requires DEC1. The question whether DEC1 and HIF1 are reciprocally regulated in response to UV irradiation is yet to be answered. We tested the hypothesis that EGFR and Rac1/PI3K/AKT mediate UV-induced HIF1α expression and its target genes and DEC1 and HIF1α are reciprocally regulated in response to UV irradiation. We found that EGFR plays critical role in UV-induced HIF1α expression and Rac1/PI3K/AKT mediates this signaling pathway. Using DEC1 knockout cells, we observed that UV- and hypoxia- but not chemical-induced HIF1α expression is DEC1 dependent. Collectively, we conclude that reciprocal regulation of DEC1 and HIF1α in response to UV radiation is mediated by EGFR/Rac1/PI3k/AKT pathway.

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Insensitivity to oxidative stress and hyper-activation of AKT contribute to anomalous invasiveness of human melanoma cells

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Among other types of skin cancer, melanoma is highly invasive and poorly manageable, with the mechanisms of invasiveness unaccountable. Human skin cells including mutated cells are constantly under oxidative stress, and yet the responding mechanisms of different cell types vary dramatically. We observed that human melanoma cells sustain oxidant treatment much better than keratinocytes in vitro, although arsenite and H_2O_2 both induced cell death of both types to certain extent. In keratinocytes, arsenite induced CDC25C degradation but not CDC25A, and H_2O_2 induced CDC25A degradation but not CDC25C. In melanoma cells, arsenite induced CDC25A degradation but not CDC25C, and H_2O_2 induced CDC25C degradation but not CDC25A. Arsenite induced MAP kinase activation in both human keratinocytes and melanoma cells. However, interestingly, H_2O_2 induced MAP kinase activation in keratinocytes, but did not induce MAP kinase activation in melanoma cells. Further, arsenite-induced MAP kinase activation was inhibited by NADPH oxidase inhibitor DPI in human keratinocytes, but not in melanoma cells. In addition, although both arsenite and H_2O_2 induced phosphorylation of AKT in both human keratinocytes and melanoma cells in a time and dose dependent manner, AKT activation occurred much earlier and stronger in melanoma cells. Arsenite- and H_2O_2 -induced AKT activation was inhibited by PI3 Kinase inhibitor LY294002 in human keratinocytes and melanoma cells. Collectively, our data indicate that melanoma cells survive oxidative stress with inactivation of MAP kinase pathways and protection from CDC25A degradation and hyper-activation of AKT cell survival pathway. These results provide insights into understanding of the molecular mechanisms that underlie the invasiveness of melanoma cells especially under oxidative stress or hypoxia.

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Nicotine modulates gene expression in oral keratinocytes

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Tobacco causes various kinds of mucocutaneous morbidly, such as precocious aging, delayed wound healing, and cancer, but the mechanisms remain elusive. Nicotine (Nic) is a likely mediator of the pathobiologic effects, since it displaces the local hormone acetylcholine from the nicotinic receptors (nAChRs) expressed by keratinocytes (KCs). To gain a mechanistic insight into tobacco-induced morbidity, we studied effects of environmental tobacco smoke (ETS) vs. pure Nic on oral KCs. Both ETS and Nic upregulated expression of the cell cycle and apoptosis regulators p53, p21, Bcl-2 and caspase-3, the differentiation marker filaggrin and the signal transduction factors NF-κB, JAK-1, STAT-1 and GATA-3 at both the mRNA and protein levels. These changes could be abolished due to transfection of KCs with anti-α3 small interfering RNA or treatment with α-conotoxin MII, an α3β2 prefererring antagonist. Functional inactivation of the α3 gene in receptor knock-out KCs also prevented most of the ETS/Nic-dependent changes in gene expression. To determine relevance of the in vitro findings to the in vivo situation, we studied gene expression in wild-type and α3-/- littermates delivered by heterozygous mice soon after their exposures to ETS or pure Nic in drinking water. In addition to RT-PCR and western blot, the ETS/Nic-dependent alterations in the gene expression were also detected by semi-quantitative immunofluorescence directly in KCs residing in the oral epithelium. Only wild-type mice developed changes in the expression of p21, Bcl-2, filaggrin, NF-κB and STAT-1. These results identified α3β2 nAChR as a major receptor mediating effects of tobacco products on KC gene expression. The ETS/Nic exposures also upregulated α5 and β2 nAChR subunits and downregulated M2 and M3 muscarinic receptor subtypes, suggesting that a switch in the repertoire of KC cholinergic receptors mediate, at least in past, pathobiologic effects of tobacco products in the mucocutaneous epithelia.

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Role of protein kinase C δ as a tumor suppressor for human squamous cell carcinomas

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Carcinogenesis is a multi-step process involving the loss or silencing of tumor suppressor genes that permit the outgrowth of malignant cells. For human skin cancers, inactivation of genes involved in UV apoptosis (i.e. *p53*) can provide a selective growth advantage since the surrounding normal keratinocytes are preferentially killed by repeated sunlight exposure. Protein kinase C (PKC) δ is required for the induction of apoptosis by UV radiation in human keratinocytes, and is down-regulated or inactivated in keratinocytes expressing the activated Ha-ras oncogene making it a candidate tumor suppressor gene for squamous cell carcinoma. To determine if PKCδ is reduced in sunlight-induced human skin cancers, we examined Bowen's Disease and squamous cell carcinomas for PKCδ expression by immunohistochemistry. We found that 29% (12/42) had lost or reduced PKCδ when compared to normal skin or epidermis surrounding the tumor. Although there was no correlation between PKCδ loss and tumor histology, PKCδ was reduced in 4 of 10 Bowen's Disease samples and one solar keratosis indicating that loss of PKCδ may be an early event. To explore the significance of PKCδ loss in transformed human keratinocytes, we used the tumorigenic HaCaT Ras-II-4 clone that forms highly differentiated, locally invasive squamous cell carcinomas in nude mice. HaCaT Ras II-4 cells have reduced PKCδ levels, and re-expression of PKCδ by retrovirus transduction caused a modest increase in apoptosis (6% increase) and growth retardation in culture. However PKCδ re-expression dramatically suppressed the tumorigenicity of HaCaT Ras II-4 cells in nude mice ($p < 0.05$), with PKCδ-transduced cells producing much smaller tumors and/or cysts only. Together, these results indicate that PKCδ may function as a tumor suppressor in human squamous cell carcinomas where its loss could provide a growth advantage to pre-malignant cells by affording them resistance to UV-induced apoptosis.

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Stage-specific regulation of skin tumor development by apoptosis: survivin enhances mutagenesis yet suppresses clonal expansion

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Apoptosis is one of many obstacles to tumor formation, but its role in regulating individual stages of multi-step carcinogenesis has not been defined. For example, it is possible that once apoptosis-resistant mutant cells have arisen, pro-apoptotic conditions will aid their clonal expansion. Using a transgenic mouse (K14-survivin Tg) with epidermis-specific expression of survivin, an inhibitor of apoptosis, we investigated the impact of apoptosis on initiation, clonal growth of p53-mutant keratinocytes, creation and growth of benign papillomas, and conversion to squamous carcinoma after ultraviolet-B (UVB) irradiation. At carcinogenic UVB doses, the survivin transgene led to a 2-fold reduction in UVB-induced epidermal apoptosis. After 5 wks of UVB irradiation, small p53-mutant keratinocyte clones (<1/3 stem cell compartment) were 1.5-fold more frequent in K14-survivin Tg mice ($p=6x10^{-4}$), reflecting the anti-mutagenic effect of apoptosis. Strikingly, the effect of survivin was reversed for existing clones growing by clonal expansion (>2 stem cell compartments), which were ~1.7-fold rarer in the Tg (p=0.047). At 10 wks, the genotypic differential for large clones approached the apoptosis differential (~2-fold, $p=4x10^{-5}$), consistent with apoptosis facilitating clonal expansion. After 20 wks, 95% of animals carried tumors, primarily papillomas. Tumors were 1.6-fold rarer in apoptosis-defective Tg mice (p=0.03), a paradoxical result if apoptosis were solely anti-tumorigenic. In contrast, a 2-fold higher percentage of tumors in K14-survivin mice attained a size of 3 mm (p=0.048) or converted to carcinoma. Thus survivin-inhibitable apoptosis suppresses initiation and malignant conversion, two stages that involve new mutations, yet enhances clonal expansion of existing p53-mutant and papilloma cells.

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Malignant transformation of human cells through an autocrine loop between platelet derived growth factor-BB (PDGF-BB) and PDGFRβ

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Tuberous sclerosis (TS) is a common autosomal dominant disorder, characterized by benign tumors of the skin, and benign and malignant tumors of the brain and kidney. We have previously established a cell line (SV7tert) from an angiomyolipoma and found that it expresses functional PDGFRβ. In order to determine whether autocrine PDGF-PDGFRβ signaling could result in malignant transformation, we retrovirally transduced PDGF-BB into SV7tert cells. Overexpression of PDGF-BB led to activation of MAP kinase and phosphoinositol-3 kinase/akt, upregulation of vascular endothelial growth factor, and reactive oxygen species, as well as resistance to apoptosis under hypoxic conditions. Implantation of SV7tert cells expressing PDGF-BB led to the development of tumors in nude mice, while tumors were not observed in vector control cells. Tumors took several months to appear, suggesting further epigenetic events. We have previously shown that reactive oxygen mediated carcinogenesis leads to downregulation of the tumor suppressor gene p16ink4a, and cells derived from tumors showed decreased expression of p16ink4a compared with cells prior to injection into mice. Thus, autocrine expression of PDGF-BB leads to transformation in part through downregulation of p16ink4a and upregulation of angiogenesis through MAP kinase and phosphoinositol-3 kinase signaling. These observations represent the first known example of malignant transformation of human cells through autocrine signaling.

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Gli1 co-operates with EGFR-Src signalling to induce a fibroblastoid phenotype in primary keratinocytes

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The transcription factor Gli1 is implicated in the development of basal cell carcinoma (BCC) and other neoplasms due to constitutive activation of the Sonic hedgehog signalling pathway. Despite the identification of many target genes, the cellular mechanisms by which Gli1 induces neoplasia are poorly understood. We show that EGF stimulation of Gli1-expressing human primary keratinocytes induces a fibroblastoid morphology characterised by cell elongation and the formation of extensive membrane protrusions that is not observed in control cells. The EGF-induced change in cell morphology was also observed in keratinocytes expressing a Gli1 mutant protein localised to the nucleus suggesting that the phenotype is dependent upon Gli1 transcriptional activity. Using pharmacological inhibitors we identified that the change in cell morphology is mediated by Src kinase(s), but not Ras-ERK or PI3K signalling. More specifically, Gli1 co-operates with EGFR-Src signalling to regulate the cytoskeleton, probably through inhibition of the Rho-ROCK pathway as shown by increased activity of the actin remodelling protein cofilin. Indeed, exposure to the ROCK inhibitor Y27632 alone is sufficient to induce a spindle phenotype in Gli1 keratinocytes. Interestingly, the phenotype of Gli1 cells exposed to EGF or Y27632 is dependent upon microtubule remodelling as it is negated in the presence of nocodazole. As cytoskeletal remodelling is required for tumour invasion, these data suggest a novel role for Gli1 that may be relevant to BCC aetiology.

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Overexpression of akt converts radial growth melanoma to vertical growth melanoma

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Melanoma is the major cause of death due to a dermatologic disorder, and transformation of radial growth melanoma to vertical growth is required for invasive disease and metastasis. We have previously shown that MAP kinase is activated in radial growth melanoma, suggesting that further signaling events are required for vertical growth melanoma. The molecular events that accompany this transformation are not well understood. We introduced active akt, a signaling molecule downstream of phosphoinositol 3 kinase into the radial growth WM35 melanoma in order to see whether akt overexpression was sufficient to accomplish this transformation. Overexpression of akt led to upregulation of vascular endothelial growth factor (VEGF), and increased production of the reactive oxygen species superoxide. Superoxide has been previously implicated in aggressive melanoma. Implantation of WM35 cells overexpressing akt lead to rapidly growing tumors in vivo, while vector control cells do not form tumors. Akt thus serves as a molecular switch that allows increased angiogenesis and generation of superoxide, thus allowing aggressive behavior. Targeting akt and reactive oxygen may be of therapeutic importance in treatment of advanced melanoma

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Up-regulation of the lymphatic marker podoplanin in human squamous cell carcinomas and germ cell tumors

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The mucin-type glycoprotein podoplanin is specifically expressed by lymphatic, but not blood vascular endothelial cells in culture and in tumor-associated lymphangiogenesis. Our previous studies showed that podoplanin deficiency results in congenital lymphedema and impaired lymphatic vascular patterning, and that podoplanin enhances migration and adhesion of endothelial cells by reorganization of the cytoskeleton. However, its expression in normal tissues and in human malignancies has remained unknown. Using tissue arrays of normal human tissues and of a number of human cancers, we found that - in addition to lymphatic endothelium - podoplanin is also expressed by bile duct cells of the liver, peritoneal mesothelial cells, osteocytes, glandular myoepithelial cells, ependyma cells and by stromal reticular cells and follicular dendritic cells of lymphoid organs. These findings were confirmed in normal mouse tissue arrays. Podoplanin was also strongly expressed by granulosa cells in normal ovarian follicles, and by ovarian dysgerminomas and granulosa cell tumors. Although podoplanin was largely absent from normal human epidermis, its expression was strongly induced in 22 out of 28 squamous cell carcinomas studied. The expression pattern of podoplanin was dependent on the level of differentiation of squamous cell carcinomas. Together, these findings suggest a potential role of podoplanin in epithelial tumor progression.

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Caspase 3 dependent apoptosis induction by resveratrol in B16F10 melanoma cells

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Resveratrol (RES), a natural phytoalexine present in red grapes and wines, has been reported to induce apoptosis in murine and human melanoma cells. Our previous studies of chromatin condensation, an early marker of apoptosis, have been observed by fluorescence microscopy using B16F10 murine melanoma cells. Previous analysis of B16F10 treatment with RES by flow cytometry using AnnexinV staining (probe to measure translocation of phosphatidyl serine) and TUNEL assay (measure of DNA fragmentation) suggested induction of apoptosis. This prompted a more detailed examination of RES induced apoptosis. Therefore, we measured the intracellular caspase activity using a cell permeable fluorogenic substrate with DEVD sequence recognized by caspase 3. We also observed cell size changes using flow cytometry. Flow cytometry analysis indicates that B16F10 cells undergo cell shrinkage and increased cell granularity after treatment with RES. We have also explored the concentration and time dependence of RES to induce apoptosis via caspase 3 meahanism. RES (150 μM) at 1 hr, 2 hr, and 3hr showed caspase 3 activation in 11.3% of cells vs. 6.7% vehicle control, 8.8% vs. 8.7% control, and 22.4% vs. 15.8% control, respectively. Data for RES (200 μM) at 1 hr showed 17.3% caspase 3 activity vs. 6.7% control, at 2hr 20.3% vs. 8.7% control, and at 3 hr 51% vs. 15.8% control. Data for RES (250 μM) at 1 hr showed 21.2% vs. 6.7% control, at 2 hr 24.9% vs. 8.7% control, and at 3 hr 73.2% vs. 15.8% control. Thus, RES induces caspase 3 activation in B16F10 cells in time and dose dependent manner. We are currently investigating RES effect on other caspase-induced mechanisms of apoptosis.

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Senescent, but not proliferating, keratinocytes suppress tumorigenesis

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The phenotype and function of senescent keratinocytes (sKCs) remains poorly understood. Clones of mutant p53 containing KCs are detected in aged skin, but the majority fail to progress to invasive carcinoma. We postulate sKCs present in aged epidermis restrain tumorigenesis in flanking regions. Thus, varying numbers of normal proliferating KCs (pKCs) vs sKCs were combined with malignant cells bearing p53 mutations at initial seeding in soft agar. KCs were derived from foreskins, grown in serum-free medium and harvested either as rapidly proliferating cells, or weeks later when they underwent replicative senescence (ceased cell division despite feeding). Addition of $3x10^4$ malignant cells to soft agar produced approximately 200 colonies/cm^2 by day 12. Addition of pKCs ($5x10^3$, $10x10^3$, $30x10^3$) at time of seeding did not significantly reduce colony formation. However, addition of sKCs impacted colony formation in a concentration dependent fashion. A typical result is as follows: tumor cells alone (213+/-15), or tumor cells containing the following number of sKCs: $5x10^3$ (167+/-20); $10x10^3$ (104+/-15); and $30x10^3$ (65+/-10). Similar results were observed in 4 independent experiments($p< 0.01$). To address mechanisms by which sKCs mediated reduction in tumorigenesis, Western blots of proliferating versus sKCs were performed, and 2 highly upregulated proteins were TGF-β1 and TGF-β2. Furthermore, addition of recombinant human TGF-β1 to tumor cells produced a dose dependent reduction in colony formation at doses of 0.1, 1.0, and 10 ng/ml comparable to degree of inhibition by sKCs. Based on these results we conclude: a) sKCs, but not pKCs, strongly suppress colony formation in a density dependent fashion, b) sKCs are not decrepit, but are metabolically active and over produce TGF-β compared to pKCs, c) TFG-β represents a potential molecular mediator by which sKCs can suppress tumorigenesis. In summary, we suggest irreversibly growth arrested sKCs accumulating during aging may not only resist transformation themselves, but by remaining viable and metabolically active serve as a barrier to expansion of flanking KCs that acquire p53 mutations.

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Telomere homolog oligonucleotide-induced signaling in adenocarcinoma

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DNA damage due to UV irradiation, oxidative stress or chemical adducts, as well as telomere shortening due to aging, is hypothesized to expose the single-stranded telomere 3'-overhang sequence (tandem repeats of TTAGGG). Exposure of this sequence is the initial signal leading to a variety of physiologic DNA damage responses, including senescence, apoptosis or adaptive differentiation. However, although these responses are present in normal cells, they are often muted in malignant cells, possibly because of failure of the cells to expose and/or recognize the exposed overhang. Interestingly, addition of an 11-base oligonucleotide (GTTAGGGTTAG), homologous to the 3'-overhang sequence (T-oligo), leads to identical DNA damage responses, presumably because it mimics the exposure of the 3' overhang. To determine the T-oligo effect on adenocarcinoma cells, 40uM T-oligo or the complementary (control) oligonucleotide or diluent alone were introduced into adenocarcinoma cell line (CRL-2119). By western blot, compared to controls, T-oligo induced p53 level and phosphorylation on serine 15 by 142% and 2160% respectively, within 48 hours, indicating p53 activation, and lead to S-phase arrest of 54%±0.1% of the cells (FACScan analysis, $p<0.001$). Within 96 hours 7.7%±0.02% of cells were apoptotic as determined by the sub-G_0/G_1 DNA content (FACScan analysis), compared to 0.6%±0.1% of controls ($p<0.001$). Furthermore, TUNEL analysis showed that only T-oligo treated cells displayed increased fluorescence, indicating DNA fragmentation and apoptosis. Also, within 96 hours T-oligo decreased cell yields by 67%±0.1% ($p<0.01$). Interestingly, even after T-oligo removal from the medium, cell growth inhibition persisted for at least 6 days, suggesting it was irreversible. These findings suggest that T-oligo mimics the exposure of the 3' telomere overhang, and in malignant cells, otherwise only partially responsive to DNA damage, it induces DNA damage responses leading to cell cycle arrest and apoptosis. We propose that T-oligo is a promising novel approach to cancer treatment.

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Inhibition of hydrogen peroxide- generated lipid peroxidation product 4-hydroxy-2-nonenal by green tea polyphenols in human fibroblast WS-1 cells

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The exact role of hydrogen peroxide generated in the human skin during oxidation events such as solar ultraviolet radiation and aging is not well understood. A novel FACS based method was used to show for the first time that hydrogen peroxide is able to upregulate cellular lipid peroxidation 4-hydroxy-2-nonenal (HNE) protein adducts in WS-1 fibroblasts derived from human skin. HNE is a highly reactive second messenger aldehyde that is generated during lipid peroxidation of fatty acids from cellular membranes, being released intracellularly and extracellularly. HNE was previously reported to have genotoxic and neutrophil chemotactic effects. HNE protein adducts have been described in the past to co-localize with areas of actinic elastosis in sun-damaged human skin. Recent reports showed that topical green tea polyphenols are able to inhibit UV-generated lipid peroxidation products in the skin of mice. Our current results suggest that green tea polyphenols are able to inhibit the formation of HNE protein adducts induced by hydrogen peroxide in human skin fibroblast cells WS-1. Unlike alpha- lipoic acid, green tea polyphenols were also able to inhibit both endogenous and hydrogen peroxide- generated reactive oxygen species as shown by FACS analysis of WS-1 cells stained with Dihydrorhodamine 123. These findings have possible mechanistic implications for the rational development of anti-oxidant agents that are able to inhibit relevant cellular oxidative damage occurring during carcinogenesis and aging in the human skin.

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Expression of chemokine receptor CXCR4 enhances tumorigenesis of basal cell carcinoma

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Beside the well-known role to regulate leukocyte trafficking, chemokines and their receptors also play important roles in the regulation of mitosis, apoptosis, survival and angiogenesis, which may enhance tumor growth or metastasis. Herein we investigated the possible involvements of chemokine receptors in the pathogenesis of basal cell carcinoma (BCC). In previous study, we found high expression of CXCR4 in a human BCC cell line and tissue samples from BCC lesions. Furthermore, we addressed whether overexpression of CXCR4 may alter BCC tumor progression. By retroviral transduction, CXCR4 gene was transferred into BCC cells (CXCR4-BCC) and functionally expressed. In WST-1-based tumor proliferation assay in vitro, CXCR4-BCC cells were cultured under low serum concentration (0.5%) and subject to CXCL12 or PBS treatments. CXCL12 treatments resulted in significant increase of CXCR4-BCC proliferation compared to PBS treatments (24-hr, ~1.2-fold $p=0.027$; 48-hr, ~2.2-fold, $p=0.02$), which could be neutralized by co-treatments with anti-CXCR4 mAb. In apoptosis-resistance assay in vitro, ultraviolet radiation B was used to induce apoptosis in CXCR4-BCC and vector-transduced BCC cells (pLNCX2-BCC), evaluated by caspase-3 activity. Treatments with CXCL12 led to decreased apoptosis level (~38%, $p=0.042$) in CXCR4-BCC, which could be negated by CXCR4-blocking peptide, T22. Moreover, xenograft tumor transplants were performed to test whether CXCR4 overexpression may affect BCC tumor progression in vivo. pLNCX2-BCC and CXCR4-BCC cells were injected subcutaneously into the flanks of nude mice. Injections of CXCR4-BCC tumor cells alone yielded significant tumor progression, whereas additional serial injections of T22 resulted in significant tumor regression. pLNCX2-BCC injections only gained insignificant tumor growth. Thus, CXCR4 expression may play critical roles in BCC tumorigenesis, and functional blockade of CXCR4 could be a potentially promising therapeutic strategy.

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Tumor as wound: gene expression signature of fibroblast serum response predicts human cancer progression

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Cancer invasion and metastasis have been likened to wound healing gone awry. Despite parallels in cellular behavior between cancer progression and wound healing, the molecular relationships between these two processes, and their prognostic implications, are unclear. Here, based on gene expression profiles of fibroblasts from 10 anatomic sites, we identify a stereotyped gene expression program in response to serum exposure that appears to reflect the multifaceted role of fibroblasts in wound healing. The genes comprising this fibroblast common serum response are coordinately regulated in many human tumors, allowing us to identify tumors with gene expression signatures suggestive of active wounds. Genes induced in the fibroblast serum-response program are expressed in tumors by the tumor cells themselves, by tumor-associated fibroblasts, or both. The molecular features that define this wound-like phenotype can be evident at an early clinical stage, persist during treatment, and predict increased risk of metastasis and death in squamous cell, breast, lung, and gastric carcinomas. Thus, the transcriptional signature of the response of fibroblasts to serum provides a possible link between cancer progression and wound healing, as well as a powerful predictor of the clinical course in several common carcinomas.

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Trans-sectional tumor microdissection mutation analysis: a new tool for evaluation of genetic instability and heterogeneity as well as tumor cell evolution

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Genetic instability is a hallmark of malignant and premalignant clonal cell proliferations. Trans-sectional tumor microdissection mutation (TTMM) analysis is a new method which has been developed in order to quantify genetic instability and heterogeneity in routine formalin-fixed and paraffin-embedded histological sections of individual tumors. After staining, histological tissue slides are embedded with a liquid coverslip. The section is then laser-microdissected at multiple (8-12) areas in a linear configuration across the tumor. Each microdissected area contains approx. 100-200 cells each. Extracted DNA is then analyzed by PCR for loss of heterozygosity (LOH) of microsatellite DNA or for the presence of mutated microsatellite alleles (MSI). The frequency of LOH or MSI is an indication of genetic instability within the lesion. 10 benign melanocytic nevi, 10 dysplastic nevi, 10 malignant melanomas as well as 10 early mycosis fungoides (MF) cases have been analyzed in order to evaluate this new technique. Moles and melanomas have been screened for MSI at microsatellite markers D9S162 and D14S53 while MF cases were analyzed at microsatellite markers D9S162 and D18S62. The frequency of MSI in microdissected tissue was higher in dysplastic melanocytic nevi (0.30) and in melanomas (0.29) as compared to benign moles (0.05, $p< 0.05$). An elevated frequency of MSI in microdissected tissue was also found in MF (0.32) as compared to control lichen planus tissue (0.05, $p<0.01$). Further studies are necessary to determine the role of TTMM analysis in the diagnosis of genetic instability in malignant and premalignant tumors.

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AKT status controls susceptibility of malignant keratinocytes to the early-activated and UVB-induced apoptotic pathway

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In previous work we demonstrated the existence of an early-activated and UVB-induced apoptotic pathway in human keratinocytes, which can be completely inhibited by IGF-1 mediated AKT activation. This apoptotic pathway stands in marked contrast to the commonly described UVB-induced pathway of apoptosis, which occurs several hours later and is impervious to IGF-1 signaling. As the IGF-1 - AKT axis is a frequent target for mutation, we now compared the apoptotic response of primary, healthy human keratinocytes with the apoptotic response of two p53-mutated squamous cell carcinoma-derived cell lines (A431 and A253) to an apoptotic UVB dose. In these cell lines, both the basal AKT phosphorylation status and the apoptotic response to UVB diverged strongly from the response of healthy primary keratinocytes. Even more, a remarkable correlation was found between the two. While a constitutive dual phosphorylation of AKT rendered the A253 SCC cell line completely resistant to the early-activated and UVB-induced apoptotic pathway, deficient T308 phosphorylation of AKT in the SCC cell line A431 led to a greatly augmented sensitivity to the early-activated, UVB-induced apoptotic pathway. These results indicate that the preservation of a healthy AKT pathway is essential for a wild-type UVB-induced apoptotic response in skin and suggest that AKT-mediated dysregulation of the early-activated apoptotic response to UVB is an important event in the oncogenic transformation of keratinocytes.

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Decreased expression of Srcasm in actinic keratoses and squamous cell carcinomas

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Srcasm, Src activating and signaling molecule, is a recently discovered substrate and activator of Src family kinases. Increased Src-kinase activity has been associated with keratinocyte differentiation in murine systems. Our recent work has shown that Srcasm can activate intracellular Src-kinases, associate with the Grb2 and the p85 subunit of phosphoinositide 3-kinase, and that it is a component of the EGF signaling pathway in human keratinocytes. As Srcasm can effect keratinocyte signaling pathways important for regulating growth and differentiation, we hypothesized that Srcasm expression may be downregulated in keratinocytic neoplasia. The purpose of this study was to determine if Srcasm expression was decreased in the spectrum of keratinocytic lesions spanning actinic keratoses (AK) to squamous cell carcinoma (SCC). Srcasm expression in formalin-fixed sections of AK, SCC in-situ (SCC-IS), and SCC from up to 15 different patients each was determined by immunohistochemisty using a polyclonal affinity-purified anti-Srcasm antibody. The expression of Srcasm in lesional tissue was compared to unremarkable peri-lesional skin of the same biopsy specimen or normal skin from separate biopsies. Srcasm expression was decreased in approximately 50% of the AK and SCC-IS samples and in over 90% of the SCC samples. This increased prevalence of Srcasm downregulation in more advanced lesions of keratinocytic neoplasia supports the hypothesis that Srcasm may be important in promoting keratinocyte differentiation, possible by increasing intracellular Src-kinase activity.

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Tuberous sclerosis skin tumors overexpress MCP-1

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Tuberous sclerosis (TSC) tumors contain multiple cell types - neoplastic cells with loss of either the *TSC1* or *TSC2* tumor suppressor gene mixed with reactive cells. The goal of this study was to identify paracrine growth factors overexpressed in TSC skin tumors that stimulate the proliferation or recruitment of reactive cells. Cells cultured from angiofibromas, periungual fibromas, and shagreen patches contained 12-28% neoplastic cells, as demonstrated by allelic deletion of *TSC2* (no sample showed deletion of *TSC1*). Nylon-membrane based cDNA arrays comparing angiofibromas cells to normal-appearing skin fibroblasts from 2 patients revealed overexpression of monocyte chemotactic protein-1 (MCP-1). MCP-1 mRNA, measured using real-time PCR, was increased in cells from 4/4 angiofibromas, 3/3 periungual fibromas, and 2/4 shagreen patches from 5 patients (2.9- to 33-fold relative to control patient fibroblasts, normalized to 18S rRNA). MCP-1 protein, measured in culture supernatants by ELISA, was increased in 4/4 angiofibromas, 2/3 periungual fibromas, and 2/4 shagreen patches (1.9- to 48-fold relative to control patient fibroblasts). The relationship between increased MCP-1 production and loss of *TSC2* gene function was investigated using Eker rat embryonic fibroblasts null for *Tsc2* (EEF *Tsc2-/-*). MCP-1 levels in 24-hour culture supernatants were 2075±280 pg/ml for EEF *Tsc2-/-* cells and 73±28 pg/ml for EEF *Tsc2+/+* cells. Transient transfection of full-length human *TSC2* into EEF *Tsc2-/-* cells inhibited MCP-1 production by 33%. Rapamycin (20nM), a drug that inhibits the mTOR signaling pathway hyperactivated upon loss of *TSC2*, inhibited MCP-1 production by 90% in EEF *Tsc2-/-* cells. In contrast, rapamycin inhibited MCP-1 production by human angiofibroma cells by only 23%. These studies indicate increased MCP-1 production by TSC skin tumor cells, possibly as a consequence of loss of *TSC2*. MCP-1 may play important roles in TSC tumorigenesis by stimulating angiogenesis, fibrogenesis and recruiting tumor-associated macrophages.

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Senescence induced by telomere 3' overhang requires either the p53 or pRb pathway

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Senescence, a major defense against cancer, is largely dependent on the p53 and pRb pathways. In normal human fibroblasts (hFb), suppressing both of these pathways is necessary to bypass senescence due to critical telomere shortening or experimental telomere disruption. We recently reported that treatment with an oligonucleotide homologous to the telomere 3' overhang (T-oligo) activates both the p53 and pRb pathways and leads to hFb senescence, reproducing the cellular and molecular phenotype resulting from serial passage or telomere disruption. To further characterize T-oligo-induced senescence, we treated a hFb line and derived cell lines lacking functional p53 and/or pRb pathways with 40 μM T-oligo (pGTTAGGGTTAG) for 7 days. T-oligo induced senescence-associated β-galactosidase (S.A. β-gal) activity in cells deficient in either the p53 or pRb pathways, 45±4% and 60±5%, respectively, compared to 65±7% for wild-type cells. However, hFb deficient in both pathways failed to induce S.A. β-gal activity compared to diluent-treated controls (6±2% and 5±3%, respectively). Therefore, inactivation of both the p53 and pRb pathways is necessary to suppress T-oligo-induced senescence. Similarly, in Saos-2 human osteosarcoma cells with non-functional p53 and pRb pathways, T-oligo failed to induce S.A. β-gal activity or the large, spread morphology characteristic of senescent cells. However, in A375 human melanoma cells expressing a functional p53 but not pRb pathway, T-oligo activated p53, (serine 15 phosphorylation), induced p21, and rapidly induced senescence. Also, in HT-1080 human fibrosarcoma cells with functional pRb but not p53, T-oligo activated pRb, shown by resistance of pRb to phosphorylation in response to serum, and induced senescence. These data demonstrate that T-oligo-induced senescence parallels that induced by telomere loss or disruption, strongly suggesting that exposure of the telomere 3' overhang is the initiating signal for telomere-based DNA damage responses. Also, T-oligos have therapeutic potential to induce senescence in cancer cells mutated in one of these pathways.

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Inhibition of C/EBP family function in the mouse epidermis suppresses skin tumor formation and induces irreversibly hair loss

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CCAAT/enhancer binding proteins (C/EBP) are members of B-ZIP family of DNA binding proteins/transcription factors that regulate the differentiation of keratinocytes. To examine the role of C/EBP in skin, we have generated a conditional transgenic mouse that expresses dominant negative form of C/EBP protein (named A-C/EBP) in epidermis under the control of doxycycline (dox) regulatable Keratin-5 promoter. There are no dramatic phenotypes following expression of the dominant-negative in either the developing or adult skin. However, following a two step carcinogenesis protocol using 7, 12-dimethylbenz(a)anthracene (DMBA) and TPA, A-C/EBP transgenic mice had dramatically fewer tumors after 10th week which completely disappeared after 30th week. Additionally, after 8 weeks of treatment, there was systemic hair loss. Histological examination of both the tumors and skin indicated a dramatic immunological infiltrate that extended into the epidermis. This is reminiscent of the human condition of alopecia areatra. The hair loss could not be reversed by suppressing A-C/EBP expression. The molecular characterization of the "hairless skin" indicates that the expression level of AP-1 (activator protein-1) protein, which is essential for tumor promotion, was decreased in transgenic mice. In contrast, the level of p53 protein, which induces apoptosis, as well as p21 protein, which is downstream target of p53, was highly up-regulated. Also, it has been demonstrated that the apoptotic level following TPA treatment was increased in transgenic mice expressing A-C/EBP protein. These findings suggest that the C/EBP family has a critical role in tumorigenesis, cell survival, and hair follicle growth through the regulation of p53 level and implicate C/EBP as a target for tumor inhibition and hair loss.

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Identification of critical domains of β4 integrin and laminin-5 required for human SCC development

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β4 integrin and its extracellular ligand laminin-5 are strongly expressed at the invading margins of squamous cell carcinoma (SCC) tumors, and expression has been shown to correlate with tumor severity and patient prognosis. Recently it has been shown that human KC transformed with Ras and IKB produce tumors that closely resemble SCC after SQ injection into nude mice. β4 integrin or laminin-5 negative KC derived from patients with epidermolysis bullosa (EB) fail to produce tumors in this system, however retroviral transfer of wild type laminin-5 or β4 integrin cDNA to these cells restored tumor formation. In the current study, we have utilized mutational analysis to identify domains on β4 integrin and laminin-5 which are critical to SCC development. A laminin α3 cDNA coding for a protein truncated at aa 1337 (the putative processing site) was introduced into laminin α3 negative KC. The truncated laminin-5 (which lacks the G4/5 domain) was expressed at normal levels in transduced cells and assembled into a trimeric structure which supported cell attachment and migration. Interestingly Ras/IKB transformed 1337Tr KC showed a complete lack of tumor formation in our animal model system. In contrast cells expressing full length laminin a3 cDNA produced large tumors equivalent to wild type controls. These results suggest that the laminin α3 G4/5 domain (which is normally absent in skin after processing) appears to play a critical role in SCC development. In addition, we introduced mutant β4 integrin cDNA constructs into β4 integrin negative EB KC and analyzed these cells after Ras/IKB transformation in vivo. Two well characterized β4 integrin mutations (R1281W and D230A/P232A/ E233A), that interfere with plectin-β4 integrin binding and laminin-5 ligation respectively, significantly inhibited SCC tumor growth. These results suggest that interaction of β4 integrin with laminin-5 and plectin is also critical for SCC tumor development.

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Molecular mechanisms of arsenic on keratinocyte cell cycle regulation

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Exposure to arsenic is associated with Bowen's disease, a carcinoma in situ of skin. The characteristics of abnormal cell cycle regulation, abnormal cell proliferation, carcinogenesis and apoptotsis are observed in the lesions of Bowen's disease. However, the molecular mechanism of arsenic in keratinocytes is poorly understood. In this study, the regulatory proteins were detected to elucidate arsenic-induced cell cycle changes and apoptosis in keratinocytes. We found increased NF-κB activity and the expression of cyclin D1/CDK4 and cyclin B1/CDK1 at low concentrations of arsenic (lower than 1 μM), which results in keratinocyte proliferation. In contrast, the high concentrations (higher than 1 μM) of arsenic suppressed NF-κB activity, enhanced AP-1 activity and caused a p53 dependent cell cycle arrest. Western blot analysis showed that G1-S check point proteins (cyclin D1/CDK4) and G2-M check point proteins (cyclin B1/CDK1) were suppressed in the high arsenic concentration treatments. Furthermore, expression of cell cycle inhibitory proteins p21 and 14-3-3σ were enhanced. Taken together, keratinocyte cell cycle was arrested at G0/G1 and G2/M phase in high concentrations of arsenic. In addition, Fas/FasL pathway plays a major role in arsenic-induced keratinocyte apoptosis. Our results indicate that arsenic-induced alternations of keratinocyte cell cycle are correlated with arsenic concentrations. At low concentrations, arsenic induces survival signals and enhances keratinocyte proliferation. At high concentrations, arsenic reveals inhibitory effects on cell cycle progression and induces keratinocyte apoptosis.

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Imaging of skin tumor-peripheral nerve interactions by laser scanning confocal microscopy

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Purpose of the study: Interactions between skin cancers and peripheral nerves are not well understood. Our purpose was to demonstrate a method for three-dimensional imaging of skin tumor-peripheral nerve interactions and to describe the patterns of nerves, neuropeptides, and blood vessels within common skin cancer types. Experimental design and methods: Immunofluorescent staining and laser scanning confocal microscopy (LSCM) were used to generate high-resolution maps of nerve structure and distribution in basal (BCC) and squamous (SCC) cell carcinomas obtained from tumor debulking prior to Moh's micrographic surgery. Patterns of nerves, neuropeptides, and blood vessels in tumors were described and compared to those in unaffected skin. Results: The BCC-selective antibodies Ber-EP4, agglutinin UEA-I, pan-neuronal marker PGP 9.5, and nerve subtype selective antibodies against Substance P and CGRP demonstrated numerous nerve fibers adjacent to (within several microns) but not clearly within BCC and SCC tumor lobules. In a micronodular BCC demonstrating perineural spread on light microscopy, tumor lobules were detected adjacent to large nerve bundles. In at least one BCC, previously undescribed large PGP 9.5-reactive cells were visualized. Summary of conclusions: LSCM can be used to image peripheral nerves in skin cancers. Results suggest that if relevant neuronal interactions occur in BCC and SCC, it appears likely to occur within the tumor-stromal interface through soluble factor signaling. This method applying immunofluorescence labeling and LSCM can be extended to studying a variety of skin tumor types and may have application in studies of perineural tumor access and spread, cutaneous sensory function, and the cutaneous neuroimmune system.

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Gene expression profiles of primary cutaneous T cell lymphoma with focus on CD 30+ anaplastic large cell lymphoma (ALCL): unique clustering pattern of ALCL with extensive limb presentation

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We used cDNA microarrays to characterize global gene expression patterns in the most common subtypes of primary CTCL including tumor stage mycosis fungoides (MF), +/- large cell tranformation (MF LT), peripheral T-cell lymphoma unspecified (PTCL), primary cutaneous ALCL and cutaneous NK-/T-cell lymphoma (NK/TCL). We also included samples of normal skin and a NK/TCL nasal-tissue specimen. Unsupervised hierarchical clustering of all 50 specimens collected from 31 patients identified two readily evident gene expression signatures unique to cutaneous NK/TCL and to normal skin samples. MF +/- LT specimens clustered separately. The case of NK/TCL nasal-tissue presented with a different gene expression profile compared to its cutaneous counterparts. The other subtypes of CTCL did not separate into distinct groups by unsupervised clustering alone. Using SAM multiclass analysis, we found 40 genes that were able to distinguish the different subtypes of CTCL. For further analysis, we focused on 14 cases of CD30+ ALCL and performed separate hierarchical clustering. Two distinct groups could be identified, that correlated with clinical data. The group with a typical indolent clinical behaviour clustered separately from the group with an uncommon, extensive limb presentation, who had a more aggressive clinical course. STAT5A and HIPK2 (both inhibitors of apoptosis) and IL2RA were among the small group of genes that were more highly expressed in the extensive limb ALCL subtype compared to the other ALCL group.

Conclusion: Unsupervised and supervised analysis of gene array studies reveal distinct subsets in a variety of CTCL.

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Nucleotide excision repair responses in UV-irradiated human keratinocytes deficient in p53

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Although the tumor suppressor protein, p53, plays a central role in coordinating the cellular response to DNA damage, its involvement in regulating DNA repair in human keratinocytes is less clear. We used an immunoblot assay of UV-induced cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts (6-4 PP) as well as quantitative real-time RT-PCR to measure the global nucleotide excision repair response of human keratinocytes deficient in p53 following UV radiation. Human keratinocytes expressing the human papillomavirus 16 E6 and E7 proteins have barely detectable p53 levels by Western blotting. These cells, however, have normal repair of 6-4 PP and near-normal repair of CPD at early times, and are not significantly different from normal keratinocytes by 24 hours following UV. Similar results were seen in SCC-25 cells possessing inactivating mutations in p53 as well as in cells treated with pifithrin-α, a chemical inhibitor of p53. When mRNA of proteins involved in damage recognition was measured following UV, induction of mRNA encoding p48 or DDB2 is diminished several-fold in E6/E7-expressing keratinocytes relative to normal cells while induction of mRNA encoding XPC is preserved. These results indicate that human keratinocytes deficient in p53 preserve basal and certain inducible DNA repair responses in keratinocytes following UV radiation. In addition, these results suggest that human keratinocytes may be able to regulate global nucleotide excision repair activity, including induction of XPC, through mechanisms in addition to p53.

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Mechanisms facilitating escape of HPV-harboring tumor cells from the host immunosurveillance

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Immunosurveillance plays an important role in the control of progression of HPV-associated anogenital tumors. We studied expression of various mediators of immune reactions and cell adhesion molecules by non-tumorigenic keratinocytes (HaCat cells and HPV16-harboring SKv-e2 human keratinocytes) and HPV16-transformed tumor cells (Skv-e1, CaSki and SiHa). The production of chemokines (IL-8, GROα? MCP-1 and RANTES) and cytokines (TNFα??IL-18), as well as expression of ICAM-1 and LFA-3 were evaluated by ELISA. Specific mRNAs were studied by ribonuclease-protection assay or Northern blot using specific RNA probes. We found that non-tumorigenic cells expressed constitutively high amounts of ICAM-1 (but not LFA-3) and all chemokines studied, as compared to tumorigenic keratinocytes. Expression of these proteins in non-tumorigenic cells could be stimulated by exogenous TNFα? whereas tumorigenic cells did not respond to this cytokine. Addition of neutralizing monoclonal antibodies against TNFα to the cultures of non-tumorigenic cells led to a significant decrease in spontaneous ICAM-1 expression suggestive of an autocrine effect of endogenous TNFα. Functional studies revealed that non-tumorigenic cells exerted strong chemotactic activity towards both neutrophils and monocytes, and were highly sensitive to natural killer (NK)-mediated cell lysis. Conditioned media from cultures of tumorigenic HPV16-transformed cells significantly decreased NK cell activity and production of immunostimulatory INFγ by normal human peripheral blood mononuclear cells stimulated by anti CD3 antibodies, due to IL-18 inactivation by E6 oncoprotein of HPV16. The study showed that HPV16-transformed tumorigenic cells have evolved various mechanisms, such as inhibition of chemoattraction of leukocytes and their cytotoxicity, which could be responsible for local immunosuppression facilitating tumor progression.

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Delta-like 4 in T cell lymphoma

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In the thirty years since the term cutaneous T cell lymphoma (CTCL) was first introduced, our knowledge of this malignancy has greatly increased; yet the molecular mechanism continues to elude the scientific community. In the case of mycosis fungoides (MF), the most common type of cutaneous T cell lymphoma, the role of oncogenes and tumor suppressor genes have been investigated but the data has been inconsistent and nonconclusive. We have a murine model of T cell lymphoma (referred to as Tg8) that resulted from the fortuitous integration of a transgene (T cell receptor alpha) into the mouse genome. 100% of Tg8 mice develop lymphoma at approximately three months of age and given the fulminant nature of the malignancy, die within one month. Analysis of Tg8 mice located the transgene insertion site in chromosome 2 between two novel genes of unknown function, MGC4504 and KIAA1259. The closest known gene to the insertion site is Dll4, a newly identified ligand of Notch receptors. Our working hypothesis is that the transgene inserted into chromosome 2 and leads to an aberrant effect on the neighboring genes. Since the notch pathway has been implicated in human and murine T cell lymphomas, Dll4 appears to be the likely oncogene in our mouse model. The expression levels of genes at the insertion site were evaluated by real time PCR. Dll4 expression is highly upregulated in Tg8 mice as compared to wildtype littermates. MGC4504 and KIAA1259 also appear to be upregulated in Tg8 mice but to a lesser extent then Dll4. In order to determine the relevance of Dll4 overexpression in the development of T cell lymphoma in Tg8 mice, human samples of T cell lymphoma were analyzed. Our preliminary results show that a fraction of T cell lymphoma samples, including MF samples, overexpress Dll4, strengthening our hypothesis that Dll4 can act as an oncogene. This data provides the first evidence for Notch ligand-mediated malignancy.

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V beta expression in the leukemic phase of cutaneous T cell lymphoma

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The goal of this study was to identify cases of leukemic phase cutaneous T cell lymphoma (CTCL) that reacted with monoclonal antibodies directed against the beta region of the variable chain of the T cell receptor (TCR Vb) for use in other studies. Viably frozen peripheral blood lymphocytes from patients with leukemic involvement (B2 rating) were studied with T cell markers (CD3, CD4, CD7, CD8, CD26) and 20 TCR Vb antibodies conjugated with fluorescein isothiocyanate: Vb1.1-2 (BL37.2), Vb2 (MPB2D5), Vb3 (CH92), Vb5.1 (IMMU157), Vb5.2 (36213), Vb5.2-3 (MH3-2), Vb7.1 (ZOE), Vb8.1-2 (56C5.2), Vb9 (AMKB1-2), Vb11 (C21), Vb12 (VER2.32.1), Vb13.1 (IMMUN222), Vb13.6 (JU74.3), Vb14 (CAS1.1.3), Vb16 (TAMAYA1.2), Vb17 (E17.5F3.15.13), Vb20 (ELL1.4), Vb21.3 (IG125), Vb22 (IMMUN546), and Vb23 (AHUT7). To clarify results, additional staining of selective cases was performed with antibodies Vb5a/Vb52-3 (1C1), Vb5b/Vb5.3 (W112), and Vb5c/Vb5.1 (LC4). Of 49 samples studied, 31(63%) were identified as having a neoplastic cell population that reacted with an anti-Vb antibody. The median percentage of Vb positive cells in the lymphocyte gate was 66% (range, 28% to 97%). Correlation was made with other measures of neoplastic T cells: Sezary cell counts and the percentage of CD4+CD7- and CD4+CD26- subsets, and the only significant correlation was between Vb positive cells and CD4+CD7- ($r= 0.501$, $p= 0.006$). The neoplastic cells were positive for Vb1, Vb7, Vb21.3 and Vb22 from one patient each; for Vb6.7, Vb8, and Vb23 in 2 patients; for Vb3.1, Vb12, and Vb13.6 in 3 patients; and for Vb5 segments in 13 patients (Vb5.1, 9 patients; Vb5.2-3, 4 patients). The high frequency of Vb5 restriction suggests that neoplastic T cell clones in CTCL may be prone to arise from skin-homing T cells that are selected or stimulated to expand through the action of staphylococcal exotoxins or other antigens in the skin on V beta 5 segments.

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Association of p53 arginine polymorphism at codon 72 with development of skin cancer in epidermodysplasia verruciformis patients from Brazil

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The prevalence of p53 polymorphism at codon 72 is proposed to be a risk factor in human papillomavirus-related carcinogenesis. We studied the prevalence of p53 polymorphism at codon 72 in skin biopsies of epidermodysplasia verruciformis patients compared to DNA samples from healthy individuals. DNA samples extracted from normal skin and tumor biopsies of 22 Brazilian patients with epidermodysplasia verruciformis and blood samples from 27 healthy Brazilian individuals were studied for p53 codon 72 polymorphisms using restriction fragment length polymorphism analysis. All epidermodysplasia verruciformis patients with the malignant form of epidermodysplasia verruciformis were homozygous for arginine (Arg/Arg) at codon 72 of the p53 gene, in contrast to none with the benign form ($P < 0.0001$). p53 arginine polymorphism is likely to be associated with the development of skin malignancies in epidermodysplasia verruciformis patients from Brazil.

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The role of ATR and the replication checkpoint *in vitro* and *in vivo* in skin

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The replication checkpoint is required for the response to UV and other forms of DNA damage by ensuring that DNA replication is complete prior to mitosis. Here we describe studies that reveal greater detail of the role of the protein kinase ATR (ataxia telangiectasia and Rad3-related) in the replication checkpoint and genomic fidelity. We have employed three approaches to modulate ATR activity: inducible expression of a dominant negative ATR mutant or, siRNA targeting ATR both diminish ATR function while a cre-lox mediated deletion of ATR ablates its function. Our initial studies showed that suppression of ATR activity increased sensitivity to caffeine (an inhibitor of the replication checkpoint), augmented premature chromatin condensation, and induced chromosomal gaps and breaks at classically defined chromosomal 'fragile sites' (Cell 111:779). Because cancer cells often have insertions and translocations at these same fragile sites, this finding suggests that ATR function may be critical in malignant cells. To study ATR's role in skin in vivo we generated transgenic mice using a keratin-14 promoter linked to dominant negative alleles of either ATR or its downstream target Chk-1. Five independent founders carrying one of these transgenes (3 of ATR; 2 of Chk-1) are viable and without spontaneous defects in skin or hair at >6 months of age. While protein expression studies suggest these initial founders display only low level expression, some lines are experiencing significantly diminished survival. We are currently generating more founders and investigating the apoptotic response to UV radiation in vivo in these mice.

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Discovery of small molecules that modulate cell cycle replication checkpoint function

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ATR is a protein kinase involved in the replication checkpoint for which a potent specific small molecule inhibitor would be of great interest. However, discovery of small molecules that inhibit specific target proteins is typically a costly process requiring abundant purified protein and a robust bioassay. We have taken a novel approach to discover inhibitors for ATR, a large (301 kDa) protein that is not readily purified and for which no simple bioassay exists. Approximately 15,000 compounds from three diversity-oriented synthetic chemical libraries were robotically arrayed onto activated glass microscope slides to form covalently captured compounds. Cellular extracts were prepared from 293T cells that over-expressed Flag (epitope)-tagged ATR and from mock-transfected cells. After sequential incubation with a mouse anti-Flag antibody and a fluorescently-labeled anti-mouse antibody, slides were read in a fluorescence scanner. Six small molecules reproducibly yielded a signal with lysates from Flag-ATR transfected cells but not with mock-transfected lysates. Positive compounds were structurally similar to each other suggesting that they might recognize a common target (presumably ATR). The six compounds were tested for functional activity in a cell-based premature chromatin condensation assay and four of the six demonstrated inhibition of the ATR pathway. A chromosomal fragility assay for replication checkpoint function also demonstrated similar effects of these compounds. Studies are ongoing to characterize the biochemical effects of these small molecules on the phosphorylation of Chk1, a key event in the replication checkpoint. This is the first application of this new technology to a target protein in a cellular lysate—a general approach that may allow discovery of small molecules relevant to diverse biological systems.

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Time course of DNA repair, cytokine release and cellular redox state in UVB-irradiated reconstituted epidermis

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The purpose of this study is to evidence strategic phases in the repair of UVB-induced DNA damages by monitoring markers indicative of DNA repair, inflammation, and cellular redox state to improve design and evaluation of new photoprotective agents. Human Reconstituted Epidermis (HRE) were exposed to UVB (300 mJ.cm-1), and a kinetic study spanning on 48h was carried out. Sun Burn Cells (SBC) and TT dimers formation, were monitored by Hematoxylin-Eosin and immunostaining. Expression profiles of P53, GADD45 and IL-12, indicative of DNA repair mechanisms progression, were analyzed by RT-PCR. Inflammatory cytokines release (IL-1?, IL-8, TNF-?) was determined by ELISA. Cellular redox-state disregulation was evidenced by the dosage of glutathione (GSH) and glucose-6-phosphate-deshydrogenase (G6PDH). Cell viability was assessed by MTT test. 3 strategic phases following UVB irradiation were identified: Phase 1, from 0 to 6h, characterized by an important increase in transcriptional activity of GADD45 and P53 genes, related to a rapid set-up of DNA repair mechanisms. TT dimers formation and GSH redox-state disbalance were immediate consequences of UVB exposure. Phase 2, from 6 to 24h, characterized by the increasing release of IL-1, TNF-a and IL-8 correlated with cells entering in apoptosis to escape malignant transformation caused by DNA damage. Phase 3, from 24h to 48h, characterized by an important release of pro-inflammatory cytokines, and a significant decrease in cell viability due to apoptotic and necrotic death. This study evidences the time course of DNA repair events and necessary destructive processes for the removal of potential cancer cells in UVB-irradiated HRE. Study of the cellular redox state outlines that DNA repair is dependant of other biological mechanisms such as energy production and detoxification. This experimental model enables to design new photoprotective associations for an improved management of the photocarcinogenesis risk associated with sun exposure.

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Cell and isoform specific roles of protein kinase C isoenzymes in regulating *in vitro* and *in vivo* proliferation of keratinocytes and skeletal muscle cells

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The protein kinase C (PKC) system plays a crucial role in the regulation of proliferation, differentiation, and tumor formation of several cell types. In this study, we examined the specific roles of the conventional cPKCα and β and the novel nPKCδ and ε isoforms in the in vitro and in vivo growth of HaCaT keratinocytes and C2C12 skeletal muscle cells stably overexpressing the above isoforms. In vitro proliferation was assessed by a bromo-deoxy-uridine cell proliferation ELISA kit and growth curve analysis, whereas in vivo growth (tumor genesis) by was investigated by intradermally injecting the PKC overexpresser cells to immunodeficient (SCID) mice. In the HaCaT keratinocytes, overexpression of cPKCα or nPKCδ decreased in vitro proliferation and resulted in smaller and more differentiated tumors in SCID mice. However, the overexpression of cPKCβ or nPKCε stimulated proliferation and (less differentiated) tumor growth yet did not alter the benign phenotype of the developed tumors. As a marked contrast, in the muscle cells, overexpression of cPKCα or cPKCβ decreased proliferation and tumor development whereas the presence of nPKCε had no effect on in vitro and in vivo growth. Of great importance, however, overexpression of nPKCδ dramatically increased proliferation rate of the cells and resulted in the development of large and malignantly transformed tumors in SCID mice. These findings, therefore, strongly argue for pivotal yet antagonistic roles of certain PKC isozymes in the regulation of cellular proliferation and tumor formation. In addition, our results clearly highlight that the regulatory role of the given PKC isoform in the above processes possesses marked cell specificity.

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Loss of cell adhesion overcomes microenvironmental suppression of early carcinoma progression in 3D models of premalignancy

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To study the role of adhesive interactions between premalignant and normal keratinocytes (NK) during early cancer progression, we developed 3-D human tissue models to investigate how loss of E-Cadherin-mediated adhesion influences the fate of intraepithelial (IE) tumor cells (HaCaT-II-4) in premalignant disease. II-4 cells were transduced with a retrovector that generated a dominant-negative disruption of E-Cadherin function (H-2Kd-Ecad). As controls, II-4 cells were infected either with retrovectors expressing an empty vector (pBabe) or an H-2Kd-Ecad fusion protein with a loss of function deletion in its β-catenin-binding domain (H-2Kd-EcadΔC25). These three II-4 variants were β-gal-marked and mixed with NK at ratios 4:1 and 12:1 (NK:II-4) and the fate of IE tumor cells was determined before and after transplantation of 3-D tissues to nude mice. Four weeks after grafting, mixtures of adhesion-competent II-4 cells (pBabe and H-2Kd-EcadΔC25) generated normal-appearing grafts. In contrast, grafts containing mixtures with adhesion-deficient II-4 cells (H-2Kd-Ecad) demonstrated rapidly growing malignant tumors after 4 weeks. To explain the ability of these H-2Kd-Ecad-expressing II-4 cells to invade, the IE dynamics between tumor cells and NK was studied by double-immunofluoresence for Bromodeoxyuridine/β-gal and β-catenin/β-gal of mixtures before grafting. H-2Kd-Ecad-expressing II-4 cells were found at the basement membrane (BM) where they showed redistribution of β-catenin and active proliferation. In contrast, adhesion-competent II-4 cells were growth-suppressed by adjacent NK and were found in the suprabasal cell layers. Adhesion assays showed that selective adhesion of H-2Kd-Ecad-expressing II-4 cells to BM was mediated by the augmented adhesion to Type IV collagen. This study suggests that loss of E-Cadherin-mediated adhesion contributes to the transition from precancer to carcinoma by overcoming microenvironmental constraints induced by adjacent normal cells.

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Acquisition and evasion of host anti-tumor immune responses following rapid accumulation of immunogenic B16 murine melanoma cells in skin-draining lymph nodes

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B16/F1 melanoma cells are poorly immunogenic and readily form tumors after dermal implantation. To understand the requirements for effective anti-B16 immune responses, we rendered B16 cells more immunogenic through retroviral transduction with luciferase (LUC). LUC-B16 cells showed rapid (as early as 5 min), pertussis toxin-insensitive accumulation at the draining cervical LN of mice following inoculation into the ear skin. Mice mounted a potent host response that prevented formation of tumor in the ear and, later, at other skin sites upon rechallenge. Interestingly, small numbers of tumor cells could be detected by in vitro LUC assay 4 weeks after injection in the absence of overt tumor formation. By contrast, B16 cells expressing the chemokine receptor CCR10 (but not CXCR4) formed progressive tumors in the ear with cervical LN metastasis within 14 days following injection, suggesting that CCR10-expressing tumor cells can escape host tumor control. By LUC analysis, accumulation of LUC-positive B16 cells in the popliteal LN following footpad injection was 8-10 fold less efficient (vs. ear skin injection) and did not result in anti-tumor responses. Indeed, MRI-lymphangiography revealed that transport of a low molecular weight, Gd (III)-labeled contrast agent was markedly slower and less intense following injection of contrast agent into the footpad as compared to the ear skin. Finally, ear vaccination of mice (N=10) with LUC-B16 cells delayed formation of parental B16/F1 tumors that did not express luciferase ($p<0.001$), suggesting that reactivity to endogenous melanoma antigens had occurred. Thus, effective host anti-tumor responses that extend to less immunogenic tumor strains can be initiated by accumulation of sufficiently immunogenic tumor cells in secondary lymphoid organs. Moreover, expression of specific chemokine receptors allows tumor cells a means of evading host anti-tumor responses.

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Correlation of hypoxia inducible factor-1α expression with worsening grades of squamous dysplasia

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Cellular hypoxia is a commonly occurring phenomenon during tumor growth. The blood vessels created within tumors are often malformed, leading to insufficient blood flow and tissue hypoxia. When exposed to hypoxic conditions, cancer cells respond by inducing a variety of signaling pathways to allow for survival and tumor progression. Hypoxia Inducible Factor-1α (HIF-1α) is a transcription factor that is induced by cellular hypoxia. In addition, many reports have found that HIF-1α upregulation can be due to genetic alterations without any intratumoral hypoxia involved. It has been linked to critical pathways involved in regulation of angiogenesis, cell proliferation, and programmed cell death. The aim of this experiment was to establish the presence or lack of HIF-1α expression in various grades of squamous dysplasia ranging from pre-malignant to malignant lesions. We evaluated HIF-1α expression in skin sections obtained from patients with confirmed histological diagnoses of Actinic Keratosis (AK), Bowen's Disease (BD), Superficial Squamous Cell Carcinoma (SCC) and Invasive SCC. HIF-1α expression was quantified with a polyclonal HIF-1α antibody using immunohistochemistry. Our results showed a consistent, gradual increase of HIF-1α, from low expression in actinic dysplasia to high levels of expression in invasive SCC. No HIF-1α expression was noted in normal human skin. We conclude that upregulation of HIF-1α is directly correlated with worsening cellular dysplasia and dermal invasion. Furthermore, we have found that immunohistochemistry is both a sensitive and specific assay for the presence of HIF-1α. As a result, it may be a useful prognostic tool and helpful in determining approaches to treatment for certain types of skin cancer. As inhibition of HIF-1α activity has been shown to decrease expression of genes associated with increased angiogenesis and cellular metabolism, this transcription factor may prove to be a useful target against carcinogenesis.

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AP-1 transcriptional activity determines keratinocyte lineage commitment during tumorigenesis

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To investigate the collective role of AP-1 transcriptional activity in skin differentiation and tumorigenesis, transgenic mice were produced that express a dominant-negative Fos protein (A-FOS) under the control of a tetracycline operon (tet-O-A-FOS). These mice were crossed with K5-tet-Transactivator (K5-tTA) mice such that A-FOS is targeted to the basal epidermis and hair follicle outer root sheath in the absence of Doxycycline (Dox) in bigenic mice (K5-tTA/A-FOS). Inhibition of AP-1 activity was confirmed in vivo and in vitro by crossing K5-tTA/A-FOS mice with TRE-luciferase mice and measuring activity in skin and keratinocytes . Transgenic mice expressing A-FOS develop sebaceous adenomas during the course of 2-stage carcinogenesis and mixed sebaceous and squamous tumors when A-FOS expression is either induced or suppressed by changing Dox treatment during tumorigenesis. A-FOS expression prevents malignant conversion of benign tumors. Two proteins were identified to be specific for sebocytes in the sebaceous lesions- Indian Hedgehog and Adipocyte Differentiation Related Protein (ADRP). Further, ADRP was noted in keratinocytes surrounding sebaceous lesions, suggesting this is an early marker for sebocyte differentiation. Both markers were found to be expressed and specific for sebocytes in normal epidermis.

In cultured transgenic keratinocytes large lipid droplets formed in A-FOS expressing cells, and these cells contained a significantly higher lipid content when quantified by flow cytometry using Nile Red. Linoleic acid treatment increased lipid content in K5-tTA/A-FOS cultures as well as in cultures expressing a ras oncogene. These results suggest AP-1 is involved in determining the lineage commitment of multipotential keratinocyte precursors and may be required to maintain a squamous cell phenotype under conditions of high proliferation such as in cultured keratinocytes or epidermal tumors.

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CD4−CD25+ tumor-infiltrating T cells are associated with enhanced cutaneous tumor growth observed in CD4-deficient mice

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We have previously demonstrated decreased tumor growth in mice genetically deficient in αβ T cells undergoing two-stage chemical carcinogenesis with DMBA initiation and TPA promotion. Other investigators have observed the enhancement of tumor development in Harvey ras transgenic mice pre-immunized to elicit an anti-ras response. Taken together, these results demonstrate the capacity of αβ T cells to stimulate tumor growth under certain conditions. Therefore, we assessed tumor growth in CD4-/- mice, deficient in CD4 but not CD8αβ T cells, relative to TCRβ-/- mice which lack all αβ T cells. While the numbers of tumors per mouse (N=14) were comparable between CD4-/- and TCRβ-/- mice (18.4±1.6 vs 14.9±1.9, $p=0.09$) at week 15 post-initiation, tumor area (190.8±23.2 vs 74.1±9.9 mm^2, $p=0.0001$) and numbers of carcinomas per mouse (11.1±1.1 vs 5.0±0.6, $p<0.0001$) were markedly greater in the CD4-/- group, implicating either the absence of a protective CD4+ population and/or the presence of a tumor-promoting CD4− population. Four-color FACS analyses of lymphocytes isolated from papillomas and carcinomas growing in CD4-/- mice revealed tumor-infiltrating CD8+ T cells as well as a striking population of CD4−CD25+αβ+ T cells (of which only a small minority were CD8+). Thus, one potential explanation for the enhanced tumor growth in CD4-/- mice is that the tumor infiltrating CD4−CD25+αβ+ T cells are suboptimal T regulatory cells, incapable of suppressing a CD8+ tumor-promoting population. Future studies will be aimed at delineating the mechanism by which T cell subsets may stimulate tumor growth in the skin as such findings have important implications for immunotherapy in strategies.

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No association between p53 codon 72 polymorphisms and ultraviolet B induced erythema

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Any association between allelic variants of the p53 gene and acute ultraviolet radiation (UVR) sensitivity is important as it may provide insight into the mechanism underpinning the reported association of p53 with cutaneous malignancy. Although there is conflicting evidence, recent work has shown a link between p53, sunburn and both melanoma and non-melanoma skin cancer, with Pro/Pro homozygotes having a higher minimal erythema dose (MED) and thus less UV sensitive (J Invest Dermatol 119:84-90,2002; J Invest Dermatol 121:1510-1514). We had the opportunity to examine the same p53 variants and UVR sensitivity, measured not as a threshold measurement (MED), but by objective reflectance spectrophotometry at a range of UVR doses in 2 groups of patients. In group 1, 74 healthy Northern European residents were irradiated on the lower back with a range of UVB doses 119-300mJ/cm2 (Philips 9w/12). In group 2, 31 psoriasis patients, prior to phototherapy, were irradiated on the inner forearm with 6 doses of UVB (0.47-1.5J/cm2) from a TL01 Philips tube. Triplicate reflectance readings from each site, including baseline, were taken at 48 hrs and 24 hrs in groups 1 and 2 respectively. Genotyping of the p53-72 polymorphism was carried out by PCR and RFLP analysis. Neither study group showed an association between erythemal response and allelic variation at codon 72. Mean erythema (+/- SEM) at 300mJ/cm2 for group 1 Arg/Arg, Arg/Pro and Pro/Pro was 145(7.3), 162(6.8) and 149(18.7) respectively ($p=0.249$). And for the same polymorphisms at 1.5J/cm2 in the second group, 162(24.4), 159(23.1) and 169(29), ($p=0.986$). We found no evidence to support any increase in sun sensitivity at either body site at any dose examined. In the absence of further study we believe there is little compelling evidence to implicate p53 codon 72 alleles with sensitivity to UVR.

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Aggressive behavior of Merkel cell carcinoma of the skin may be related to tumor microvasculature density

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This study was undertaken to: (1)assess Langerhans cell population, that of their precursor, stromal microvasculature density, and epidermal proliferative index in Merkel Cell Carcinoma (MCC) and (2) to evaluate the prognostic value of these parameters in its metastasis occurrence. This is a retrospective study investigating twenty-five subjects aged between 20 and 90 (mean 70) years with biopsy-proven MCC. Patients were distributed into several groups according to the occurrence of metastasis. Diagnosis was based on the positive identification of a specific immunohistochemical profile including CK 20, NSE and Chromogranin. Langerhans cells were identified using an antibody directed against membrane CD1a while their precursors were characterized by human CD34 positive immunostaining. Proliferation index was quantified using MIB-1 antibody. As expected, surface density of CD1a+ cells was significantly reduced in epidermis overlaying the tumor, as compared with normal skin. CD34+ dendritic cells were not present within the tumoral stroma. On the other hand, proliferation index of MCC cells and overlaying keratinocytes was very high (41% and 13%, respectively). No correlation was found between these parameters and the clinical outcome of patients. Immunohistochemical staining labeling with CD34 also showed a higher number of intratumoral blood vessels compared with other skin malignancies such as melanoma among others. The median observation period was 38 months. Together, these results indicate that intratumoral microvasculature density may be the one of these quantitative parameters that correlates with metastasis occurrence.

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ΔNp63 isotypes differentially regulate keratinocyte proliferation and differentiation

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The p53 homologue p63 is required for squamous development and is expressed as multiple isotypes of two major subclasses: TA and ΔN. We have previously shown that *in vitro* overexpression of the ΔNp63α isotype in primary murine keratinocytes inhibits Ca^{2+}-induced growth arrest and differentiation. These findings, along with the reported overexpression of ΔNp63 in squamous cell carcinomas, suggest a role for ΔNp63α in the maintenance of the basal cell phenotype in normal and neoplastic epithelium. Here we address the importance of the α-terminus within ΔNp63α, and compare the effects of TA and ΔN isotypes. Using primary murine keratinocytes as a model of squamous epithelium, we overexpressed ΔNp63α, $\Delta Np63^{p40}$, TAp63α, TAp63γ, or β-gal *via* adenoviral transduction. Both ΔN isotypes inhibit G1 growth arrest mediated by elevated (0.12mM) extracellular [Ca^{2+}], as determined by FACS analysis. These findings are supported by western blotting using an anti-PCNA antibody, and suggest that the p63 core domain and ΔN terminus are sufficient for promoting keratinocyte proliferation. However, unlike ΔNp63α, $\Delta Np63^{p40}$ overexpression allows Ca^{2+}-mediated induction of the differentiation markers keratin 10 and filaggrin. Thus, aberrant expression of $\Delta Np63^{p40}$ dissociates growth regulation from differentiation. No alterations in cell cycle distribution or expression of keratin 10 were observed in keratinocytes overexpressing the TAp63 isoforms TAp63α or TAp63γ. Thus the C-terminal α-terminus is capable of modulating early markers of keratinocyte differentiation only within the context of the ΔNp63 subclass of isotypes. Ongoing analysis of the downstream similarities and differences between the ΔN isotypes may provide mechanistic insight into hyperproliferative skin disorders.

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Activation of TNFα/TRAF-2 and downstream effector molecules by 12-O-tetradecanoylphorbol-13-acetate (TPA) in mouse skin

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In animal models, TPA is reported to promote skin carcinogenesis by upregulation of ornithine decarboxylase (ODC) activity and difluoromethyl-ornithine (DFMO), a suicidal inhibitor of ODC, partially inhibits it, thus suggesting involvement of other pathways in skin tumor promotion. In this study we investigated alternate pathways that are activated by TPA and contribute to the process of tumorigenesis in mouse skin. CD-1 mice were treated topically with TPA and skin samples were collected 6, 12, 24 and 48 h later. In another group, mice were pretreated with DFMO 30 min prior to TPA application and skin samples were collected for immunoblot analysis. We observed that TPA induces expression of TRAF1/2 through the induction of TNFα. While the induction of TRAF-1 was observed only at 6 hr post TPA treatment, there was a sustained expression of TRAF-2 observed at all time points. We observed that concomitant with the inhibition of ODC expression DFMO treatment inhibited TRAF-1 expression. However, no change in the expression of TRAF-2 was observed in DFMO treated mice. We next evaluated the role of TNFα receptors viz., TNFR1 and TNFR2 in TPA induced TNFα signaling pathway. While a sustained induction of TNFR1 was observed, TNFR2 showed an increased expression only at 6 h post TPA, suggesting that TRAF2 interacts with TNFR1 in TPA induced signaling. Pretreatment of mice with DFMO showed no inhibition of either receptor suggesting that TNFα/TRAF1/2 signaling continues to be active despite the inhibition of polyamine biosynthesis. TRAF-2 is reported to associate with TRADD and RIP to activate the MAPKs pathway. We observed increasing expression of TRADD, RIP, p38 and JNK by TPA application. Treatment of mice with DFMO prior to TPA application inhibited TRADD and RIP expression, however, p38 and JNK were not affected by DFMO pre-treatment suggesting that TRAF-2/TNFR1 complex activates MAPK pathway independent of TRADD and RIP in TPA induced tumor promotion. In summary, we identified a novel pathway of TPA-induced skin tumor promotion.

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Mast cells contribute to host anti-tumor defense

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Mast cells (MC) accumulate at sites of tumor formation where they are suspected to be involved in growth promotion and angiogenesis. To test whether MC modulate the development of epidermal tumors, MC-deficient Kit^{W}/Kit^{W-v} mice, normal *Kit+/+* mice and Kit^{W}/Kit^{W-v}+MC mice that had been repaired of their cutaneous MC-deficiency by adoptive transfer of bone marrow-derived cultured MC to the skin of Kit^{W}/Kit^{W-v} mice were subjected to classical two-stage chemical carcinogenesis. Unexpectedly, *Kit+/+* mice proceeded to develop tumors significantly slower than Kit^{W}/Kit^{W-v} mice when treated topically with a carcinogen (DMBA, once) followed by a promotor (TPA, 2x/week for 15 weeks). After 12 weeks of treatment only 48.6 ± 4.2% of *Kit+/+*-mice, but 78.2 ± 4.7% of Kit^{W}/Kit^{W-v} mice showed ≥ 1 papilloma ($p<0.005$). Interestingly, *Kit+/+* mice also exhibited reduced numbers of papillomas per treatment site (0.31 ± 0.05 papillomas/cm^2 skin as compared to Kit^{W}/Kit^{W-v} mice (0.62 ± 0.09 papillomas/cm^2 skin, $p<0.005$). Susceptibility to skin carcinogenesis in Kit^{W}/Kit^{W-v}+MC mice was comparable to that seen in normal *Kit+/+* mice (week 12: ≥ 1 papilloma in 35.6 ± 10.9% of Kit^{W}/Kit^{W-v}+MC mice, 0.22 ± 0.05 papilloma/cm^2 skin). Most notably, MC appear to also regulate tumor growth. After 20 weeks of treatment the average tumor volume per papilloma was markedly increased in Kit^{W}/Kit^{W-v} mice as compared to *Kit+/+* mice and Kit^{W}/Kit^{W-v}+MC mice: 9.2 ± 2.8 vs. 3.8 ± 0.8 and 6.6 ± 3.0 mm^3/papilloma, respectively. Suspecting that MC release products required for resistance to *de novo* carcinogenesis, we tested whether MC undergo degranulation after TPA application. As assessed by histomorphometric analysis, virtually all subepidermal MC exhibited signs of extensive (54.2 ± 10.4%) or moderate (43.8 ± 10.4%) degranulation. These data support a dual role for activated MC in murine epithelial carcinogenesis: MC protect from skin tumor development and suppress tumor growth.

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Effects of ultraviolet B exposure on the expression of proliferating cell nuclear antigen in murine skin

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Proliferating Cell Nuclear Antigen (PCNA) is an active nuclear protein involved in DNA replication, recombination, and repair. PCNA is found throughout the basal layer in normal skin, and seen in all layers of the epidermis in malignancy. This study evaluates PCNAs patterns of expression at specific timepoints following acute and chronic UVB irradiation. Mice exposed to 1.5 kJ/m2 and 4.5 kJ/m2 were sacrificed at 6, 12, 24, 48, 72hrs, and 7days. Single and repeated exposure groups were maintained for the aforementioned doses. Immunohistochemical analysis performed on paraffin sections revealed PCNA expression throughout the basal layer of normal skin with diminished expression upon initial ultraviolet exposure. In subsequent timepoints, in the1.5 kJ group, PCNA immunoreactivity progressively increased, with appreciable upward epidermal migration upon chronic exposure. The 4.5 kJ group exhibited prolonged recovery in staining and also demonstrated this altered migratory pattern with chronic exposure. We hypothesize that the diminished expression in PCNA immunoreactivity associated with initial ultraviolet exposure, is representative of the immediacy of harmful effects that ultraviolet light imposes on cutaneous cellular repair mechanics. Progressive reactivation of PCNA expression results from protective events, elicited by the cells in response to ultraviolet damage. PCNA migration to upper layers of the epidermis, indicate proliferation, and subsequent transformation toward an increased malignant potential. We conclude that PCNA can serve as a marker of DNA repair and indirectly as an indicator of UVB induced damage, expression being time dependent and dose related. Specific immunoreactivity patterns and observable atypia in keratinocytes are relevant in elucidating malignant potentiation.

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Human skin equivalent: a reliable *in vitro* model for carcinogenic experiments

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EpiDerm FT (MatTek Co., MA) is an in vitro model for human skin derived from keratinocytes of human neonatal foreskin. The epidermal component is cultured to form a highly differentiated model of metabolically and mitotically active cells that are arranged analogous to those found in human epidedrmal skin. This advanced full-thickness model incorporates a fibroblast-containing dermis with epidermal keratinocytes. Epiderm has been used in dermatological research to test the irritancy of various consumer products, and the reactivity of specific biomarkers. In this study, we evaluate the capacity of this human skin equivalent to express cyclobutane-pyrimidine dimers(CPD)and Proliferating Cell Nuclear Antigen(PCNA)using immunohistochemical analysis. CPD represents one of the most abundant mutagenic and cytotoxic DNA photoproduct lesions, and current literature supports this biomarkers reliability in assessing ultraviolet-induced cutaneous DNA damage in human skin. PCNA is an active nuclear protein involved in cellular proliferation and repair that can be used indirectly to assess damage to cutaneous cellular mechanics. This report is the first to describe the immunohistochemical expression profiles of these biomarkers in human reconstituted skin. We observed a dose dependent increase in CPD expression from 20 mJ/m2 to 60 mJ/m2 of UVB for Epiderm specimens harvested six hours post-irradiation, with no CPD immunoreactivity in sham radiation specimens. PCNA expression was noted throughout sham radiation control specimens, with subsequent diminished expression upon ultraviolet exposure from 20 mJ/m2 to 60 mJ/m2, consistent with our previously reported results with similar UVB exposure levels in mouse skin. All specimens showed disrupted architecture at 100 mJ/m2. We conclude that Epiderm FT represents a formidable alternative to human and animal skin models. This viable in vitro option offers compelling relevance in the elucidation of ultraviolet induced biomarker evaluation and stands to serve as suitable substitute in cutaneous carcinogenesis experimentation.

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Lupeol inhibits TPA-induced activation of nuclear factor kappa B (NFκB) and phosphatidyl inositol 3-kinase (PI3K) /Akt and skin tumor promotion in CD-1 mouse

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Since primary prevention such as the use of sunscreens or wearing of protective clothing has proved inadequate in reducing skin cancer incidence, chemopreventive agents that act on multiple molecular targets may prove useful in association with primary prevention. Lupeol [Lup-20 (29)-en-3 ?-ol] is abundant in fruit plants such as olive, mango, strawberry and fig. In current study, we show that Lupeol modulates NFκB and PI3K /Akt pathways and inhibits skin tumorigenesis in mouse skin. We first determined the effect of topical application of Lupeol to CD-1 mouse against 12-O-tetradecanoyl- phorbol-13-acetate (TPA) -induced alterations in various molecules involved in tumor promotion and then determined its effect on mouse skin chemical carcinogenesis. We found that topical application of Lupeol (1- 2 mg / mouse) 30 min prior to TPA (3.2 nmole/mouse) application onto the skin of mice afforded significant inhibition, in a time and dose-dependent manner, against TPA-mediated increase in (i) skin edema and hyperplasia (ii) ornithine decarboxylase (ODC) activity and (iii) protein expression of ODC, cycloxygenase-2 and nitric oxide synthase. We also found that Lupeol treatment to mouse skin resulted in inhibition of TPA-induced (i) activation of PI3K, (ii) phosphorylation of Akt at Thr^{306}? (iii) activation of?NFκB and IKKα and (iv) degradation and phosphorylation of IκBα. The animals pretreated with Lupeol showed significantly reduced tumor incidence, lower tumor body burden and a significant delay in the latency period for tumor appearance. At the termination of the experiment at 28 weeks, 100% of the animals in TPA treated group exhibited 6-7 tumors / mouse whereas only 53% of the mice receiving Lupeol prior to TPA treatment exhibited 1-2 tumors /mouse. These results for the first time provide evidence that Lupeol possesses anti-skin-tumor promoting effects in mouse and could be used as an emollient or patch for chemoprevention or treatment of skin cancer.

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Induction of apoptosis by procyanidins from grape seeds is mediated by p53, Bcl-2 and caspase pathways

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The use of dietary botanical supplements has received considerable interest to prevent the risk of skin disorders including skin cancer. We have shown that dietary feeding of procyanidins from grape seeds (GSP) prevents ultraviolet (UV) light induced tumor promotion in mice. Since tumor promotion stage is reversible, it is better to target the molecular and cellular events for the prevention of skin cancer at this stage. Therefore, we determined the molecular mechanism involved in prevention of tumor promotion events by GSP treatment. For this purpose, we used mouse epidermal JB6 Cl41 cells, a well developed cell culture model for studying the molecular events in tumor promotion. JB6 cells are sensitive to the promotion by phorbol esters and other tumor promoters. Here we report that treatment of GSP dose (20-100 µg/ml)-dependently resulted in significant induction of apoptosis (12-70%) which was determined by FACS analysis, DNA ladder assay and immunofluorescence staining. Western blot analysis showed that treatment of GSP dose-dependently (20-80µg/ml) induced phosphorylation of p53 at Ser15 in JB6 Cl41 cells, and thus induction of apoptosis appeared to be p53 dependent because cells lacking p53 (p53-/-) showed no signs of apoptosis whereas p53+/+ cells underwent apoptosis after treatment with GSP. Further, treatment of GSP to JB6 cells resulted in dose-dependent decrease in anti-apoptotic proteins Bcl-2 and Bcl-xl levels accompanied with the increased expression of caspases. These data indicated that GSP induced p53-dependent apoptosis in JB6 Cl41 cells through Bcl-2 and caspases pathways, and suggesting that procyanidins from grape seeds could be developed as a chemopreventive agent for skin disorders which are associated with the dysregulation of p53, a tumor suppressor protein.

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Activation of oncogenic KrasG12D induces oral tumor formation

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Mouse models for sporadic human cancer represent powerful tools to analyze how mutations frequently found in human cancer regulate specific properties of tumor development. One of the key features of sporadic human cancer is the accumulation of mutations in the tumor cells but not in the surrounding normal tissue. We have developed a novel system that allows us to activate mutations in selected areas of stratified epithelia. This technology also overcomes the embryonic or neonatal lethality associated with the activation of oncogenes or inactivation of tumor suppressor genes using conventional transgenic/knockout approaches. We confirmed that gene activation/inactivation in this system occurs in the stem cells that regenerate stratified epithelia, including skin, oral mucosa, tongue and palate. We are currently using this system to assess the consequences of focal accumulation of somatic mutations such as activation of a mutant Kras allele and/or a mutant p53 allele. We found that inducible activation of the oncogenic KrasG12D allele in stratified epithelia induces oral tumor formation in all mice that were analyzed. These tumors arise in the oral mucosa, tongue and palate. However, skin tumors only appear after treatment with the tumor promoter TPA. Interestingly, preliminary genetic analysis of the oral tumors showed activation of the mutant KrasG12D allele in the presence of the wild type allele. However, only the mutant allele is present in skin tumors, suggesting that loss of the wild type allele is required for the appearance of skin tumors but not for oral cancers. We have initiated studies to assess the impact of activation of the gain of function mutant p53R172H allele in the presence or absence of oncogenic Kras. Preliminary data suggest that expression of mutant p53 accelerates the appearance of KrasG12D-dependent oral tumors. We are currently monitoring these mice for tumor progression.

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The capacity for Ras-driven tumorigenesis in primary human skin cells is lineage-dependent

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A central question in cancer biology concerns the relative roles of tissue-specific tumor induction mechanisms versus universally acting oncogenic pathways that drive neoplasia in all cell types. In human epidermis, oncogenic Ras can act in concert with IkBa to trigger invasive epidermal neoplasia indistinguishable from squamous cell carcinoma (SCC), thereby inducing cancer by directly altering only 2 signaling pathways. The efficiency with which deregulation of these 2 pathways drives tumorigenesis, coupled with the fact that both pathways play functionally critical roles in nearly all cells, raises the possibility that Ras-IkBa might serve as globally active oncogenes capable of facilitating malignant transformation of any primary human cell type. To address this, we studied primary human skin cells from a number of discrete lineages, including endodermal (endothelial cells), mesenchymal (fibroblasts), neuroectodermal (melanocytes) as well as epithelial control (keratinocytes). Ras and IkBa were co-expressed in each of these cell types via high efficiency retroviral transduction, as confirmed by immunoblotting. Each of these transduced cell populations was injected subcutaneously into nude mice (1x106 cells/mouse) to assay for tumor formation (n=5 mice/cell type). While transduced keratinocytes formed tumors within 2-4 weeks, no tumors were observed with any of the other cell types over the course of 6 months, indicating that Ras and IkBa are not universally active triggers of malignant transformation in human cells. To identify potential mechanisms contributing to these cell lineage differences, cell types are being examined for resistance to Ras triggered growth arrest, alterations in cell cycle regulators and the ability to invade extracellular matrix. These data indicate that requirements for carcinogenesis in skin vary depending on cellular lineage and indicate that, within human cutaneous tissue, keratinocytes are uniquely susceptible to tumorigenesis via Ras induction and NF-kB blockade.

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Specific sequences within type VII collagen are required for epidermal carcinogenesis

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Collagen VII disruption causes recessive dystrophic epidermolysis bullosa (RDEB), a disorder often accompanied by early-onset squamous cell carcinoma (SCC). The mechanism underlying increased SCC in RDEB is unknown and has been attributed to factors such as chronic wounding or a role for collagen VII in tumor suppression. However, the lack of comparable increases in SCC in other inherited chronic blistering diseases and the observation that collagen VII protein is overexpressed in sporadic SCCs, casts doubt on these explanations. To determine directly whether collagen VII enables or antagonizes carcinogenesis, we examined Ras-driven tumorigenesis in primary epidermal cells from 12 unrelated RDEB patients using co-expression of Ras and IkBa, which transforms normal keratinocytes into invasive SCC tumors in immune-deficient mice. Unexpectedly, cells from 4/12 patients failed to form SCCs in vivo. Collagen VII protein was entirely absent in this non-tumorigenic subset, in contrast to cells from the 8 tumorigenic RDEB patients, which all retained expression of a specific collagen VII sub-domain. Re-expressing both full-length collagen VII as well as this sub-domain restored tumorigenicity to collagen VII-null cells in a non-cell autonomous fashion. Collagen VII sequences lacking this sub-domain failed to restore the capacity for tumorigenesis to null cells, indicating they are required for neoplasia in this setting. Purified sub-domain fragments enhanced cell invasiveness and proliferation in a manner dependent on specific integrins, while retroviral sub-domain co-expression with Ras induced epidermal tumors in vivo. Polyclonal and monoclonal antibodies raised to sub-domain epitopes abolished invasion and hyperproliferation by transformed keratinocytes in culture and, when administered in vivo, blocked tumor formation by Ras and IkBa expressing wild-type human keratinocytes. These data indicate that specific collagen VII sequences are necessary for invasive human epidermal neoplasia and underscore their therapeutic potential in SCC.

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The effects of chemopreventive agents on DNA damage and PCNA expression on mouse skin treated with BaP-UVA

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We have previously reported that topical administration of BaP substantially enhances UVA-induced formation of oxidized DNA bases, DNA photoproducts, and potentiates skin tumorigenesis by UVA. The goal of our study was to assess the initial protective benefits of several naturally occurring chemopreventive agents: Genistein, Baicalein, and sandalwood oil (SWO), against synergistic enhancement of skin carcinogenesis by BaP-UVA. This was done using immunohistochemical staining for several molecular markers such as PCNA (proliferating cell nuclear antigen), 8-OHdG (8-hydroxy-2-deoxyguanosine), and (6-4) DPs (dipyrimidine photoproducts). PCNA is a marker for cell proliferation and DNA repair after damage. 8-OHdG is a marker for oxidative DNA damage. 6-4 DPs are a marker for UV-induced DNA damage. Eight groups of mice (5 female SKH-1/group) were treated with BaP(2g) and 60 min. later followed by different comparable concentrations of either Genistein, Baicalein, SWO, or acetone (control), which was applied 30 min prior to, or 5 min post appropriate doses of UVA (20 kJ/m2). Mice were sacrificed 24 hrs post-UVA irradiation, and the mouse skin was paraffin embedded for immunohistochemical analysis. In conclusion, all the chemopreventive agents showed significant inhibition of DNA damage caused by BaP-UVA, in comparison to the acetone (control) groups. More specifically, SWO showed to be the most potent agent for the inhibition of DNA damage and activation of DNA repair as demonstrated by having the most PCNA positively stained cells. Baicalein was the most potent agent for the inhibition of 6-4 DP formation, and Genistein was the most scavenging of ROS, as was demonstrated by having the least amount of 6-4 DP and 8-OHdG positively stained cells, respectively. These results suggest, that the naturally occurring genistein, baicalein, and SWO may have a protective benefit against chemical and photocarcinogenesis in vivo, by interfering at the level of DNA damage.

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Role of suprabasal α6β4 integrin expression in squamous cell carcinoma formation

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Squamous cell carcinoma (SCC) is the second most common malignancy in Caucasians and has a relatively high capacity for metastasis making the current rise in SCC incidence a major health issue. Inappropriate α6β4 integrin expression correlates with a high risk of tumor progression in stratified squamous epithelial tumors such as SCC. However, the impact of aberrant α6β4 expression in otherwise normal epithelium on the initiation and course of epithelial tumorigenesis is relatively unknown. Targeted expression of α6β4 in the suprabasal layers of transgenic mouse epidermis dramatically increases the frequency of papillomas, squamous cell carcinomas and metastases induced by chemical carcinogenesis. Suprabasal α6β4 expression also perturbs transforming growth factor β (TGFβ) signal transduction and relieves TGFβ-mediated growth inhibition of basal cells. The disruption of TGFβ signaling by suprabasal α6β4 has been shown to require E-cadherin-mediated intercelluar adhesion and PI 3-kinase activity. Here we show evidence that α6β4 integrin is linked to the actin cytoskeleton in suprabasal cells suggesting a possible role for Rho GTPases and actin cytoskeleton reorganization in the effects of α6β4 on TGFβ signaling and epidermal tumor formation. In addition, Rac1 expression and activity is dramatically upregulated in the basal and suprabasal layers in α6β4 transgenic epidermis compared to wt epidermis in response to tumor promoter treatment. Manipulation of Rho GTPase-mediated actin cytoskeleton reorganization may represent a novel mechanism of regulation of growth factor signaling pathways by epidermal tumor cells. Overall these results illustrate how differentiated cells can influence the tumor microenvironment and the impact of changes in the tumor microenvironment on the outgrowth and progression of SCC.

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Withdrawn

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Hypoxia-inducible factor 1α (HIF-1α) involves in activation of transcription responses to tumor growth as well as cellular apoptosis

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In the process of tumorigenesis, lack of oxygen (hypoxia) often suppresses the tumor growth. Unfortunately, many tumors adapt to this situation through the activation of hypoxia inducible factor-1α (HIF-1α), a transcription factor which controls a series of genes (VEGF, p53, ras, p21, bcl-2 etc) that play a key role in tumors survival and development. Chronic exposures to UV radiation, involves the loss of wild type p53 function, subsequently forming p53 mutation. Furthermore, unrestrained cell proliferation and clonal expansion leads to skin cancer. p53 has also been implicated in the regulation of Bcl-2 family expressed apoptotic pathways. We hypothesize that HIF-1α promotes tumor growth by regulating transcription factors and also induces apoptosis by modulating balances between p53 and bcl-2 genes. A series of observations on UVB induced mice skin tumors (Squamous cell carcinomas), were stained with HIF-1α, p53 (wild type & mutant) and bcl-2 family (i.e. bcl-2, bax and bcl-x) using immunohistochemistry and immunofluorescence. The results showed that the expression of HIF-1α correlates with wild type p53 as well as induction of mutanted p53. The anti-apoptotic member bcl-2 diminished significantly with an increased bax and bcl-x expression, the pro-apoptotic members. These results show a strong correlation that HIF-1α modulates p53 transcriptional activities promoting uncontrolled cell proliferation and tumor growth, furthermore activating apoptotic responses by regulating the balances of bcl-2 family anti and pro-apoptotic members. Based on the observations, HIF-1α activation promotes tumor growth as well as cellular apoptosis.

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Mre11 is central to telomere-based DNA damage responses

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Telomeres are repeats of TTAGGG, ~ 10, 000 base pairs long, that cap and protect the ends of chromosomes. Critically short or experimentally disrupted telomeres lose their protective function and initiate DNA damage signaling, leading to apoptosis or senescence. Telomeres end in a 3' overhang normally protected within a loop structure in intact, "capped" telomeres. "Uncapped" telomeres associate with many proteins involved in DNA repair and/or damage signaling and elicit DNA damage responses. One such protein is the 3' to 5' exonuclease Mre11, mutated in the radiation-sensitive, cancer-prone disease, Ataxia-Telangiectasia (AT)-Like Disorder (ATLD). We have shown that treatment of human cells with DNA oligonucleotides homologous to the telomere overhang act through ATM to induce DNA damage responses, presumptively by mimicking exposure of the overhang. Only T-oligos with hydrolysable internucleotide bonds are active, suggesting a role for a nuclease in mediating their effects. We investigated the role of Mre11 in T-oligo responses using RNAi technology, selectively "knocking-down" this protein by 50-90% compared to a negative control siRNA, as shown by Western blot analysis. In the Mre11-depleted cells, T-oligo-induced phosphorylation of the ATM substrates p95/Nbs1 and p53 was reduced: p95/Nbs1 serine 343 by 42% and p53 serine 15 by 90%. Furthermore, T-oligo-induced phosphorylation of p53 serine 37, a substrate for the ATR and DNA-PK kinases, but not for ATM, was reduced in Mre11 knockdown cells by ~ 40%, suggesting a role for these signaling kinases as well. Also, the induction of senescence by T-oligo was blocked; senescence-associated β-galactosidase activity increased 18-fold with T-oligo treatment in control siRNA cells ($p<0.01$, compared to diluent-treated controls) but decreased by 7% in Mre11 siRNA cells ($p>0.8$). These data strongly suggest that Mre11 "senses" and hydrolyses the exposed telomere overhang at times of telomere loop disruption, signaling through ATM and likely through ATR and DNA-PK. T-oligos provide a useful model for studying these responses.

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DNA homologous to the telomere 3' overhang induces cell senescence independent of genomic telomere structure

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Telomeres are located at the end of linear chromosomes and consist of ~ 10,000 base pairs of TTAGGG repeats and an ~ 100-400 base 3' single strand overhang (ssOH) that inserts within the telomere duplex, forming a large "T-loop" at the chromosome end. Disruption of the telomere loop structure exposes the ssOH and rapidly induces apoptosis or senescence, both acknowledged anti-cancer mechanisms. It was recently demonstrated that telomeres of fibroblasts (fb) serially passed to senescence lack the ssOH, suggesting that a blunt end may constitute the critical feature of an "uncapped" telomere in senescent cells. However, treatment of human fb with oligonucleotides homologous to the telomere overhang (T-oligos) also induces apoptosis or senescence, suggesting that exposure of the overhang itself "uncaps" a telomere. In order to distinguish between these two possibilities, we examined the ssOH on telomeres of fb rendered senescent by treatment with T-oligo for one week, and in fb then maintained an additional week in the absence of T-oligo. In contrast to diluent-treated controls, over 60% of fb treated with T-oligo for one week exhibited senescence-associated β-galactosidase (S.A. β-Gal) activity; expressed increased levels of p53, p53 phosphoserine 15, p21 and p16; and were unable to phosphorylate pRb after serum stimulation. Even after an additional week in the absence of T-oligo, the fb continued to express these protein markers and S.A. β-Gal positivity, characteristic of senescent cells. Analysis of the ssOH showed that the telomeres of these cells, in contrast to fb serially passed to senescence, were not disrupted and the 3' overhang was intact in T-oligo-treated fb. These data demonstrate that a blunt-end telomere is not a prerequisite for senescence and suggest that exposure of the overhang is likely the critical event in telomere "uncapping" that initiates DNA damage responses.

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Chemical inhibition of nuclear factor kappa B (NFκB) prevents UVB-induced squamous cell carcinomas (SCCs) in SKH-1 mice

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Constitutive activation of NFκB augments tumor resistance to apoptosis and may contribute to the induction of carcinogenesis. Paradoxically in skin, blockade of NFκB by overexepessing IκB and ras promotes the growth of lesions resembling SCCs. We tested the chemopreventive effects of sulfasalazine and pyrrolidine dithiocarbamates (PDTC) chemical agents known to inhibit NFκB against UVB-induced SCCs in SKH-1 mice. Oral administration of these agents substantially delayed tumor formation, resulting in 1.6-fold and 4-fold decreases in tumor incidence after 25 weeks in mice receiving sulfasalazine and PDTC respectively, as compared to UVB-irradiated, non-treated animals. By week 35, the UVB-irradiated, non-treated group had more than 20 tumors per mouse whereas the average tumor number in the sulfasalazine and PDTC-treated groups was 9 and 2, respectively. Furthermore, UVB-induced NFκB DNA binding activity was decreased by sulfasalazine and PDTC treatment as assessed by electrophoretic mobility shift assay (EMSA). Both NFκB inhibitors decreased the levels of cell cycle regulatory proteins (cyclins A/B1/D/E, cdk2/4/6, p53, and chk1) and proliferation markers (ODC and PCNA) to levels comparable to unirradiated controls. Our results demonstrate that pharmacological inhibition of NFκB substantially abrogates UVB-induced skin carcinogenesis in tumor-susceptible mice.

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Identification of putative novel genomic loci involved in sporadic basal cell carcinoma tumorigenesis

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Mutations in the PTCH or SMO genes, which are components of the SHH signaling pathway, have been observed frequently in sporadic basal cell carcinoma (BCC). However, a significant proportion of sporadic BCCs lack mutations in these or other known SHH pathway genes. We hypothesize that alternative or additional genes may contribute to the pathogenesis of some BCCs. We screened a panel of 24 tumors using array-based comparative genomic hybridization (aCGH) to identify loci that are commonly lost or gained in sporadic BCCs, which may harbor tumor suppressor genes or oncogenes, respectively. Tumor-derived genomic DNA and patient-matched normal reference DNA were differentially-labeled with Cy3 and Cy5 fluorescent dyes and co-hybridized to a human genomic DNA bacterial artificial chromosome (BAC) library spotted in triplicate on a glass slide. Each BAC clone carries roughly 150 Kb of insert DNA and the genomic map position of each clone is known. The library, which was optimized specifically for aCGH, is composed of 4134 clones that span the human genome at a spacing interval of approximately 1 Mb. After hybridization, slides were scanned to detect fluorescence signal intensities for each BAC clone. The ratio of the fluorescence intensities is used to calculate DNA copy number, enabling analysis of genome wide copy number in tumor DNA. Using aCGH, we observed loss of the PTCH region at 9q22.3 in 8 of 24 (33%) samples. This finding is comparable to previously reported rates for loss of heterozygosity for PTCH in sporadic BCC and validates our capacity to detect alterations in copy number in tumor DNA. In addition, we detected losses at 20q (29%), 17p (25%), and 19q (25%), as well as gains at 15q (25%). These results indicate that changes in copy number at regions other than the PTCH locus are common in sporadic BCC and are likely to be non-random. We are screening additional BCC samples to define the minimal critical regions of gain or loss at these loci and identify candidate genes that may contribute to BCC tumorigenesis.

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UVA modifies UVB-induced skin carcinogenesis *in vivo*

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Ultraviolet (UV) irradiation has long been involved in photocarcinogenesis. UVB (290-320 nm) in particular has been extensively reported as an initiating and promoting agent for skin carcinogenesis. On the other hand, UVA (330-400 nm) is considered to be a very weak initiator and promoter of photocarcinogenesis. There have been reports showing that long wavelength UVA exposure can increase levels of certain antioxidant enzymes. The present study has investigated combinational effects of non-carcinogenic UVA (>360 nm) and UVB on mouse skin carcinogenesis. SKH1 hairless mice were irradiated with non-carcinogenic UVA at 10 and 30 KJ/m^2 respectively 2 hr prior to UVB (0.3 J/m^2) treatment. The exposure combination was repeated thrice a week for 25 weeks. No tumors were observed in UVA alone groups, whereas UVB alone groups exhibited significant tumor incidence and multiplicity. In UVA and UVB combination groups, both tumor incidence and multiplicity were remarkably lower than the UVB alone group. This study suggests that UVA treatment prior to UVB exposure significantly decreases skin carcinogenesis. The mechanism of photoprotective action of UVA remains unknown and requires further investigation.

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Multiple cutaneous and uterine leiomyomata: identification of germline fumarate hydratase mutations and evidence for a founder splicing mutation

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Germline mutations in the fumarate hydratase gene (FH) predispose to multiple cutaneous and uterine leiomyoma syndrome (MCL) and MCL associated with renal cell cancer (HLRCC). MCL is inherited in an autosomal dominant manner and is characterized by the presence of concurrent benign tumors of smooth muscle origin (leiomyoma) in the skin and uterus. In HLRCC, patients can develop papillary renal cell carcinoma and uterine leiomyosarcoma in addition to leiomyomas. The genetic locus for MCL and HLRCC was recently mapped to chromosome 1q42.3-43 and subsequently, mutations in the fumarate hydratase gene (FH) were identified. The analysis of the FH gene in tumors of MCL/HLRCC patients suggests that FH may function as a tumor suppressor gene. Here we report the clinical and genetic analysis of 16 families with MCL. Mutational analysis of the FH gene revealed 13 different mutations accounting for the disease in all families. All of them are unique to single families, with the exception of the splicing mutation 905-1G>A, identified in four families of Iranian origin. The analysis of highly polymorphic microsatellite markers in the vicinity of the FH gene showed a shared haplotype in these four families, suggesting that 905-1G>A represents a founder mutation. Collectively, we have identified 10 novel and three previously described FH mutations, providing further evidence for the role of FH in the pathogenesis of MCL.

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Combined treatment with cisplatinum and HA14-1, a small molecule Bcl-2 inhibitor, results in synergistic cell death of WM35 melanoma cells

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We have previously shown that stable transfection of activated H- or N-ras into the low invasive potential, radial growth phase (RGP) melanoma cell line, WM35, produces cell lines with characteristics typical of vertical growth phase (VGP) melanoma. Ras expression decreases both spontaneous apoptosis and cisplatinum-induced apoptosis when WM35 cells are grown in spheroid culture. Growth of melanoma cells on tissue culture plastic induces expression of Bcl-2, while anchorage independent growth in spheroid culture does not. Ras expression, however, maintains elevated levels of Bcl-2 protein and differences in susceptibility to apoptosis correlate inversely with the level of Bcl-2 expression. HA14-1 is a small molecule nonpeptidic ligand that binds and inhibits the anti-apoptotic activity of Bcl-2. It has also been described that HA14-1 induces translocation of Bax, a proapoptotic Bcl-2 analog, from the cytosol to the mitochondria. We used HA14-1 to pretreat WM35 control and mutant ras-expressing cells grown in spheroid culture before exposing them to low-dose cisplatinum. Cell death was measured using an MTS assay for viability. Apoptosis was measured using annexin V and ethidium bromide acridine orange staining. When exposing WM35 cells to either cisplatinum or HA14-1 alone, the level of cell death was always less than 30%. However, combining HA14-1 and cisplatinum resulted in greater than 60% cell death. Mutant ras-expressing cells, while sensitive to the effects of the Bcl-2 inhibitor on cisplatinum-induced death, appeared to be somewhat resistant compared to the control cells. Malignant melanoma is resistant to therapy using single drugs or radiotherapy. However, these experiments raise the possibility that Bcl-2 inhibition may make melanoma susceptible to low-dose chemotherapy.

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Ultraviolet B radiations cause an upregulation of survivin in human keratinocytes and mouse skin

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Excessive exposure to solar UV radiation, particularly its UVB component (290-320 nm), to human skin is the major cause for more than a million new cases of non-melanoma skin cancer diagnosed annually in the USA. Understanding of the mechanism of UVB-mediated cutaneous damages is far from complete. Here, we evaluated the involvement of survivin, which is a structurally unique member of the inhibitors of apoptosis protein family and is known to be involved in the control of cell division and apoptosis. The cancer-specific expression of survivin, coupled with its importance in inhibiting cell death and in regulating cell division, makes it a target for cancer treatment. This study was designed to investigate role of survivin in UVB-mediated damages in normal human epidermal keratinocytes (NHEK) (*in vitro*) and in SKH-1 hairless mouse model (*in vivo*). For *in vitro* studies, NHEKs were treated with UVB (50 mJ/cm^2) and samples were processed at 5 min, 15 min, 30 min, 1 h, 3 h, 6 h, 12 h and 24 h post-treatment. Immunoblot and real-time quantitative RT-PCR analyses showed a time-dependent increase in the levels of survivin protein and mRNA. We also observed a time-dependent i) decrease in Smac/DIABLO, and ii) increase in p53, in NHEK. To study the relevance of our *in vitro* findings to *in vivo* situations, we employed SKH-1 hairless mice as a model. The mice were subjected to a single exposure of UVB (180 mJ/cm^2) and samples were processed at 3 h, 6 h, 12 h and 24 h post-UVB exposure. UVB treatment resulted in a time-dependent increase in protein and RNA levels of survivin, phospho-survivin and p53 and a concomitant decrease in Smac/DIABLO in mouse skin. We also found that UVB treatments to NHEK as well as SKH-1 hairless mouse skin resulted in a time dependent increase in protein tyrosine kinase phosphotransferase activity, a marker for cell cycle progression. Our results, for the first time show the involvement of survivin (and the associated events) in UVB response. We suggest that survivin could serve as a target for the management of UVB exposure-mediated damages including skin cancer.

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Correlative analysis of microarrays for characterizing basal cell carcinoma: NDUFA1 and the anaerobic tumor environment

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Numerous mechanisms have been implicated in the development of basal cell carcinoma (BCC). These include aberrations in tumor suppressor (*PTCH* and *p53*) and oncogene (*ras*) activity, UV-induced DNA mutation, DNA mismatch repair defects, immune dysregulation and free radical induction. Furthermore, BCCs demonstrate heterogeneity with genetic polymorphisms, cytogenetic differences and histological diversity. To elucidate the genes dysregulated in BCC while addressing these forms of heterogeneity, we developed a novel, correlative analysis of microarrays (CAM) for the selection of candidate genes that are commonly (among all tumors) differentially expressed. CAM is a nested, nonparametric method designed to qualitatively select candidate genes based on their individual, similar effects upon an array-wide closeness measure. We applied the CAM method to a two-channel microarray experiment where individual arrays were performed on 21 BCC and patient-matched, normal skin specimens. We selected 19 candidate genes (7 over- and 12 under-expressed) in all represented BCCs versus normal skin. The expression of several genes in the oxidative phosphorylation pathway, including the 7.5kDa NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1 (NDUFA1), was consistently downregulated among tumor samples. NDUFA1 is an accessory component of complex I that is essential for functional respiratory activity. Our findings further support the hypothesis that irregularities in normal mitochondrial function are involved in neoplastic disease. Our data indicates suppression of NDUFA1 represents a common and potentially key pathogenic mechanism in the development of BCC. Maintaining this cornerstone of cellular aerobic energy production could prevent the hypoxic stress and free radical induction that is associated with the malignant growth and tumor progression.

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Conditional expression of *Gli2* in skin reveals a requirement for sustained hedgehog signaling in basal cell carcinoma growth

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Transient hedgehog signaling regulates epithelial growth during hair follicle morphogenesis, and constitutive activation of this pathway has been implicated in the development of essentially all basal cell carcinomas (BCCs). We have previously shown that the major transcriptional effector of this pathway in skin is *Gli2*, which is sufficient to produce BCCs when overexpressed in mice. To examine the role of continued hedgehog signaling activity in tumor maintenance, we generated tetracycline transactivator (tTA) responsive *Gli2* transgenic mice (*TRE Gli2*). While *TRE Gli2* mice were indistinguishable from controls, bitransgenic mice that also harbored a K5 promoter driven tTA (tet off) transgene produced multiple BCCs by 6 months of age with 100% penetrance. Administration of doxycycline resulted in efficient deinduction of transgenic *Gli2* mRNA; reduced expression of the upregulated hedgehog target genes *Ptch1, Gli1, and Hip1*; and dramatic tumor regression, characterized by a 90% reduction in tumor volume by three weeks. Tumor regression was associated with a rapid inhibition of cell proliferation and striking increase in apoptosis. Although tumors did not recur even after 5 months of transgene deinduction, a small fraction of residual keratinocytes was detected in regressed BCCs. These cells were non-proliferative and expressed keratins K5 and K17, similar to hair follicle keratinocytes in the resting phase of the hair cycle, but could resume proliferation to form BCCs upon reactivation of *Gli2* expression. Our findings demonstrate the requirement of sustained hedgehog/Gli2 signaling for continued growth of established BCCs. Moreover, the residual, non-proliferative keratinocytes in regressed BCCs share several features in common with epithelial hair follicle stem cells, and may represent tumor stem cells.

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Cripto-1 induces keratin 8 and 18 expression in primary mouse keratinocytes

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Cripto-1, an EGF-CFC family member that is frequently overexpressed in human epithelial cancers, is both a coreceptor for the TGFbeta superfamily member nodal and a direct inhibitor of activin signaling. Cripto-1 also stimulates mitogen-activated protein kinase and AKT phosphorylation. We have identified Cripto-1 as a target of Smad7 that could mediate Smad7 induced malignant progression of v-rasHa expressing keratinocytes. Cripto-1 is rapidly induced in keratinocytes infected with a Smad7 adenovirus, and in tumors from keratinocytes transduced with a Smad7 retrovirus. TGFb1 superfamily members downregulated Cripto-1 expression, while Smad7 induced promoter activity from a cotransfected human Cripto-1 promoter-luciferase construct, suggesting that Smad7 counteracts inhibitory effects of TGFb superfamily signaling on Cripto-1 expression. Similar to overexpression of Smad7, treatment of primary mouse keratinocytes with soluble Cripto-1 induced expression of keratins 8 and 18, markers of simple epithelial dedifferentiation and malignant conversion. However, Cripto-1 did not block the induction of keratin 1 or 10 by elevated calcium. Since keratinocytes do not express nodal and activin A and activin B suppress expression of keratin 8, it is possible that the effect of Cripto-1 on keratin 8 expression is through inhibition of endogenous activin signaling. However, treatment of primary keratinocytes with soluble Cripto-1 induced phosphorylation of AKT and ERK1/2. The PI3-kinase inhibitor Wortmannin but not the MEK inhibitor UO126 blocked keratin 8 induction by Cripto-1, suggesting additional involvement of the PI3 kinase pathway in the regulation of keratin 8 by Cripto-1. These results suggest that the simple epithelial conversion in Smad7 expressing cells may be mediated through induction of Cripto-1.

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T-Oligo treatment down-regulates constitutive and UV-induced COX-2 expression in cultured cells and in murine and human skin

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Chronically irradiated murine skin and UV-induced SCC overexpress the inducible isoform of cyclooxygenase (COX-2), and COX-2 inhibition reduces photocarcinogenesis in mice. p53 has been reported to regulate COX-2 expression both positively and negatively, with discrepancies likely related to protein level vs activity. We have previously reported that DNA oligonucleotides substantially homologous to the 3' telomere sequence (T-oligos) enhance DNA repair capacity (DRC) and multiple other cancer prevention responses, in part through up-regulation and activation of p53. To determine if T-oligos affect COX-2 expression, human newborn fibroblasts were pretreated with T-oligos (pTT or pGAGTATGAG) or diluent alone for 24 hours, then UV irradiated and processed for western blotting. Within 24 hrs T-oligos markedly down-regulated baseline COX-2 expression, coincident with p53 activation (phospho- p53ser15). Transcriptional repression of COX-2 gene expression by p53 was further confirmed by transfecting WT and p53DN cells with a COX-2/Luciferase reporter plasmid. In WT but not in p53DN cells, within 48 hrs T-oligos decreased constitutive COX-2 expression (luciferase activity) by >50% (WT - 125±30 vs 58±8, $p<0.04$; p53DN - 100±6 vs 94±30, p=NS, diluent vs T-oligos n=3/group in triplicate). To examine the effect of T-oligos on COX-2 expression in intact skin, mice were pretreated topically and human skin explants were provided with T-oligos or diluent alone in culture medium, and then irradiated 24 hrs later. Compared to controls, T-oligos markedly decreased COX-2 immunostaining in murine and human skin both pre- and post-UV. This coincided with increased p53 immunostaining. We conclude that T-oligos transcriptionally down-regulate COX-2 expression by activating and up-regulating p53. This may contribute to the observed ability of T-oligos to reduce photocarcinogenesis.

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Benign and malignant mouse epidermal tumors have distinct transcriptional responses to TGFβ1 *in vivo* reflecting tumor suppressor to oncogene switch

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Transforming growth factor beta (TGFβ1) has both tumor suppressive and oncogenic roles in epithelial cancer progression that is dependent on the cancer stage. Our aim is to test the hypothesis that this switch in outcome reflects altered responsiveness at the transcriptional level. We have generated tumors in mice expressing a conditionally regulated active TGFβ1 and identified TGFβ1 regulated targets in papillomas and squamous cell carcinomas (SCC) using microarray analysis. With the TGFβ1 transgene suppressed the latency and yield of benign and malignant tumors was similar in single transgenic (ST) and double transgenic (DT) mice. Tumor bearing mice were given dox for 48 hrs to induce TGFβ1 in tumors. Nuclear localization of Smad3 was observed in double transgenic papillomas and some SCC after induction of TGFβ1, suggesting that the TGFβ1 signaling pathway was intact. In papillomas induction of TGFβ1 caused a rapid shutdown of DNA synthesis while there was no effect or a slight increase in SCC. Microarrays were done comparing RNA from DT to ST papillomas and DT to ST SCC, 48 hrs after addition of dox. Surprisingly, very few genes were similarly regulated by TGFβ in both tumor types. Of 211 genes identified as differentially expressed ($p<.02$), 63% were upregulated in papillomas expressing TGFβ1 but downregulated in SCC, while 37% were upregulated in SCC expressing TGFβ1 but downregulated in papillomas. In SCC, elevation of TGFβ1 rapidly caused an expression profile consistent with a more progressed phenotype: upregulation of genes involved in wnt signaling, a reactive stroma and invasion; downregulation of genes involved in adhesion, apoptosis, inflammation and interferon signaling. These data are the first to show that at the gene expression level, benign and malignant tumors respond differently to TGFβ1, and that the acquisition of a more aggressive phenotype is a rapid transcriptional response of malignant tumors to elevated TGFβ1.

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Deregulated p63 expression accelerates skin carcinogenesis

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Genes that are active during normal development are frequently found to be deregulated during neoplastic transformation. An example of a gene that is both required for normal development and involved in tumorigenesis is p63, a transcription factor that is required for the initiation of a stratification program. While mutations in p63 are rare in human cancers, deregulated expression of different p63 isoforms is frequently observed in human epithelial cancers. To better understand the role of p63 in carcinogenesis, we used inducible mouse models to target TAp63α expression to single-layered lung epithelia or the epidermis. We found that ectopic expression of TAp63α in single-layered lung epithelia resulted in hyperproliferation. In addition, squamous metaplastic lesions developed in the bronchioles, suggesting that TAp63 expression predisposes to neoplastic transformation. We further targeted TAp63α expression to the basal layer of the epidermis and hair follicles. Skin biopsies demonstrated that deregulated TAp63α expression resulted in hyperproliferation, hyperplasia, and a delayed onset of terminal differentiation. To determine if deregulated p63 expression predisposed mice to develop skin cancer, we subjected our inducible mouse model to a two-stage chemical carcinogenesis protocol. We found that deregulated TAp63α expression greatly increased susceptibility to skin carcinogenesis. In addition, deregulated TAp63α expression caused accelerated conversion to squamous cell carcinoma (SCC). Moreover, the tumors that developed in this model rapidly progressed to spindle cell carcinoma (SPCC) and metastasized to the lung. Interestingly, we found that tumor progression in this model does not appear to involve genetic instability. Taken together, these data demonstrate that deregulated TAp63α expression induces hyperproliferation and inhibits terminal differentiation, resulting in accelerated tumorigenesis associated with rapid progression and metastasis.

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Activation of p44/42 MAP kinase in keratinocytic neoplastic lesions

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Squamous cell carcinoma (SCC) arises from a series of genetic changes that result in a clone of keratinocytes with enhanced growth characteristics. The p44/42 Map kinase (Erk 1/2) pathway is a highly conserved growth regulatory pathway that helps relay critical signals from the cell membrane to the nucleus. Evidence demonstrating activation of the p44/42 pathway in the development of human cutaneous SCC has not been established. This study was conducted to determine if p44/42 MAP kinase is activated in lesions of keratinocytic neoplasia. Lesions from the defined stages of keratinocytic neoplasia, normal skin, actinic keratoses, proliferative actinic keratoses, squamous cell carcinoma in situ, and squamous cell carcinoma, were randomly selected from archived material and studied. Antibodies that detect human p44/42 (phosphorylated and unphosphorylated) and only phosphorylated, activated, human p44/42 were applied to lesional tissue. The intensity and prevalence of cytoplasmic and nuclear staining was evaluated in the lesional cells. The results suggest that there is a not a simple linear relationship between the amount of nuclear staining and the type of lesion. However, the results suggested that there was a significant increase in the level of nuclear phosphorylated p44/42 staining progressing from a proliferative actinic keratoses to a sqaumous cell carcinoma in situ. These findings suggest that p44/42 MAP kinase may be activated in the latter stages of keratinocytic neoplasia.

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Early occurrence of H-ras gene mutations by synergistic benzo(a)pyrene and UVA at subcarcinogenic levels in the skin of SKH-1 mice

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Melanoma and Non-melanoma squamous carcinoma are closely associated with UV and certain chemicals such as benzo(a)pyrene (BaP), one of polycyclic aromatic hydrocarbons. Both UV and BaP can cause gene mutations which are believed to be the part of initial events of carcinogenesis. The pattern of mutations of oncogene ras remains unclear in chemo-photocarcinogenesis model. In this study, we demonstrated that subcarcinogenic BaP and UVA caused the very early H-ras gene mutations in skin hairless SKH-1 mice. The epidermis were collected after mice were treated with UVA (40 kJ/m^2) and/or BaP (8 nmol/mouse, topical application) thrice a week for 2, 6, and 10 weeks. UVA or BaP alone did not induce any tumors over 25-week experiment but the combination of two agents synergistically increased tumor incidence and multiplicity. For comparison, DNA from tumors induced by UVB (30 mJ/cm^2 thrice a week for 25 weeks) or the combination of UVA and BaP were also investigated. Our results indicated that four types of point mutations, GAC, GCC, GTC and CGC, occurred in UVB-induced tumors at H-ras codon 13, compared with the wild type GGC and one type of point mutation GAA at codon 12, compared with wild type GGA. The treatment of BaP alone and the combination of UVA plus BaP for 10 weeks have caused one type of mutation, GGA to GAA at codon 12, and GGC to GAC at codon 13 in normal-appearance skin tissues, respectively, as well as in BaP-UVA induced tumors. However, there were no mutations detectable in the treatments with UVA alone during the 30-week period of experiment and in all the treatments with BaP or the combination of BaP plus UVA for 2 or 6 weeks. No mutations were detected at codon 61 in all 10-week period of treatments. In conclusion, H-ras mutation is a very early event 8 weeks before the emergence of tumors by subcarcinogenic BaP and UVA. G—>A transversion at ras gene codon 13 was not reported previously.

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Phorbol 12-Myristate 13-acetate (TPA) inhibits the induction of basal cell carcinomas in Ptch1+/- mice

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Ptch1+/- mice exposed to UV irradiation or ionizing radiation (IR) from Cesium 137 develop basal cell carcinomas (BCCs). Ptch1 wildtype mice subjected to a standard 2-stage chemical carcinogenesis protocol using the polycyclic hydrocarbon 7,12-dimethylbenz[a]anthracene (DMBA) and TPA develop papillomas and carcinomas of the squamous lineage, but not BCCs. We investigated the tumorigenic effects of topically applied DMBA and/or TPA on the skin of Ptch1+/- mice. We have found that a single application of DMBA (40?g) enhanced induction of BCCs, thus paralleling the effects of UV irradiation or IR. TPA (2.5?g 3X/week) applied to otherwise untreated skin of Ptch+/- mice had no effect on tumorigenesis, whereas application to Ptch1+/- mice previously initiated with DMBA induced benign papillomas as occurs in Ptch1 wildtype mice. Unexpectedly, TPA reduced the number of microscopic BCCs by ~50% in standard skin biopsies as compared to treatment with DMBA or IR alone. The effect of TPA as an inhibitor of the induction of BCCs appears to be directly opposite to its enhancement of SCC carcinogenesis. Elucidation of the mechanism of BCC inhibition by TPA may provide clues for development of novel chemopreventive and chemotherapeutic agents for this most common of human cancers.

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Deregulated hedgehog signaling in extracutaneous basaloid squamous cell carcinomas

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There is compelling evidence implicating uncontrolled activation of the hedgehog signaling pathway, operating through Gli transcription factors, in the pathogenesis of basal cell carcinoma (BCC). In contrast, little is known about the molecular basis of the biologically distinct basaloid tumors arising in non-cutaneous sites. We have generated mice carrying a conditional *Gli2* transgene, *TRE-Gli2*, which is activated in the presence of the tetracycline transactivator tTA. Bitransgenic *K5-tTA;TRE-Gli2* mice thus express *Gli2* in the proliferative compartment of several epithelia. In addition to BCCs in skin, a substantial proportion of these mice develop rapidly-growing tumors in the maxilla, mandible, or nasopharynx by 4-5 months of age. These tumors consist of monomorphous, basaloid cells with a high mitotic index; scattered foci of keratinization; and comedo necrosis: features that are strikingly similar to those seen in human basaloid squamous cell carcinomas (BSCCs), which also arise in the upper aerodigestive tract and elsewhere. In contrast to BCC, BSCC is a high-grade malignancy with frequent regional metastasis and a 40-50% mortality. Like human BSCC, the basaloid cells in odontogenic tumors from *K5-tTA;TRE-Gli2* mice express keratins K5 and K17, and cells in keratinizing foci express K1. *In situ* analysis revealed high-level expression of mRNA encoding *Gli2* and the hedgehog target genes *Gli1* and *Ptch1* in mouse BSCCs. Moreover, in two of six human BSCCs examined, *GLI1* and *PTCH1* mRNA levels were upregulated to a level comparable to that seen in BCCs. Early tumors in *Gli2*-expressing mice arose either from quiescent epithelial rests within the periodontal ligament or from gingival epithelium. Our data support the concept that in addition to typically indolent BCCs arising in skin, constitutive hedgehog pathway activity may play a role in the pathogenesis at least some highly-aggressive extracutaneous BSCCs, which carry a particularly poor prognosis.

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Identification of putative genomic loci involved in the pathogenesis of Sezary syndrome

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Sezary syndrome is the leukemic phase of cutaneous T cell lymphoma and represents an advanced stage of disease. However, little is known about specific genes involved in the pathogenesis of Sezary syndrome. Our goal is to identify genomic loci that commonly undergo gains or losses in malignant Sezary T cells and which harbor oncogenes or tumor suppressor genes, respectively, that may contribute to the pathogenesis of Sezary syndrome. Towards this end, we are using the approach of array-based comparative genomic hybridization (aCGH). Genomic DNA from patient blood samples and sex-matched, pooled reference DNA from normal blood samples are differentially-labeled with Cy3 and Cy5 fluorescent dyes and co-hybridized to a human genomic DNA bacterial artificial chromosome (BAC) library spotted in triplicate on a glass slide. Each BAC clone carries approximately 150 Kb of insert DNA and the genomic map position of each clone is known. The library, which was optimized specifically for aCGH, is composed of 4134 clones that span the human genome at a spacing interval of approximately 1 Mb. After hybridization, slides are scanned to detect fluorescence signal intensities for each BAC clone. The ratio of the fluorescence intensities is used to calculate DNA copy number, enabling analysis of genome wide copy number in tumor cell-derived DNA. To date, we have screened a panel of 14 high tumor burden blood samples from Sezary syndrome patients using aCGH. We have observed gains at 8q and 17q in 50% of the samples tested, as well as losses at 17p (50%), 10q (43%), and 11p (29%). These results indicate that some genomic loci are frequently subject to copy number changes in malignant Sezary T cells. We are screening additional samples to define the minimal critical regions of gain or loss at these loci and identify candidate genes that may contribute to the pathogenesis of Sezary syndrome.

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Coenzyme Q10 inhibits the proliferation of oncogenic cells while stabilizing growth in primary cells *in vitro*

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Coenzyme Q10 (Q10) is present in most human cells and is essential for the production of ATP required for cellular activities. Q10 is a lipophilic substance thereby making it difficult to dissolve in cell culture media. For this reason, *in vitro* experiments involving Q10 have often been unsuccessful. Our laboratory has discovered a novel protocol that permits solubilizing Q10 in cell culture media resulting in a homogeneous mixture. This allows studies to test the effects of various concentrations of Q10 on different cell lines. Human neonatal fibroblasts (nFIB) and keratinocytes (KC) were used as primary cell models while human malignant melanoma (SKMEL28) and squamous cell carcinoma (SCC) were used as oncogenic models. Each cell line was seeded into 6-well tissue culture plates containing media supplemented with various concentrations of Q10 (0-100µM) under physiologic conditions. After appropriate incubation, cells were trypsinized and counted using a Coulter cell counter. A significant ($p<0.01$) increase in cell number as compared to the control for nFIB and KC was observed, yielding a maximum increase of 20% at 50µM Q10 and 25% at 20µM Q10, respectively. In contrast, a significant ($p<0.01$) inhibition of proliferation was shown to correlate with Q10 concentrations in the malignant cell lines. Optimal inhibition was observed at 100µM Q10 for SKMEL28 (63%) and at 50µM Q10 for SCC (50%). The vehicle that allows for the solubilization of Q10 exhibited no influence on the proliferation of any cell type. Our results demonstrate that Q10 exerts an inhibitory effect on oncogenic cell lines while stabilizing growth of normal cells. This implies that Q10 may be used as an effective treatment for malignancies.

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Retinoid receptor expression in basal cell carcinoma of the skin

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Basal cell carcinoma (BCC) of the skin is the most common form of human cancer. Humans with basal cell nevus syndrome (BCNS) develop large numbers of BCCs, and the PATCHED 1 (PTCH1) gene is the site of the causative mutations. PTCH1 protein functions by inhibiting hedgehog signaling. BCNS patients are heterozygous for a functioning PTCH1 allele, and Ptc1 heterozygous mice develop cutaneous BCCs after exposure to ionizing or ultraviolet radiation. We have identified strong chemopreventive efficacy of the RARβ/γ-specific retinoid, tazarotene: topical tazarotene application reduced UV-induced BCC number and size by approximately 85%. Similarly, in a controlled study, we found that tazarotene was also highly effective against ionizing radiation (IR)-induced BCCs. To begin to investigate the mechanism of this responsiveness, we assessed murine BCCs and Ptc1+/- skin for retinoid receptor gene and protein expression. Ptc1 +/- mice were exposed to a single dose of 5 Gy IR and skin biopsies were taken 7 months after exposure, a time when all skin specimens contained microscopic BCCs. Visible BCC tumors that developed on the same animals were excised, and RNA and protein extracts from all samples were analyzed by real-time PCR and Western blotting, respectively. As reported by others, we find that in human and mouse skin, the predominant retinoid receptors expressed are RARα, RARγ and RXRα. Unlike the reported decrease in RAR gene expression in SCCs, we find that the majority of BCCs retained expression of RARα, RARγ and RXRα. Receptor mRNA levels did not differ between untreated skin of Ptc1+/- and Ptc1 wildtype mice and receptor mRNA levels in treated skin with microscopic tumors were intermediate between those of visible tumors with increased expression and those of untreated skin. Similarly, at the protein level, all SCCs examined showed loss or RARα and RXRα expression, while the majority of BCCs maintain expression of both receptors. Retention of receptors is consistent with the strong chemopreventive effects of tazarotene against BCCs and suggests that at least part of tazarotene′s anti-BCC effect may be via a receptor-mediated mechanism.

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Non-denatured soybean extracts enhance skin elasticity

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Elastic fibers are essential extracellular matrix components of the skin, which contribute to its resilience and elasticity. During chronological aging, synthesis of elastic fibers is reduced, and the existing elastic fibers are degraded by elastases. Non-denatured soybean extracts were found to protect the elastic network from degradation, and to restore elastic fiber synthesis. Using a luciferase reporter assay, non-denatured soybean extracts were shown to induce elastin promoter activity in a dose dependent manner. Non-denatured soybean extracts inhibited the activity of human leukocyte elastase *in vitro*, and protected elastic fibers of fibroblast cultures from degradation by exogenous elastase. Human facial skins were treated with non-denatured soy extracts for 8 and 12 weeks, respectively, in two independent clinical trials. Monthly cutometer measurements demonstrated statistically significant increase in skin firmness as early as 4 weeks, and sustained to the end of treatment in each study. These data suggest the possible use of non-denatured soybean extracts in skin care, to improve skin elasticity.

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Co-expression of p16INK4A and laminin 5 gamma 2 in keratinocytes: a normal wound healing response that goes awry during neoplastic progression to carcinoma

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We recently reported that p16INK4A (p16) and the gamma 2 chain of Laminin 5 (Lam5g2) are coordinately expressed in early invasive regions of neoplastic epithelium in vivo, at the edges of wounds made in confluent cultures of normal primary keratinocytes, and by senescent keratinocytes, associated with directional hypermotility (Natarajan et al. (2003) Am J Path 163: 477-491). These results led us to investigate whether p16/Lam5g2 coexpression occurs in wounds in vivo and to determine the cause and effect relationships between expression of these proteins and the hypermotile phenotype. We examined 16 skin ulcers immunohistochemically for p16 and Lam5g2. In 13 of the specimens, p16 and Lam5g2 were coordinately expressed at the normal epithelium/ulcer border and in reepithelialized regions lacking normal basal cell layer morphology. To investigate causal relationships between Lam5 and p16 expression and hypermotility, we plated keratinocytes at low density on surfaces coated with extracellular matrix proteins secreted by the tumor cell line 804G, shown by others to be comprised predominantly of Lam5. Normal keratinocytes plated on such a surface expressed p16, arrested growth, and exhibited enhanced directional motility. Two cell lines cultured from neoplastic epithelium—-one p16-deleted and the other expressing mutant p16, became directionally motile when plated on such Lam5 surfaces. In contrast, precoating culture dishes with serum, which contains fibronectin, produced no effect. We conclude that contact with endogenously produced or exogenous Lam5 induces p16 expression and hypermotility in normal keratinocytes, but that p16 is not essential for the hypermotile phenotype. These results suggest that p16/Lam5g2 coexpression is an important feature of normal epidermal wound healing, causing growth arrest of the motile cells that lead wound closure. Loss of p16 during neoplastic progression permits motile cells to continue proliferating, thereby resulting in invasive growth.

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Ganglioside GM3 exerts reciprocal effects on MMP-9 and MMP-2 expression in the presence of uPA

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Proteolytic degradation of the extracellular matrix plays a crucial role in cancer invasion and wound healing, in which expression of urokinase-type plasminogen activator (uPA), its receptor, and matrix metalloproteinases (MMPs) is known to be up-regulated. Two MMPs produced by squamous carcinoma cells, MMP-9 and MMP-2, are increased during early and late wound healing, respectively. Yet, the factors that differentially regulate the expression of these MMPs are unclear. We hypothesized that endogenous alterations in ganglioside expression may differentially affect the expression and activity of these MMPs. Expression of GM3, the predominant ganglioside of epithelial cell membranes, was modified genetically or biochemically in SCC12 squamous carcinoma cells, and the effect of GM3 expression on uPA-induced MMP expression and activity was studied. In the presence of uPA, endogenous overexpression of GM3 inhibited, and depletion of GM3 stimulated, MMP-9 expression and activity. In contrast, endogenous overexpression of GM3 augmented MMP-2 expression and activity, but depletion of GM3 had no effect. The increase in MMP-2 expression by GM3 was inhibited by incubation of cells with either 100 nM rapamycin (inhibits p70 S6 kinase activity) or 20 uM LY294002 (inhibits PI3K activity). The augmentation of MMP-2 expression by GM3 was also suppressed by transient transfection with mutated protein kinase B (PKB)/Akt or kinase-dead integrin-linked kinase (ILK), while transient transfection with wild type PKB/Akt or ILK promoted the stimulation by GM3. Neither PKB/Akt nor ILK affected the expression of MMP-9 triggered by depletion of GM3 in the presence of uPA. Instead, depletion of GM3 increased MMP-9 expression and activity via activation of EGFR/Ras/mitogen-activated protein kinase signaling. These studies implicate gangliosides as regulators of the temporal expression of MMP-9 and MMP-2 during wound healing.

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Desmogleins 1 and 3 in the companion layer are necessary to anchor the anagen hair to the follicle

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Desmogleins (Dsgs) 1 and 3 are critical for maintaining the structural integrity of epidermis through cell-cell adhesion. Recent studies showing that, in areas where both are normally expressed, one Dsg can compensate if the other is inactivated either by antibodies in pemphigus or exfoliative toxin (ET) in bullous impetigo, have elucidated the pathophysiology of these diseases. Much less is known about the role of Dsgs in hair follicles. Dsg3-/- (knockout) mice lose hair during telogen, but their anagen hairs remain anchored to the follicle. To determine if Dsg1 compensates for the loss of Dsg3 in the anagen hair of these Dsg3-/- mice, we used ET to inactivate their Dsg1. Four hours after injection of ET, Dsg3 -/- mice, but not Dsg3 / or Dsg3 /- mice, showed striking loss of anagen hair, which was confirmed and quantitated by gentle tape stripping. Histology of the skin of these mice as well as of the tape-stripped hair showed separation between the outer root sheath and inner root sheath of the hair follicle, at the plane of the companion layer. Immunostaining with AE13 for trichohyalin and K6, a companion layer marker, in skin and stripped hair confirmed the plane of separation. Furthermore, labeling of proliferating cells by BrdU demonstrated that the matrix keratinocytes responsible for producing the hair shaft were below the split and remained in the follicle after anagen hair loss. Consistent with the companion layer being the major target for loss of anchorage of anagen hair in these mice, immunofluorescence of normal mouse follicles showed Dsg1 and Dsg3 overlapping only in the companion layer. These findings demonstrate the importance of the companion layer, and particularly the Dsg 1 and 3 in this layer, in anchoring the anagen hair to the follicle. In addition, these results may have implications for the human disease loose anagen hair syndrome, in which anagen hair may also separate at the companion layer.

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MMP-26 (matrilysin-2/endometase) co-localizes with laminin-5 in migrating epithelial cells and is up-regulated by cell-matrix interactions in culture

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MMP-26 was recently cloned from placenta/endometrial tumor libraries. It degrades in vitro fibronectin, type IV collagen, vitronectin and gelatins. In vivo MMP-26 has been reported in human cytotrophoblasts, normal endometrial glands and prostate cancer and it is widely expressed in cancer cell lines of epithelial origin in culture. It is autocatalytically activated and activates MMP-9. Our objective was to investigate the expression and role of MMP-26 in skin biology. We assessed by immunoanalysis expression of MMP-26 in benign hyperproliferative disorders of the epidermis, during wound repair, and in basal (BCC) and squamous cell cancers (SCC). Both primary keratinocytes and HaCaT cells were cultured in the presence of various cytokines and mRNA levels were measured using quantitative Taqman RT-PCR. In normally healing wounds, MMP-26 was detected in migrating keratinocytes colocalizing with cytoplasmic staining for laminin-5. Mere keratinocyte hyperproliferation, as seen in psoriasis, did not induce MMP-26. Analogously, in various tissue samples its expression did not correlate with Ki67 staining. In actinic keratoses, MMP-26 was expressed by keratinocytes with cellular atypia in E-cadherin negative areas. BCC islands were negative, but intensive staining was found in the invasive front of grade I SCCs, where MMP-26 also colocalized with laminin-5. More dedifferentiated SCCs were negative for MMP-26. Unstimulated keratinocytes and HaCat cells do not express MMP-26 mRNA. It is upregulated in keratinocyte cultures via cell-matrix interactions. We conclude that MMP-26 may function in wound repair and influence the initial phase of skin carcinogenesis.

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FLICE/caspase-8 activation triggers anoikis induced by β_1-integrin blockade in human keratinocytes

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Anoikis is a mode of cell death that occurs after detachment from the extracellular matrix. Keratinocyte stem cells (KSC) leave their "niche" after disruption of β_1-integrin signal. We have previously shown that KSC, but not transit amplifying (TA) cells are protected from anoikis via an integrin signaling pathway. The aim of the present study was to further evaluate the mechanisms involved in keratinocyte anoikis. KSC were selected through adherence to type IV collagen and cultured in serum-free medium. We first demonstrated that, upon suspension, poly(ADP-ribose)polymerase (PARP) cleavage was present in TA cells but not in KSC. In suspended cells, caspases are activated earlier in TA than in KSC. All caspases were activated to a greater extent in keratinocytes treated with anti-β_1-integrin antibody, as compared to suspended cells. We evaluated the hierarchical activation of caspases after treatment with anti-β_1-integrin. Caspase-8 activation and cytochrome c release from mitochondria were observed at 3 hrs, while caspase-9 was activated at 6 hrs. Caspase-9 inhibitor zLEDH-fmk failed to block caspase-8, -10, -3 and Bid activation. On the contrary, caspase-8 inhibitor zIETD-fmk delayed the activation of caspase-9 and -3, and blocked the activation of Bid. Anti-β_1-integrin failed to cleave BID, to release cytochrome c and to activate caspase-9 in c-FLIP (FLICE/caspase-8 inhibitory protein) overexpressing keratinocytes. Finally, anti-β_1-integrin induced anoikis in c-FLIP keratinocytes to a lesser degree, as compared to mock cells ($p<0.01$). These results demonstrate that anoikis induced in keratinocytes by b1integrin disruption is triggered by caspase-8, which in turn activates the executioner caspase-3 (extrinsic pathway). Anoikis seems also to be amplified through the caspase-8-induced mitochondrial release of cytochrome c and the activation of caspase-9 (intrinsic pathway).

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Withdrawn

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Pemphigus autoantibody binding to keratinocyte cell surfaces disrupts desmosomes and causes Dsg3 endocytosis through an endo-lysosomal pathway

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Pemphigus vulgaris (PV) is an autoimmune disease characterized by the loss of epidermal cell-cell adhesion and by the presence of autoantibodies directed against the desmosomal cadherin Dsg3. To investigate the mechanisms by which PV autoantibodies compromise keratinocyte cell-cell adhesion, primary cultures of human keratinocytes were exposed to normal human sera or to PV sera. In addition, monoclonal antibodies directed against various epitopes of the Dsg3 extracellular domain were used to verify that keratinocyte responses were caused by antibody ligation of Dsg3. Using an in vitro assay to measure adhesive strength, both PV sera and a pathogenic monoclonal antibody specific to Dsg3 were found to severely compromise keratinocyte cell-cell adhesion. In contrast, normal human sera and a control Dsg3 monoclonal antibody had no effect on keratinocyte adhesion. Immunofluorescence analysis demonstrated that PV sera caused internalization of cell sufrace Dsg3 and trafficking of Dsg3 to early endosomes. Similarly, biotinylation experiments demonstrated that PV IgG caused a dramatic depletion of cell surface Dsg3. By 6 hrs after exposure to PV sera, Dsg3 co-localized with markers for late endosomes and lysosomes. Importantly, only PV sera and the pathogenic monoclonal Dsg3 antibody caused Dsg3 endocytosis, wheras a control Dsg3 monoclonal antibody failed to cause Dsg3 internalization. Both plakoglobin and Dsc2 were co-internalized with Dsg3, whereas desmoplakin did not colocalize with the internalized cadherin complex. However, Dsg3 internalization was associated with the disassembly of desmosomes and with the retraction of keratin filaments from the cell surface. Furthermore, sequential detergent extraction experiments revealed a loss of both membrane and cytoskeletal associated Dsg3 by 24h. These findings raise the possibility that PV autoantibodies cause changes in Dsg3 membrane trafficking and that these alterations are mechanistically tied to PV pathogenicity.

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Overexpression of MsrA protects WI38 SV40-T fibroblasts against oxidative stress

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Proteins are modified by reactive oxygen species and oxidation of specific aminoacid residues can impair their biological functions leading to an alteration of cellular homeostasis. Oxidized proteins can be rapidly eliminated through either degradation or repair. Degradation of oxidized proteins is achieved by the proteasome, a multicatalytic proteolytic system, while repair is limited to few modifications such as methionine oxidation that can be reversed by the Msr system. This system is composed of MsrA and MsrB, which reduce the methionine-S-sulfoxide and the methionine-R-sulfoxide, respectively. However, accumulation of oxidized proteins occurs during aging, replicative senescence, neurological disorders or after an oxidative stress while MsrA activity is impaired. MsrA was shown to be involved in antioxidant defense in several organisms such as bacteria, yeast, drosophila as well as mammals. To analyse more precisely the relationship between oxidative stress and MsrA, we stably overexpressed the MsrA full-length cDNA in SV40 T antigen-immortalized human fibroblasts WI38. We showed that MsrA overexpressing cells are more resistant than control cells to hydrogen peroxyde-induced oxidative stress at concentrations from 250 up to 500 µM. Moreover, in the transfected cells, Msr activity is stimulated by addition of H2O2 while an H2O2-dose dependent inhibition of the Msr activity is observed in control cells For higher concentration of hydrogen peroxide (750 µM), the overexpression of MsrA is no more able to protect cells from oxidative damage and apoptosis. These results suggest that MsrA may play an important role in cellular defenses against oxidative stress and in protection against apoptosis by limiting, at least in part, the accumulation of oxidatively modified proteins.

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Differential expression of cutaneous lymphocyte antigen in lymphomatoid papulosis and lymphomas is a potential marker for diagnosis and tumor progression

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LyP is a self-healing CD30+ cutaneous lymphoproliferative disorder (CLPD) associated with lymphoma in 10-20% cases. Anaplastic large cell lymphoma (ALCL) is a tumor of CD30+ lymphocytes. In normal T lymphocytes, the CLA carbohydrate epitope, expressed primarily on the PSGL-1 protein backbone, is believed to be a skin-homing receptor. We compared CLA expression in LyP, ALCL and Hodgkin lymphoma (HL).CLA, defined as immunoreactivity with mAb HECA 452, was examined in biopsies of 15 LyP cases, 7 primary cutaneous ALCL, 2 ALCL secondary to LyP and one HL of an LyP patient, by immunohistochemistry. CLA expression was studied in CD30+, anaplastic lymphoma kinase (ALK)+ systemic, and ALK- cutaneous, ALCL lines by FACS and Western blot. In 17 LyP skin lesions, CLA was expressed on 82+/-6% of CD30+ cells. In contrast, CLA was displayed on 13+/-7% of tumor cells in 13 ALCL lesions, (p<.002, Mann-Whitney U test. CLA was absent on HL tumor cells. CLA was low on ALCL lines. ALK- Mac-1 and 2B, but not 2A cutaneous lymphoma cells, expressed PSGL-1. On blots, PSGL-1 migrated at the expected MW (240 kDa dimer/140 kDa monomer), but all observed CLA immunoreactivity migrated at >300 kDa. CLA expression is high on CD30+ cells in LyP but is usually low or absent in cutaneous and systemic ALCL and HL which may provide a differential diagnostic marker for LyP and lymphoma. CLA on ALCL lines is expressed as a >300 kd glycoprotein not yet identified but of higher MW than PSGL-1 and CD44. Decreased CLA expression can precede progression of LyP to ALCL and should be investigated as a new prognostic marker.

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Fibronectin expression is post-trancriptionally up-regulated by epidermal growth factor in human dermal fibroblasts via protein kinase C-delta signaling pathway

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In the present study, we demonstrated that epidermal growth factor (EGF) up-regulates the expression of fibronectin mRNA and protein in human dermal fibroblasts. Actinomycin D, an RNA synthesis inhibitor, significantly blocked basal mRNA expression, but the addition of EGF compensated the blockage. In addition, the treatment with EGF significantly reduced the degradation rate of fibornectin mRNA. However, EGF did not increase fibronectin promoter activity. EGF-mediated induction of fibronectin expression was inhibited by the treatment of fibroblasts with non-selective protein kinase C (PKC) inhibitor, Calphostin C, and selective PKC delta inhibitor, Rottlerin. The transfection of a dominant negative mutant of PKC delta into fibroblasts significantly reduced the induction of fibronectin protein expression by EGF. Rottlerin blocked the EGF-mediated reduction of mRNA degradation rate. These results indicate that EGF-mediated induction of fibronectin expression occurs at the post-transcriptional level and involves PKC delta signaling pathway.

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Expression of cutaneous lymphocyte-associated antigen on human bone-metastatic prostate tumor cells

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Human prostate tumors characteristically metastasize to bone marrow (BM), where prostate tumor cells exhibit an enhanced adhesiveness to BM endothelial cells compared with adhesion to other microvascular endothelial cells. Since E-selectin is constitutively expressed on BMEC, we hypothesized that E-selectin ligand(s) on metastasizing prostate tumor cells help initiate their adhesion to BMEC under blood flow conditions. To identify human prostate tumor cell lines expressing E-selectin ligand activity, we performed parallel-plate flow analysis and observed robust rolling of human bone-metastatic prostate MDA PCa 2b cells on E-selectin-Ig and on native E-selectin expressed by BMEC cells. Furthermore, to identify prospective E-selectin glycoprotein ligands, we performed flow cytometric analysis of known human hematopoietic E-selectin ligands CD44 and PSGL-1 and found that MDA PCA 2b cells express PSGL-1 (75% positive) and low levels of CD44 (1% positive). By Western blot analysis, we found that anti-PSGL-1 immunoprecipitate from MDA PCa 2b membrane protein was stainable with moAb HECA-452, which defines cutaneous lymphocyte-associated antigen (CLA) expression, E-selectin-binding function and capacity of T-cells to enter skin. We also identified PSGL-1 expression on five other bone-metastatic prostate tumor cell lines; however, they did not express HECA-452-reactive PSGL-1 and coincidentally did not show remarkable E-selectin ligand activity. These data show for the first time that human bone-metastatic prostate tumor cells express E-selectin ligand activity and, interestingly, express CLA. Studies examining chemokine receptor expression are underway to further elucidate BM-homing specificity. These results establish a new prospective into the migratory mechanism of human prostate tumor metastasis to BM.

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Development of human skin equivalent by using a culture of keratinocytes and fibroblasts without exogenous materials

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During the past decade, several skin equivalents have been widely used in various fields of skin biology. However, they contain exogenous materials for dermal substrates, which may be difficult to obtain and a source of infections. The purpose of this study was to reconstruct a human skin equivalent (HSE) consisting of both epidermis and dermis by using only a culture of keratinocytes and fibroblasts without exogenous materials. After culturing dermal fibroblasts in the medium containing several supplements for only 3 weeks fibrous matrix was formed. Then, keratinocytes were cultured on the top of the fibrous matrix in air-liquid interface for 2 weeks. Histology, immunohistochemistry and electron microscopy for the fibrous matrix and HSE were performed. In the fibrous matrix, many fibroblasts were found between extracellular matrix. Collagen fibers were present by Masson-trichrome staining and electron microscopy. In HSE multilayered epidermis with horny layer was formed similarly to normal epidermis in vivo. Differentiated markers (keratin 1 and 10, involucrin) were expressed in the epidermis and the components of basement membrane (beta 4 integrin, laminin 5, type 4 and 7 collagens) were all expressed at the dermo-epidermal junction. Ultrastructurally, BMZ such as hemidesmosomes, lamina lucida and lamina densa were formed, although it was still incomplete. These findings show that a fibrous matrix can be made by using a culture of dermal fibroblasts for a period of 3 weeks. In addition, HSE can be constructed by culturing keratinocytes on this fibrous matrix as a dermal substrate without exogenous materials, suggesting that this model may be useful for the study of skin biology.

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Differences between papillary and reticular fibroblasts in reconstructed skin

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The dermis of human skin - whose major resident cell is the fibroblast - is made of two compartments : the papillary dermis (thin and close to the epidermis) and the reticular dermis (deeper and thicker). It is possible to isolate specific fibroblast population (papillary versus reticular) from each of these compartments. Only a few reports describe differences between papillary and reticular fibroblasts in terms of proliferation, gel contraction and macromolecule synthesis. After isolation of both fibroblast populations from the same site, we have reconstructed an in vitro skin system containing in the dermal part either papillary or reticular fibroblast populations in order to investigate and compare their biological properties. Several unexpected differences were observed, in particular epidermal or dermal markers were differently expressed depending on the fibroblast population. I) In the epidermis we observed a delay in terminal differentiation when the dermal compartment contained only reticular fibroblasts (granular layer, filaggrin and loricrin immunostainings decrease, while keratin 10 was delayed in the upper layer) as compared to the epidermis obtained when the dermal part contained only papillary fibroblasts. The expression of syndecan 4 in the basal layer was also more pronounced in the presence of papillary fibroblasts as compared to reticular fibroblasts. II) In the dermis reticular fibroblasts seemed to express more macromolecules of basement membrane zone and extracellular matrix than papillary fibroblasts (a more intense staining of collagen IV, procollagen I and procollagen III was observed in the matrix containing reticular fibroblasts as compared to the matrix containing papillary fibroblasts). These studies strongly suggest a different influence and specialized role of these populations in the organization and differentiation of both dermal and epidermal tissues in skin.

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Proliferation and onset of differentiation in cultured epidermal keratinocytes : pre-programmed pathways independent of calcium?

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Cultured mouse and human keratinocytes are believed to require low extracellular Ca^{2+} to proliferate, and conversely to depend on high extracellular Ca^{2+} to commit to differentiation. We initially observed that cultured canine keratinocytes proliferate equally well in low (0.09 mM) or high (1.8 mM) Ca^{2+}-containing medium. This prompted us to complete in depth analyses on the effects of extracellular Ca^{2+} on proliferation and onset of differentiation in cultured canine as well as mouse keratinocytes. A proliferation assay confirmed that canine keratinocytes proliferate without significant difference under both high and low Ca^{2+} conditions. Most interestingly, results obtained with the cultured canine cells were reproduced with murine keratinocytes. Moreover, at confluency, both canine and murine keratinocytes exited the cell cycle and initiated differentiation independent of the Ca^{2+} concentration, as exemplified by upregulation of mRNA encoding cell cycle regulators like $p21^{WAF}$ and differentiation markers like involucrin and desmosomal cadherins. Steady-state levels of the corresponding proteins also increased in both high and low Ca^{2+}, with the only difference that protein levels remained lower in canine and murine cells held in low extracellular Ca^{2+}. In conclusion, our results demonstrate that extracellular Ca^{2+} is not crucial for proliferation and the onset of differentiation of cultured murine and canine keratinocytes. In contrast it is the cell-cell contact that appears to be in control of the switch from proliferation to onset of the epidermal differentiation program.

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Age-related differences in reconstructed skin containing fibroblasts provided by donors of various ages

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Skin aging can be explained (at least in part) by the glycation reaction leading to AGEs products (Advanced Glycosylation Ends Products) which modify the dermal architecture. To study the effect of chronologic aging in skin, we have isolated different fibroblast populations (papillary and reticular) from donors of various ages. These fibroblasts were incorporated separately in dermal equivalents made either with native collagen or collagen modified by glycation. Several modifications were observed in this system which were age dependent. I) In the epidermis, the glycation reaction had an effect on the thickness if the dermal compartment was populated with fibroblasts extracted from a young donor as compared to the unglycated control. The thickness of the epidermis increased in the presence of papillary fibroblasts and decreased in the presence of reticular fibroblasts. No modifications were observed in the thickness with fibroblasts extracted from donors of advanced age. II) While terminal differentiation was delayed in the presence of reticular fibroblasts in the dermis especially with a young donor, we observed normal filaggrin immunostaining with old donor only when collagen modified by glycation was used. In the dermal compartment, several markers were differently expressed and were fibroblast type, age and glycation dependent. For example, chondroitin dermatan sulfate chains were more abundant in the native collagen of the dermal compartment populated by reticular fibroblasts as compared to papillary fibroblasts. The detection was more obvious when the dermal compartment was made of collagen modified by glycation, especially when reticular fibroblasts extracted from young donors were used. These results demonstrate, that AGEs in the skin in vitro may modulate responses in a cell type and age dependent manner. These results also suggest that modifications provoked by aging in skin in vivo could be explained, in part, by changes in fibroblasts of the two populations (papillary versus reticular) like their relative proportions or their reactivity with the environnement.

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Collagen-like peptide exhibits a very interesting stimulating effect on the extra cellular matrix of human skin: histological studies

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Recent studies of a newly developed synthetic oligo collagen-like peptide have shown that it enhances cultured cell adhesion and differentiation, and improves ex vivo skin morphology. Consequently, we were interested in furthering the investigation of the effects of collagen-like peptide using histological studies on human ex vivo skin, and by comparing the peptide with vitamin C, which is known for its ability to enhance the synthesis of ECM molecules such as collagen. In time course studies that included 16h to 72h of application, we performed various immunostaining studies on human ex vivo skin samples treated with collagen-like peptide at 1%. Comparative experiments with vitamin C at 20 ?g/ml were conducted in parallel. The immunostaining studies included evaluations of collagen I, III, IV, laminin 5, beta 1 integrin, filaggrin and pan keratin. The results showed that application of collagen-like peptide to the skin enhanced the synthesis of these ECM molecules, and this effect was seen as early as 16h after application for some molecules such as laminin 5, collagen III, IV and pan keratin. The expression of the other molecules increased after different periods of time of application of the peptide. Interestingly, comparative studies with vitamin C showed that the synthesis response of some ECM molecules, such as laminin 5, collagen III, IV and pan keratin, was greater and more rapid when prompted by collagen-like peptide than by vitamin C. The studies also demonstrated that after a longer period of time, both active ingredients stimulated ECM molecule synthesis to a similar degree, with the exception of some molecules, such as collagen IV and beta 1, which maintained a superior synthesis after a longer period of time in response to the peptide. These histological studies demonstrate the remarkable and rapid stimulating effect of the collagen-like peptide on ECM molecule synthesis, revealing a property that can be of great use in antiaging and photoaging skin care products.

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Laminins 8 and 10 in skin basement membrane reconstitution and wound healing

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Laminins are the major non-collagenous extracellular matrix components in basement membrane zones (BMZ). Laminins influence a variety of important biological processes including cell attachment, migration and tissue development. Two newly discovered laminins, laminin 8 (alpha4, beta1 and gamma1) and laminin 10 (alpha5, beta1 and gamma1), are found the major laminins produced by human dermal microvascular endothelial cells. Laminin 10 is also found in human skin keratinocytes and is present at the dermal-epidermal junction. We examined the role of laminin 8 and laminin 10 in cutaneous wound repair. In vitro analysis showed that over-expression of laminin 8 in dermal microvascular endothelial cells strongly promoted cell attachment, migration and capillary tubule formation via various integrin receptors. Purified laminin 10 protein promoted keratinocyte spreading and attachment, also mediated by integrins. Using a laminin 10 alpha5 chain knockout mouse, excisional wounds in this model had decreased granulation tissue formation as well as abnormal reepithelialization. In human wound lesions, immuno-peroxidase staining, with specific antibody 4C7, stained strongly positive for laminin 10 in newly formed microvascular blood vessels. In a porcine burn wound model, Reverse Transcription and Polymerase Chain Reaction analysis found significant increased expression of laminin 8 shortly after wounding and reached peak at the day 4. The higher expression of laminin 10 was also seen soon after wounding and persisted through day 21, peaking at day 10. These expression patterns are consistent with the reconstitution of BMZs in the epidermis and microvascular blood vessels within the dermis. These studies indicate laminins 8 and 10 play an important role in wound healing by promoting angiogenesis and reepithelialization.

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Plakoglobin strengthens adhesion and suppresses keratinocyte motility via both adhesion-dependent and -independent mechanisms

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Plakoglobin (PG) is a member of the Armadillo family of adhesion/signaling proteins that is critical in the organization of desmosomes. We analyzed its role in regulating intercellular adhesive strength and cell motility using keratinocytes derived from PG-null mice. PG-/- cells exhibited weakened adhesion compared to PG+/- cells in a mechanical dissociation assay, which correlated with a reduction in desmoglein1/2 but not adherens junction proteins at cell-cell contacts. We then tested whether weakened adhesion in PG-/- cells leads to increased motility. PG-/- cells migrated up to 9-times faster than the PG+/- cells at 0.2mM calcium and ~4-times at 1.2mM calcium in Transwell migration assays. Re-expressing PG via adenoviral infection restored adhesive strength and desmosome status, and reduced motility in PG-/- cells. Surprisingly, the effect of PG on keratinocyte motility was not due solely to its effect on cell-cell adhesion, as single PG-/- cells migrated ~6-times faster than PG+/- cells in colloidal gold migration assays, regardless of calcium concentration. Alterations in FAK distribution, elevated Src activity and decreased matrix adhesion were observed in PG-/- cells, possibly contributing to the increased motility, which was inhibited by the Src kinase inhibitor PP2. Furthermore, N-terminally truncated and full-length PG, but not C-terminally truncated PG, reversed motility of PG-/- cells to the level of PG+/- cells. Thus, PG regulates cell-cell adhesion and motility in mouse keratinocytes. Its suppression of motility involves both adhesion-dependent and -independent mechanisms, the latter requiring the PG C-terminus previously shown to harbor transcriptional transactivation activity.

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(-)-muscone up-regulates hyaluronan synthesis at hyaluronan synthase 2 mRNA level in cultured human fibroblasts

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Musk, a dried secretion from the preputial follicles of male musk deer, has been reported to have the general pharmacological properties. In this study, we discovered that (-)-muscone, a major odoriferous constituent of natural musk, specifically up-regulated hyaluronan (HA) synthesis at a μM order of concentration in cultured human skin fibroblasts, but not in keratinocytes. Northern blot analysis showed that the expression of HA synthase 2 (*HAS2*) mRNA was induced in fibroblasts immediately after the stimulation with (-)-muscone. So far, we reported that *N*-methyl-L-serine up-regulated HA synthesis by increasing membrane-associated HA synthase activity of cultured fibroblasts[1)] without *HAS2* mRNA up-regulation. We found that the co-presence of (-)-muscone with *N*-methyl-L-serine exerted a synergistic effect of HA production in fibroblasts. To date, we reported that TGF-β1 up-regulated HA synthesis in human skin fibroblasts by inducing the expression of *HAS* mRNAs, generally *HAS2* mRNA[2)]. For that reason, we expect that the regulation of *HAS2* expression by (-)-muscone might be concerned with a TGF-β signaling pathway. In order to confirm this possibility, we investigated that the effect of (-)-muscone on collagen synthesis in fibroblasts which has been known to be up-regulated by TGF-β1. But the stimulation of fibroblasts with (-)-muscone did not increase the production of collagen, suggesting that (-)-muscone might control the HA synthesis by the different mechanism from that of TGF-β. Study on the control of *HAS2* expression by (-)-muscone may provide a new regulation system of HA synthesis in human skin fibroblasts.

1) Sakai S., *et al.*, (1999) *Skin Pharmacol. Appl. Skin Physiol.* **12**, 276-283
2) Sugiyama Y., *et al.*, (1998) *J. Invest. Dermatol.* **110**, 116-121

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Stretch induced transactivation of EGF-receptors by integrins

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Testing the hypothesis of a functional and spatial association of integrins and epidermal growth factor (EGF)-receptors in cell adhesion, we studied the distribution and co-localization between beta-1-integrins and EGF-receptors under cell stretch using immunocytochemistry and confocal laser scanning microscopy (CLSM). Cell adhesion was tested in an usual adhesion assay and the phosphorylation of both, MAP kinase ERK 1/2 and EGF receptor was measured using western blotting. We found that in most of the stretched cells beta-1-integrins were assembled in clusters at the basal cell membrane. Such clusters were seen rarely in controls. In stretched cultures of human keratinocytes the frequency of beta-1-integrin-clusters increased about 3 fold compared to controls. Immunocytochemically, we found a co-localization of beta-1-integrins and EGF receptors after stretching, which occurred mostly in cluster areas. Concomitantly, the application of cell stretch increased cell adhesion and induced the phosphorylation of both, EGF receptors and ERK 1/2. Blocking antibodies against beta-1-integrins as well as an inhibitor of EGF receptor tyrosine kinase prevented the stretch induced phosphorylation of ERK 1/2. Our results show an enhancement of the adhesion properties of stretched keratinocytes resulting in an increase of focal contacts. Co-localization of clustered beta-1-integrins with EGF receptors indicates possible receptor interactions. From functional experiments it could be assumed that EGF receptor activation is mediated by integrins. Finally, these findings suggest the regulation of integrin adhesiveness via an “inside-out signaling”, namely the shift from a low affinity to a high affinity state of the fibronectin-receptor.

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Study of basement membrane regeneration and epithelial cells migration during wound healing process

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Because the basement membrane is an important structure of human skin, our study investigated its reconstruction in addition to epithelial cell migration. Epithelial cell migration was first studied by seeding keratinocytes in six well plates coated with collagen or polylysine. After cell confluence is reached, wounds are made with a pipet tip and healing is observed under three different conditions: with human serum, with human plasma or calf serum as control. Cell migration was visualized on movie from Time Lapse results. We have developed a new *in vitro* model of human skin reconstructed by tissue engineering and its use simplifies the study of the wound healing process as well as reconstruction of the basement membrane. The human reconstructed skin was first built by seeding keratinocytes on two superposed fibroblast sheets. The keratinocytes differentiated and formed an epidermis histologicaly similar to normal human skin. We next used a punch biopsy to create wounds in our reconstructed tissues. Each wounded reconstructed skin was then deposited on an intact fibroblast sheet in order to allow healing and re-epithelialisation by keratinocytes. The basement membrane formation was analyzed and characterized by immunohistochemistry. The Time Lapse results permitted to characterize and compare the wound healing process for the three culture conditions tested. The healing process was found to be slower in human plasma in comparison to that observed with human serum that contain more growth factors. Expression of various constituents (laminin, collagen, nidogen and perlecan) of the basement membrane indicated a reconstitution of a functional basement membrane that occurs in an orderly step-by-step sequence. For example, the first constituent to be deposited was laminine 5, followed by integrin α6β4, collagen IV, perlecan, collagen VII and, nidogen. The results of the present study should help better understand the mechanisms involved in wound healing.

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Identification of a mutation in β4 integrin in tumor keratinocytes *in vivo* and its impact on α6β4 integrin-ligand binding and hemidesmosome assembly

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In normal keratinocytes, the α6β4 integrin regulates cell adhesion to laminin-5 in the basement membrane zone (BMZ) and nucleates formation of hemidesmosomes. A motif termed the specificity determining loop (SDL) composed of 25 amino acids in the extracellular domain of the β4 integrin subunit regulates α6β4 integrin interaction with laminin-5. Moreover, engineered β4 integrin subunits containing point mutations within the SDL act as dominant negative regulators of hemidesmosome assembly in cultured cells. Here we tested an hypothesis that tumor cells express β4 integrin containing one or more mutations within the SDL. Human oral tumor specimens were subjected to laser capture microdissection to obtain homogeneous keratinocyte tumor cell populations from which RNA was isolated. Single stranded cDNA, generated from this RNA, was amplified by PCR using primers encoding a portion of the extracellular domain of the β4 integrin containing the SDL. Sequence analyses reveal that three out of four tumor cell specimens contained a Q to K mutation at residue 155 in the SDL of the β4 integrin subunit. We propose that expression of the mutated β4 integrin leads to a loss of hemidesmosome-mediated adhesion in tumor cells. This facilitates escape of carcinoma cells from the constraints of the BMZ. Furthermore, unligated α6β4 integrin containing a mutated β4 integrin subunit likely triggers signaling cascades that enhance tumor cell survival.

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Hyaluronan synthase 2 compensates for deficiency of hyaluronan synthases 1 and 3 in skin

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Hyaluronan (HA) is a glycosaminoglycan expressed in both epidermis and dermis. HA is synthesized by HA synthases (HAS) 1, 2 and 3 which differ from one another in terms of catalytic activities and molecular sizes of products. HA has a number of roles in skin including maintenance of architecture, wound healing and inflammation. Despite the importance of HA, little is known regarding the relative contributions of the individual HAS enzymes. Using HAS1/3 double knockout (DKO) mice, we have studied the functions of HAS2. Unlike HAS2 KO mice, showing embryonic lethality, HAS1/3 DKO mice exhibited no apparent abnormality. Skin morphology of HAS1/3 DKO mice was indistinguishable from that of wild-type (WT) animals as assessed by histology. Organ cultures of HAS1/3 DKO skin and WT skin showed similar capabilities to synthesize HA as measured by ^{3}H-glucosamine uptake. By contrast, HA content was reduced in the epidermis of HAS1/3 DKO animals compared with WT counterparts based on the staining of cryostat sections with a HA-binding peptide (termed Pep-1). Since keratinocytes (KC) represent the major HA expressing cell type in the epidermis, we established KC lines from HAS1/3 DKO animals and WT mice to assess their ability to synthesize HA. We observed that: a) KC from HAS1/3 DKO mice expressed mRNA only for HAS2 whereas KC from WT animals expressed HAS1 and HAS3 (but not HAS2) mRNA, b) HAS1/3 DKO KC were morphologically indistinguishable from WT KC, c) growth rates of HAS1/3 DKO KC were comparable to WT KC, d) HA synthesis by HAS1/3 DKO KC and WT KC were virtually identical, and e) surface expression of HA was comparable between HAS1/3 DKO KC and WT KC. Our findings that HAS1/3 DKO mice had less HA in the epidermis compared with WT animals may suggest that HAS1 and/or HAS3 products are more efficiently maintained in the epidermis than HA synthesized by HAS2. On the other hand, failure to detect differences in skin architecture, HA synthesis and KC phenotype suggests that HAS2 can compensate for HAS1 and 3 deficiencies under basal conditions.

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Elevated cystein-rich 61 (CYR61/CCN1) mediates reduced collagen production in chronologically-aged human skin fibroblasts *in vitro* and *in vivo*

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Chronological aging results in decreased synthesis of type I procollagen and increased matrix metalloproteinase (MMP)-mediated degradation of fibrillar collagen in human skin. Aged human skin is thin and fragile as a consequence of this age-related reduction of collagen. Molecular mechanisms responsible for the observed aberrant collagen homeostasis are not fully understood. Cysteinrich protein 61 (CYR61/CCN1), a novel mediator of collagen homeostasis, is substantially increased in chronologically aged, compared to young, human skin *in vivo*. We report that CYR61 mRNA is predominantly expressed in the dermis of normal adult human skin (11-fold vs epidermis), measured by laser capture microdissection coupled quantitative real-time RT/PCR ($p<0.05$). Immunohistology revealed that CYR61 protein was expressed primarily in dermal fibroblasts and vascular cells. In human skin fibroblasts, overexpression of CYR61 caused reduced type I procollagen expression (60%, $p<0.05$) and increased MMP-1 (6-fold) expression. To explore the relationship between age-related increases of CYR61 and aberrant collagen homeostasis, we employed replicative senescent dermal fibroblasts as an *in vitro* aging model. Type I procollagen mRNA and protein were reduced 83% and 81%, respectively, in senescent cells, compared to subject-matched pre-senescent cells ($p<0.05$). In contrast, MMP-1 mRNA and protein were increased 3.7-fold and 3.5-fold, respectively, in senescent cells, compared to subject-matched pre-senescent cells ($p<0.05$). Importantly, CYR61 mRNA and protein were increased in senescent cells 2.9-fold and 3.1-fold, respectively, compared to subject-matched pre-senescent cells ($p<0.05$). Thus, *in vitro* cellular aging, like aging *in vivo*, is associated with elevated CYR61 expression. Given the ability of CYR61 to regulate both synthesis and degradation of collagen, it is likely that elevated-expression of CYR61 is a critical mediator of collagen deficiency observed in chronologically aged human skin.

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Keratinocytes deficient in β4 integrin assemble normal arrays of laminin-5, display hypermotility but fail to show directed migration

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The α6β4 integrin plays a crucial role in epidermal cell adhesion by binding to laminin-5 in the basement membrane zone (BMZ) and by inducing assembly of hemidesmosomes. It has also been proposed that α6β6 integrin mediates formation of the BMZ as well as epidermal cell migration since b4 integrin-null keratinocytes show deficiencies in motility in vivo and in vitro. To investigate these α6β4 functions further, we have induced expression of a YFP-tagged β3 laminin subunit in normal and β4 integrin-deficient keratinocytes and followed the fate of the labeled laminin subunit in live cells using a spinning disk confocal microscope and by immunochemistry. YFP-β3 molecules are secreted with their γ2 and α3 subunit partners by both cell types. Moreover, the resulting tagged laminin-5 trimers are organized into the same intricate arrays in the matrix of the cells suggesting that β4 integrin is not involved in laminin-5 matrix assembly. However, the most prominent feature of the β4 integrin-deficient keratinocytes is their epidermal growth factor-dependent hypermotility in comparison with their wild-type counterparts. Indeed, groups of the β4 integrin-deficient keratinocytes show rotation around a central axis. Dramatically, they lack movement in a directed fashion. In summary, our data reveal that the β4 integrin is involved in the signaling pathway(s) regulating directed cell migration that is necessary for re-epithelialization of wounds.

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Is desmoglein 4 involved in the blister formation in pemphigus or impetigo?

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Desmogleins (Dsgs) are cadherin-type transmembrane components in desmosomes. In addition to the three known isoforms of Dsg (Dsg1, Dsg2 and Dsg3), Dsg4 has been recently identified as a new isoform. The purpose of this study is to determine the pathogenic involvement of Dsg4 in the blister formation of pemphigus and impetigo. A secreted form of the entire extracellular domains of Dsg4 (Dsg4-His) was produced by baculovirus expression. Dsg4-His was immunoprecipitated by 17/20 (85%) pemphigus foliaceus sera and 13/19 (68%) mucocutaneous pemphigus vulgaris sera, both of which contained anti-Dsg1 IgG autoantibodies, but was not immunoprecipitated by either the 16 mucosal dominant pemphigus vulgaris sera that contained only anti-Dsg3 IgG autoantibodies or the 34 normal control sera. The Dsg4 immunoreactivities of these sera were completely abolished by immunoadsorbing anti-Dsg1 IgG with Dsg1-His. IgG affinity-purified on Dsg4-His from pemphigus foliaceus sera immunoprecipitated Dsg1. Conversely, the removal of anti-Dsg4 IgG from pemphigus foliaceus sera did not significantly reduce the immunoreactivity against Dsg1. Furthermore, the removal of anti-Dsg4 IgG from pemphigus foliaceus sera did not significantly affect the pathogenic activity of blister formation as determined by passive transfer model using neonatal mice. IgG affinity-purified on rDsg4-His did not cause apparent blister formation in neonatal mice. In addition, Dsg4-His was not cleaved by the three known isoforms of exfoliative toxin (ETA, ETB, and ETD) that are known to cause bullous impetigo and staphylococcal-scalded skin syndrome. These data indicate that many of pemphigus foliaceus and mucocutaneous pemphigus vulgaris sera react with Dsg4 due to cross-reactivity by a subset of anti-Dsg1 IgG, and that Dsg4 is not directly involved in pathogenic process of blister formation in pemphigus or impetigo.

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Establishing the molecular determinants for desmoglein 1-mediated adhesion

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The targeting of desmoglein 1 (Dsg 1) by autoimmune antibodies or bacterial exfoliative toxins (ET) promotes epidermal blister formation, presumably by disrupting desmosomal-based intercellular adhesion. Previous attempts to reconstitute adhesion in normally non-adherent fibroblastic L cells by overexpressing a variety of desmosomal proteins, including Dsg 1, have yielded mixed results. We adopted two complementary approaches to better resolve the molecular determinants for Dsg 1-mediated adhesion: 1) stable L cell lines containing desmocollin 1 (Dsc 1) and plakoglobin (Pg) along with a tetracycline-inducible Dsg 1 construct were used to tightly regulate the ratio of desmosomal cadherins expressed by these fibroblasts and 2) adhesion-competent primary human keratinocytes were forced to overexpress Dsg 1. The ability of single L cell suspensions expressing Dsc 1 and Pg to form aggregates was directly dependent on the level of Dsg 1 expressed by these cultures and occurred in a time- and calcium-dependent fashion. ETA, which specifically cleaves the Dsg 1 ectodomain, abrogated this Dsg1-mediated adhesion but did not alter the ability of L cells expressing E-cadherin to aggregate. The simple introduction of Dsg 1 alone in keratinocytes was sufficient to increase the aggregation of single cells and further enhanced the adhesive strength of intact epithelial sheets in a manner that could be inhibited by ETA. Whereas the induction of Dsg 1 expression had little effect on the levels of Dsc 1 present in aggregated L cells, the introduction of Dsg 1 was capable of increasing endogenous Dsc 1 expression in keratinocytes. Taken together, these observations demonstrate that Dsg 1 promotes the formation of functional intercellular adhesion complexes and suggest that the relative ratio of desmosomal cadherins expressed at the cell surface regulates this adhesive process.

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Role of reactive oxygen species in TGF-β1 -induced tropoelastin gene expression in dermal fibroblasts

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Photoaged skin contains markedly increased levels of elastin but the mechanisms underlying this accumulation are only poorly understood. In vitro, TGF-β1 induces tropoelastin synthesis by dermal fibroblasts and, in a murine model of photoaging, antioxidants inhibited solar elastosis. Since UVB induces TGF-β1 production in skin and binding of TGF-β1 to its receptor stimulates rapid formation of reactive oxygen species (ROS) in many cell types, we hypothesized that these ROS may contribute to upregulation of tropoelastin (TE) by TGF-β1 in dermal fibroblasts. Treatment of fibroblasts with TGF-β1 (3 ng/ml) stimulated TE mRNA accumulation, measured using real time PCR, with a maximum at 6-8 h and measurable levels to 24 h. Accumulation of TE mRNA was inhibited by DRB, a transcription inhibitor, but not by cyclohexamide, a translation inhibitor, suggesting transcriptional regulation without a role for new protein synthesis. The antioxidant, N-acetylcysteine, at a non-toxic level (10 mM) delayed and inhibited the TGF-β1 -induced accumulation of TE mRNA but did not influence the mRNA level of TGF-β1 induced early gene (TIEG). An inhibitor of flavin-containing enzymes including NADPH oxidase, DPI (10μM), delayed the TGF-β1-induced TE mRNA accumulation and reduced the level by 70% when added within 1 h of TGF-β1. DPI did not affect the TIEG mRNA level under the same conditions. Pre-treatment of cells with NAC or DPI did not reduce the level of TGF-β1-induced phosphorylation of Smad2. These results indicate that ROS participate in the non-Smad signaling pathways leading to TGF-β1 upregulation of TE gene expression in human skin fibroblasts.

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Urokinase plasminogen activator (uPA) receptor/α5β1 integrin interaction and uPA-dependent epithelial cell migration is regulated by gangliosides

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The interaction of the urokinase-type plasminogen activator receptor (uPAR) with integrins plays a critical role in the regulation of cell proliferation, adhesion and migration. However, the molecular events underlying the formation of a complex comprised of uPAR and integrin are poorly understood. Gangliosides have been implicated in regulating cell proliferation, adhesion and migration by modulation of epidermal growth factor receptor (EGFR) and α5β1 integrin signaling. Both uPAR and gangliosides are known be localized to lipid rafts, sphingolipid-cholesterol microdomains on the plasma membrane that concentrate signaling molecules. We hypothesized that gangliosides act as modulators of uPAR signal transduction by integrin and/or EGFR. Expression of gangliosides GM3 and GT1b was modified genetically or biochemically in SCC12 squamous carcinoma cells, and the effect of GM3 and GT1b expression on the association of uPAR with both α5β1 integrin and EGFR was studied. Using immunoprecipitation and immunoblotting techniques, we have found that endogenous increases in the expression of ganglioside GT1b or GM3 affect uPA-dependent cell migration by preventing the complex formation of uPAR with α5β1 integrin or of uPAR/α5β1 integrin with EGFR, respectively, thereby modulating focal adhesion kinase (FAK) or integrin-mediated EGFR signaling. Although both gangliosides GT1b and GM3 inhibit uPAR-stimulated migration through inhibition of phosphotidylinositol 3-kinase activation, GT1b affects uPAR-directed FAK activation while GM3 inhibits uPAR-stimulated EGFR phosphorylation. Elucidation of the mechanisms by which specific gangliosides modulate uPAR signaling will help us to understand uPAR function and to develop novel ganglioside-based cancer therapies.

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Platelet-activating factor down-regulates E-cadherin expression of melanoma cells

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Platelet-activating factor (PAF) has been reported to have a motogenic effect on some tumor types, leading to tumor cells invasion into connective tissue and distant metastasis. Although the putative involvement of PAF in the progression of malignant melanoma has been shown, the mechanism responsible for the process of invasion and metastasis has not fully been elucidated. In this study, the possible role of PAF in melanoma progression was assessed by analyzing the regulatory effect of PAF on E-cadherin expression and the motile activity in melanoma cells. Flow cytometry analysis showed that PAF dose- and time-dependently reduced cell surface expression of E-cadherin in melanoma cell lines expressing PAF-R but failed to regulate that of a PAF-R negative cell line. The down-regulation of E-cadherin expression was maximal at a concentration of 10-10 to 10-9 M and after 36 h exposure of PAF. In accordance to the down-regulation of E-cadherin expression, 10-9 M PAF reduced cohesive activity of melanoma cells, as shown by aggregation assay. Furthermore, PAF effect on E-cadherin expression enhanced the motile activity of PAF-R positive melanoma cells in the invasion assay using modified Boyden chambers. PAF-R antagonist TCV309 drastically attenuated the regulatory effects of PAF on E-cadherin expression, cellular cohesion and motile activity. E-cadherin is an adhesion molecule important for cell-cell binding and maintaining the structure of epidermal tissue. Its regulation may be responsible for pathogenesis of melanoma cells invasion into dermis, which is initiated by the dissociation from the primary tumor nest and surrounding keratinocytes in epidermal tissue. Our results suggest that PAF is a possible candidate promoting dermal invasion of melanoma cells via E-cadherin down-regulation, and that PAF-R antagonist can be a useful agent to suppress PAF-mediated activation of melanoma progression.

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Pemphigus vulgaris IgG induces desmosomal transmembrane signaling

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Pemphigus vulgaris (PV) is a human autoimmune blistering disease in which pathogenic autoantibodies directed against the desmosomal cadherin desmoglein-3 (dsg3) cause acantholysis, the loss of cell-cell adhesion. Using human keratinocytes in tissue culture, we have previously demonstrated that PV autoantibodies rapidly induce phosphorylation of intracellular proteins in a time and dose dependent fashion. To investigate the biologic significance of these phosphorylation events, we have approached the identification of these phosphoproteins by MALDI-TOF mass spectrosocopy of in gel tryptic digests. Identification of these cellular phosphoproteins has enabled us to extend these studies to an *in vivo* model. Using both one and two dimensional gel electrophoresis and immunochemical techniques, we now show that the proteins first identified in tissue culture are similarly phosphorylated when PV IgG is passively transferred to neonatal mice. The observation that the same phosphorylation events occur both in human keratinocyte tissue culture and *in vivo* mouse models provide support for the hypothesis that structural changes in the desmosomes induced by PV autoantibodies activate cellular signal transduction cascades. Additionally, it is possible that the observed transmembrane signaling may have a pathogenic role in PV IgG induced acantholysis.

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Epidermal morphogenesis and survival require basement membrane components in 3-D, organotypic culture

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The role of basement membrane components (BMC) or extracellular matrix components (EMC) on the normalization of epidermal phenotype is not well-understood. The goal of this study was to develop human organotypic tissue models to study the role of these components on epidermal architecture and keratinocyte differentiation, survival and growth. Human keratinocytes were grown on polycarbonate membranes coated with purified BMC or EMC and placed on contracted Type I collagen gels populated with dermal fibroblasts. Only keratinocytes grown on membranes coated with BMC (Type IV collagen or laminin 1) generated well-stratified epithelia demonstrating all morphologic layers. In contrast, tissues grown on EMC which are usually not found in basement membrane (fibronectin, Type I collagen and fibrillar Type I collagen) demonstrated aberrant tissue architecture. Furthermore, only keratinocytes grown on BMC demonstrated a normalized, linear pattern of deposition of laminin 5 and an elevated synthesis and processing of the $\gamma 2$ chain of laminin 5 upon Western analysis. This demonstrated that while pre-existing BMC enabled proper organization of basement membrane, EMC were not permissive for such assembly. In situ TUNEL assay revealed that no TUNEL-positive keratinocytes were seen in the basal layer when cultures were grown on BMC in comparison to high levels of TUNEL-stained cells seen in tissues generated on EMC. This showed that BMC supported keratinocyte survival. Expression of keratinocyte differentiation markers were normalized for tissues grown on both BMC and EMC, suggesting that keratinocyte differentiation was independent of these components. These studies have demonstrated that BMC are critical microenvironmental factors that sustain keratinocyte survival and optimize epithelial architecture. Moreover, this human culture model allows dissection of the role of connective tissue components that regulate epidermal phenotype and is a valuable tool for future studies in epidermal biology.

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Characterization of the initial response of engineered human skin to sulfur mustard

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Sulfur mustard (SM) is a chemical warfare weapon known to cause blisters in human skin. Models used in skin biology may play an important role in further understanding the pathogenesis of SM-induced skin lesions and lead to new prophylactic measures to prevent SM injury. We have used a new approach to identify initiating events in SM-induced injury by exposing three-dimensional (3-D), engineered, human skin to SM and determining the morphologic, apoptotic, ultrastructural and basement membrane (BM) alterations that lead to dermal-epidermal separation. Skin equivalents transplanted to nude mice were exposed to SM vapor for 5,8,10 and 12 minutes and grafts were removed 6, 24, 28 and 120 hours after exposure. Distinct prevesication and post-vesication phases of damage were identified 6 and 24 hours after SM exposure respectively. Prevesication injury (6 hr), characterized by apoptotic cell death, was restricted to focal groups of basal cells in a dose-independent manner. Electron microscopy and immunofluorescent studies have revealed the presence of intact BM during the prevesication stage, suggesting that BM may be associated with the initiating events in SM injury. The post-vesciation stage (24hr) demonstrated dermal-epidermal separation, dose-dependent induction of apoptosis in basal and supra-basal cells and loss of BM integrity. In vitro skin equivalents also demonstrated similar dermal-epidermal separation and basal cell apoptosis when exposed to 150 ug/ml SM for 7 minutes. These studies provide important proof of concept that these human tissue models mimic tissue alterations previously observed in animal models of SM injury and further our understanding of the mechanisms that initiate SM-induced blisters that can advance the development of new countermeasures designed to limit SM injury.

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K6PC-5 stimulated the proliferation and collagen synthesis in cultured human fibroblast, but suppressed HaCaT cell proliferation *in vitro*

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Sphingosine-1-phosphate (S1P), the initial product of catabolism of sphigosine by sphingosine kinase, has been implicated as an intracellular second messenger in many studies. Previous studies suggested a positive role of S1P in the process of cutaneous wound healing, such as stimulation of proliferation and matrix formation in fibroblast, and differentiation and migration of keratinocytes. Since the S1P is increased by activation of the sphingosine kinase, we have synthesized many novel molecules, and examined the effect on the activity of sphingosine kinase. Among the various tested molecules, K6PC-5 showed high activating effect on the sphingosine kinase. In this study, we have investigated the effects of K6PC-5 on the proliferation and differentiation of fibroblast and keratinocyte in vitro. The proliferation of cultured normal human fibroblast was enhanced by the K6PC-5 in a dose-dependent manner and more that 30% of enhancement was observed at a concentration of 3mg/ml. The collagen synthesis in fibroblast was also increased and about 3-folds increase was seen at a concentration of 10mg/ml. While the treatment of K6PC-5 on fibroblast showed a significant increase in proliferation and collagen synthesis, the addition of K6PC-5 to HaCaT cells at 10mg/ml concentration significantly suppressed the proliferation of the cells. Involucrin expression, as a marker for terminal differentiation of keratinocytes, was increased by K6PC-5 in a dose-dependent manner. These results suggest that the newly synthesized K6PC-5 act as a novel sphingosine kinase activator.

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Viability and apoptosis of dermal fibroblasts after cryopreservation

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The optimal serum concentration for cryopreserving cells has not been properly defined. In this study, using flow cytometry, we determined whether human dermal fibroblasts from adult skin display differences in viability and apoptosis depending on the percent of fetal calf serum (FCS) used for their cryopreservation. Cultures were cryopreserved for 4 weeks in DMEM plus DMSO and 0,10,20,50, or 80% FCS. After thawing, cells were allowed to attach to tissue culture plastic for 4 hours, and flow cytometry using propidium chloride (PI) to test for cell viability and annexin V (AV) for early apoptosis were performed on attached cells and those still in the culture supernatant. Attached cells showed no differences in viability (>90%) or apoptosis (<5%) at the various FCS concentrations. However, in unattached fibroblasts, increasing FCS concentrations were associated with with up to 30% more viability and less apoptosis ($p=0.0203$ and $p=0.0317$; two-way ANOVA). Dermal fibroblasts cultured from chronic wounds have a low proliferative capacity and are difficult to cryopreserve. Using the same methodology with these cells, we found that high FCS concentrations had no effect on viability and apoptosis. We conclude that maximum FCS concentrations, up to 80%, may be best for cryopreserving normal dermal fibroblasts. Alternate strategies are needed to properly cryopreserve wound cells.

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Ultrastructural details of a protein membrane that forms at the medium surface of keratinocyte cultures closely resemble elements of basement membranes

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Recently we have reported that a previously undetected proteinaceous membrane forms at the medium/air interface of keratinocyte cultures (Eckhart, L, Reinisch, C, Inoue, S, Messner, P, Dockal, M, Mayer, C, Tschachler, E (2003) A basement membrane-like matrix formed by cell-released proteins at the medium/air interface supports growth of keratinocytes. Eur J Cell Biol 82, 549-55). The immunohistochemical detection of collagen IV, laminins 1 and 5, the results of preliminary ultrastructural investigations and the observation of keratinocyte growth on this matrix indicated its similarity to basement membranes (BMs) *in vivo*. Here we describe the detailed ultrastructural characterization of the novel membrane with the method of negative staining. A dense network of 8-10 nm wide irregular rod-like elements formed the basic structure of the membrane. Immunolabeling showed that these rod-like structures were composed of a core of a type IV collagen filament and that type IV collagen filaments were arranged as a network. Heparan sulfate proteoglycan (HSPG), in the form of 4.5-5 nm wide ribbon-like "double tracks", was associated with the rod-like structures as a sheath material. These features strongly resembled the structure of basement membranes *in vivo*. In addition to this basic structure, the membrane also contained other structures including numerous small aggregates of HSPG "double tracks" and small loose assemblies of laminin 5 with their mode of integration still to be clarified. The similarities in the ultrastructure of the novel keratinocyte-derived membrane to BMs suggest that membranes forming at the medium/air interface of *in vitro* cell cultures may be useful models for the investigation of BM structures and functions.

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Delta-tocopheryl glucopyranoside (tocopherol precursor) in the skin: an antioxidant that protects the extracellular matrix

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The extracellular matrix of the dermis, undergoes permanent remodeling due to the balance between the synthesis and degradation of the matrix macromolecules. Matrix proteins can be broken down by the matrix metalloproteinases (MMPs). These MMPs belong to a family of zinc-dependent endopeptidases with a highly conserved structure. They are involved in the physiological or pathological remodeling processes of the extracellular matrix. The expression and the activity of the MMPs are closely controlled to avoid unordered degradation of the extracellular matrix. Numerous studies have shown a correlation to occur between the overexpression of the MMPs and intrinsic skin aging skin. MMP-1 is a collagenase that degrades dermal collagen of types I, III, VII, VIII and X. Its expression and activity are increased during time-dependent and photo-induced aging. It is also known that the massive release of reactive oxygen species (ROS) causes oxidative stress and that the ROS are precocious mediators of tissue aging. Overexposure to ROS, whether of intrinsic or external origin, damages essential constituents of the skin. To analyze the activity of δtocopheryl glucopyranoside in comparison to that of α and δtocopherol, we explored the expression of the MMP-1 gene in human skin fibroblasts stimulated with PMA. In addition, we evaluated its effect on the peroxidation of the membrane lipids and on genomic DNA damage caused by various oxidative stimuli on cutaneous cells. In our experimental conditions, δtocopheryl glucopyranoside lowered MMP-1 gene expression by more than 50%. In addition, the antiradical action of δtocopheryl glucopyranoside was also shown with a massive 40% reduction of lipoperoxidation and 65% inhibition of DNA strand breaks. These results support δtocopheryl glucopyranoside as an important antioxidant and highlight its ability to protect the dermal matrix from external aggression. Overall, these findings argue in favour of a probable role of δtocopheryl glucopyranoside in the prevention of cellular skin aging.

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Characterization of epiplakin as an intermediate filament-associated cytolinker

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Epiplakin is a member of the plakin protein family of cytolinkers and it contains 13-16 plakin-repeat domains (PRD) homologous to the B subdomain of desmoplakin. The last 5-8 copies of PRD together with the linker domain are highly conserved in both human and mouse epiplakin. Epiplakin is expressed in various tissues such as liver, digestive tract, salivary gland, as well as skin. We studied the subcellular distribution and interaction of human epiplakin by overlay binding assays, immunostaining and transfection in keratinocytes and HeLa cells. Epiplakin distributed in a filamentous pattern and decorated the K8/K18, K5/K14 networks, but also partially co-aligned with vimentin intermediate filament (IF) networks. Epiplakin was also found in cell-cell contacts and focal adhesions where it co-localized with actin. *In vitro* binding assays showed that the PRD of epiplakin interacted modestly with keratins and vimentin whereas the linker alone did not show any binding. However, the presence of both linker and PRD fragments showed strong interaction and preferentially associated with the assembled IF rather than keratin monomer. In addition, transient transfection of EGFP-tagged PRD resulted in the formation of perinuclear aggregates in HeLa cells. On the other hand, expression of linker alone produced an even distribution throughout the cytoplasm, accentuated at focal adhesion contacts. The expression of PRD plus linker gave a filamentous pattern suggesting that both domains are required to decorate IF networks. Furthermore, Western blotting showed that the level of epiplakin increased with time in primary epidermal human keratinocytes after the calcium concentration of the media was raised. By using real-time RT-PCR, we showed that the expression of epiplakin was up-regulated at the transcription level. Together, these results indicate that epiplakin is a versatile cytolinker involved in (1) the interaction with keratins and vimentin IFs, (2) the formation of focal adhesion complex, and (3) the process of keratinocyte differentiation.

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Truncation of the distal C-terminus of collagen XVII impacts the physiologic adhesion functions

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Collagen XVII is a transmembrane protein in the hemidesmosomes, which mediates the adhesion of the epidermis to the basal membrane by binding to laminin 5. Genetic defects of collagen XVII are associated with epidermal detachment in junctional epidermolysis bullosa (JEB), but little is known about the molecular disease mechanisms. Here we analyzed the biologic consequences of a spontaneous deletion of the distal C-terminus of collagen XVII in a JEB patient. The underlying mutation was a homozygous duplication of four nucleotides in exon 54 of the COL17A1 gene. It resulted in a frame shift, an adjacent nonsense sequence of 18 amino acids, and a premature termination and elimination of 43 most C-terminal amino acids of collagen XVII, including the collagenous subdomain COL1. Unexpectedly, the mutation did not lead to nonsense-mediated mRNA decay, but allowed expression of a truncated molecule, albeit at a somewhat lower level. IF staining with antibodies to N-terminal domains of collagen XVII resulted in a positive signal in JEB skin, but the use of an antibody directed against the distal C-terminus of collagen XVII remained negative, indicating that a deleted molecule was stable in situ. Immunoblot analysis revealed both truncated collagen XVII forms in JEB keratinocyte cultures, the transmembrane form and the shed ectodomain. Analysis of the thermal stability by limited trypsin digestion showed that the helix-to-coil transition temperature was significantly lower for the truncated collagen XVII than for wild type controls, indicating abnormal folding of this molecule. These findings demonstrate that the deletion of the distal extracellular domain of collagen XVII causes abnormal folding, inhibits physiologic ligand binding of collagen XVII to laminin 5, and possibly increases the susceptibility to proteolytic degradation. The findings underline the role of the distal ectodomain of collagen XVII as a basement membrane ligand and its role in epidermal adhesion and etiopathogenesis of JEB.

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The β2 integrin CD11b/CD18 plays a critical role in neutrophil infiltration and elimination in the skin in experimental autoimmune bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune bullous disease characterized by subepidermal blisters with an inflammatory infiltrate and in vivo deposition of autoantibody and complement components at the basement membrane. BP autoantibodies recognize two hemidesmosomal proteins, the BP230 and BP180. Inflammatory cells, including eosinophils, neutrophils, lymphocytes, and monocytes/macrophages are identified in the upper dermis and bullous cavity. Using an IgG passive transfer model, we previously showed that neonatal mice injected with rabbit anti-mouse BP180 IgG developed a BP-like disease. Infiltrating neutrophils play a key role in subepidermal blistering in this animal model. β2 intergrins are crucial for neutrophil recruitment in inflammation. In this study we investigated the role of the β2 integrin CD11b/CD18 (Mac-1, CR3) in experimental BP. Wild-type (WT) and Mac-1-deficient (Mac-1-/-) mice were injected i.d. with pathogenic anti-mBP180 IgG and examined 24 h later. We found that Mac-1-/- mice were resistant to experimental BP with significant reduction of neutrophil infiltration at the skin. Pretreatment of WT mice with neutralizing antibodies against CD11b or CD18 block the pathogenic activity of anti-BP180 IgG. In addition, Mac-1-/- mice reconstituted locally with neutrophils from Mac-1-/- mice became susceptible to experimental BP. Time course study showed that although neutrophil infiltration in Mac-1-/- mice were severely impaired at 24 h more infiltrating neutrophils were found in the skin of Mac-1-/- mice compared to WT mice at early time points (2-4 h). Moreover, more neutrophils survived in the skin of WT mice reconstituted with Mac-1-/- neutrophil than WT neutrophils. Thus, Mac-1 mediates neutrophil recruitment to the inflamed skin and regulates inflammation by accelerating the clearance of infiltrating neutrophils.

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Genetic ablation of syndecan-1 increases proliferation and cell adhesion and delays senescence of mouse keratinocytes

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Syndecan-1 null mice show signs of delayed corneal and epidermal healing (Stepp, et al., J. Cell Science, 115, 4517-4531, 2002). Using primary epidermal keratinocytes derived from syndecan-1 null mice, we have begun to investigate the mechanisms underlying the epithelial healing defects. Primary mouse keratinocytes from wt and syndecan-1 null 1-3 day old pups on a Balb/c background were cultured in standard low Ca^{2+}(0.05mM) medium and grown for times ranging from 1 to 18 days. Cell proliferation, adhesion, and cellular senescence were assessed as well as the response of the cells to Ca^{2+} induced cell differentiation. Increased cell proliferation, assessed by 3H-thymidine incorporation, was observed in null cells compared to wt cells and was accompanied by increased cell density at confluence. Cell adhesion was assessed on several different matrices and found to be greater in the null keratinocytes, and this difference was accompanied by altered integrin localization. Integrin β1 and β4 expression in null cells was increased and keratinocyte migration was reduced in vitro, as measured by time lapse microscopy. Between 10 and 14 days after plating, the number of senescent wt cells increased from 20% to over 90% as determined by senescence associated β-galactosidase staining. In contrast, the senescent fraction of null keratinocytes increased from 18% to 58% during the same time period and reached 70% only at day 18. Paradoxically, TGFβ1 secretion into the media, previously associated with increased senescence, is greater in the null cells than the wt cells suggesting that responsiveness to TGFβ1 may be altered by the loss of syndecan-1. These data suggest that the loss of syndecan-1 impacts on several responses required for wound healing but further studies will be required to determine which of these pathways is responsible for the delay in healing observed when this heparan sulfate is absent.

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Alcohol dehydrogenase decreases the ability of *C. albicans* to form biofilm on tunneled catheters

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Biofilm formed by *Candida albicans* (CA) at catheter skin insertion sites, hubs, skin wounds and chronic skin ulcers is the major cause of bloodstream infections. Recently, using proteomics and Western Blot analyses, we showed that the levels of CA alcohol dehydrogenase (Adh1p) was 3 times lower in CA biofilms than in planktonic cells. Based on these data, we hypothesize that Adh1p plays an inhibitory role in CA biofilm formation. To test this hypothesis, we investigated expression of *ADH1* gene (encoding Adh1p) in biofilm and planktonic CA cells (grown to 6, 12, 24 and 48h) using Northern Blot analyses (NBA) and determined the effect of: (a) disulfiram (DF, Adh1p inhibitor), and (b) disruption of *ADH1* (△*adh1* strain) on CA biofilm formation. Biofilm formation was quantitated using tetrazolium-based metabolic activity (MA)/dry weight assays, and visualized using confocal microscopy (CM). NBA revealed that *ADH1* levels decreased temporally in biofilm and reached a minimal level at 48h. At the early phase (6h), the presence of DF increased biofilm formation by 5 times (Mean±SD; 0.964±0.259 MA/mg) compared to control biofilms (CB, 0.193±0.029 MA/mg, P= 0.007). CM showed that biofilm formed in the presence of DF has more polysaccharide-rich extracellular material (ECM), were much thicker and with more complex architecture than CB. Quantitative analysis of 6h biofilm formed by the △*adh1* strain showed that deletion of the *ADH1* gene resulted in significantly greater biofilm formation than the parent strain (0.093±0.011 vs. 0.049±0.007 MA/mg respectively, P = 0.005). CM revealed that biofilm formed by the △*adh1* strain secreted more ECM, was thicker and had more complex architecture (with more filaments and metabolically active cells) than the wild type strain. Similar pattern in the increase in biofilm formation was detected at later time points (12, 24 and 48h). These results clearly demonstrate that Adh1p has an inhibitory role in CA biofilm formation and suggest an anti-biofilm role for this protein.

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Epithelial-mesenchymal interactions of cylindroma cells

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The dermal cylindroma, a tumour thought to be derived from sweat duct epithelium, is characterised by an excess of basement membrane(BM). Laminin 5 is present throughout the BM-like zone and accumulates in an unprocessed form, which might promote migration. In this study, the migration of normal keratinocyte and cylindroma cell lines on a collagen substrate was investigated. Both cell lines were immortalized with HPV16, E6 and E7. Migration and the migratory response to an inhibitory laminin 5 antibody were assessed by the colloidal gold assay. Expression of laminin 5, MMP-2, MMP-9 and MT1-MMP was assessed using immunostaining, gelatin zymography and Western blotting. The expression of BM markers, keratins and wnt signalling components was studied by immunostaining of three-dimensional organotypic cultures generated on de-epidermalized dermis (DED). Cylindroma cells detached faster and migrated further when compared to the control cell line (p<0.001). The increased migration of the cylindroma cell line was inhibited by a functional antibody to laminin 5, but not by a control vimentin antibody. Western blotting for MT1-MMP demonstrated increased expression of a self-processed form of MT1-MMP in cylindroma cell lysates. Immunostaining of the raft cultures demonstrated cylindroma cells were able to form an epithelium and generate hair follicle-like structures that descended into the dermis. Increased expression of laminin 5 and type VII collagen was observed in the cylindroma BM. Cylindroma raft cultures demonstrated strong K1, K14, and K16 expression suggesting that the cylindroma cell was transdifferentiating into an epidermal keratinocyte on de-epidermalized dermis with intact BM. Increased lef-1 expression was observed in cylindroma epithelium suggesting activation of the wnt pathway. In summary, these data demonstrate that laminin 5 is necessary for cylindroma cell migration, possibly linked to the proteolytic activity of MT1-MMP. The expression profile of the cylindroma cells on BM also suggest a role for the cylindroma gene in wnt signalling and transdifferentiation.

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Effect of NGF on matrix metalloproteinases and tissue inhibitors of metalloproteinases produced by human dermal fibroblasts and myofibroblasts

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Neurotrophin (NT) family comprises a group of functionally and structurally related proteins that, together with their receptors, play a fundamental role in the development and survival of neuronal cells. NT family includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5. NGF is synthesized and released by human keratinocytes as a neurotrophic molecule for skin innervation and as a growth/survival factor for keratinocytes themselves. We demonstrated previously that human dermal fibroblasts (DF) and myofibroblasts (MF) synthesize and release all neurotrophins. They express the neurotrophin high affinity receptor p75, and the low affinity forms trkA and trkB, but not trkC. Focusing on the functional role of these neurotrophins in the dermis, we observed that NGF decreased collagen I and III secretion by these cells. We investigated therefore the effects of NGF (100 ng/ml, 48 h) on matrix metalloproteinases (MMP) -1, -2, -3, -9, -10, -13, and their inhibitors TIMP-1 and -2 both on DF and MF using a multiplex proteome ELISA array coupled with chemiluminescent signal detection. MF were obtained in vitro after 6 days treatment of DF with TGFb1 (1 ng/ml) expressing then alpha-smooth muscle actin marker. DF release higher levels of MMP-3, MMP-10, MMP-13 and TIMP-1 than MF. NGF decreases significantly MMP-2 secretion on both DF and MF since MMP-9 was only decreased in myofibroblasts. Regarding MMP-3, NGF exhibits opposite effects, increasing and decreasing it in DF and MF respectively. We conclude that DF and MF express different MMPs and TIMPs profiles and that NGF and more generally neurotrophins, are involved in the extracellular matrix remodelling and repair, by regulating collagens, MMPs and TIMPs synthesis and may therefore play a key role in fibrotic processes and skin aging.

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Estradiol down-regulates matrix metalloproteinase expression in dermal fibroblasts

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Aged fibroblasts (FB) display a matrix degrading phenotype with increased expression of matrix metalloproteinase-1 (MMP1) and matrix metalloproteinase-3 (MMP3) leading to a loss of dermal collagen. It is known that estrogens increase collagen dermal content but the effect of estrogens on dermal FB function and MMP expression is not well elucidated. By Western blot analysis, we showed that dermal fetal fibroblasts (FB) expressed both estrogen receptors (ERs): 66 kDa- ERα and 59 kDa-ER β proteins. To determine the estrogen effect on FB proliferation, normal human fetal FB were placed in either DMEM supplemented with 1% dextran charcoal fetal bovine serum (FBS) or DMEM with serum replacement (human serum albumin, human transferrin, human recombinant insulin) and stimulated with estradiol at physiologic concentration (10-10M). Proliferation was assessed by cell counts on day 1, 3, 6. Estradiol had no effect on FB proliferation in either DMEM supplemented with 1% FBS or DMEM with serum replacement. Then, we studied by RT-PCR the effect of estrogen treatment on c-fos mRNA level. In basal conditions, FB expressed low level of c-fos mRNA, addition of 20% FBS for 20 min induced significantly c-fos mRNA, addition of estradiol (10-10M) did not induce c-fos mRNA demonstrating that estradiol had no direct effect on proliferation of dermal FB. By RT-PCR, we found that in basal conditions the expression of MMP3 in normal human fetal FB was low. Treatment with interleukine 1 beta (IL1 beta) for 24 hours increased significantly MMP-3 mRNA level and estradiol treatment (10-10M) down-regulated significantly the IL1 beta induced MMP-3 mRNA level. MMP1, MMP2, tissue inhibitor of metalloproteinase -1 (TIMP1), collagen I and collagen III mRNA levels were not modified by 24 hours treatment with estradiol (10-10M). Our study suggests that estrogens could mitigate the age associated extracellular matrix degradation by regulating the transcription of some matrix metalloproteinases.

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Assembly of elastic fibers: elastin and MFAP3 interactions determined by yeast two-hybrid genetic system

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Elastic fibers comprise a central elastin core associated with microfibrils which consist of 10-12 distinct proteins. The microfibrils play a critical role in elastin fibrillogenesis during development and tissue repair by forming a scaffold into which the elastin molecules align, however, the interactions between elastin and the microfibrillar proteins are not well characterized. In this study, we examined the interactions of elastin and MFAP3, a major microfibril-associated protein, by yeast two-hybrid genetic system. First, full-length human elastin cDNA was cloned into the GAL4 DNA binding domain vector and MFAP3 cDNA into the GAL4 activation domain vector. Interactions were detected by selection in Ade-, His-, Trp-, Leu- plates and using assay of β-glactosidase as reporter. Secondly, subclones of elastin, including the C-terminal half as well as 3′-deletion clones devoid of one or both of the two cysteines residing at the carboxyl-end of the protein, were tested. The results showed that the elastin/MFAP3 interactive sites reside at the C-terminal half of elastin but the interactions do not require the presence of cysteines. The elastin/MFAP3 interactions were confirmed by GST-pull down assays. Thirdly, MFAP3 cDNA was divided into four approximately equal subclones (A-D) each corresponding to a distinct α-helical region, and tested with the C-terminal fragment of the elastin cDNA. Strong interaction was detected with the N-terminal component (A) of MFAP3. The results indicate that defined protein domains are critical for elastin/MFAP3 interactions, and perturbations in these sequences may result in diseases of abnormal elastin fibrillogenesis.

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Withdrawn

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Abrogation of E-cadherin function initiates MMP-mediated transition from premalignancy to highly invasive carcinoma

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The goal of the current study was to understand how loss of E-cadherin-mediated adhesion influences the earliest stages of carcinoma progression in three-dimensional (3D) tissue models of premalignant disease. This was accomplished by characterizing the behavior of early-stage, tumor cells (HaCaT-II-4) after abrogation of E-Cadherin function in 3D, organotypic cultures containing structured basement membrane (BM) and later transplanted to nude mice. Retroviral expression of a fusion protein consisting of an H-2K^d extracellular domain and cytoplasmic domain of E-cadherin (H-2K^d-Ecad) generated a dominant-negative (DN) effect on E-Cadherin function in II-4 cells resulting in the complete disruption of cell-cell adhesion, cytoplasmic redistribution of β-catenin and increased cell migration. In contrast, cell-cell adhesion and subcellular distribution of β-catenin were not altered either in cells transduced with an empty vector (pBabe) or in a fusion protein with a loss of function deletion in its β-catenin-binding domain (H-2K^d-EcadΔC25). When grown in 3D cultures, cells expressing H-2K^d-Ecad showed loss of adherens junctional proteins from cell junctions and underwent transition to invasive squamous cell carcinoma, which was associated with loss of Type IV collagen and laminin 5 at the BM interface. Tumor cell invasion was blocked by the presence of MMP inhibitors, demonstrating that loss of E-cadherin function activated MMP-mediated degradation of BM proteins. Surface transplantation of 3D cultures to nude mice demonstrated that only H-2K^d-Ecad cells underwent accelerated tumorgenesis and generated highly invasive, aggressive carcinomas that dissected through the connective tissue as single cells in vivo. These findings provide the first evidence that loss of microenvironmental control, resulting from abrogation of E-Cadherin-mediated adhesion, can direct the transition from precancer to highly aggressive squamous cell carcinoma.

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Skin cell attachment to an implanted transcutaneous biomaterial: a mouse model

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Transcutaneous devices such as catheters and cannulas have become integral in the management and treatment of disease. Currently, keratinocytes (KC) travel down along the transcutaneous devices, creating a space between the epidermis and the device, and establishing a haven for bacterial invasion and biofilm formation. Up to 250,000 cases of catheter-associated blood stream infections occur annually with an attributable mortality rate of 12%-25% for each infection. "The development and application of new technology holds the greatest promise for a quantum reduction in the incidence of nosocomial infections in general, and bloodstream infections derived from devices used for intravascular access in particular" (Safdar N et. al., 2002). The purpose of this study was to develop advanced biomaterials that could be studied in an *in vivo* model that would permit attachment of skin cells where transcutaneous devices penetrate the skin. Porous poly(2-hydroxyethylmethacrylate) (pHEMA) rods of approximately 2-mm in diameter were implanted at two separate sites on the backs of twelve, 8-week-old C57 black mice. The pHEMA rods were treated with: 1) PBS, 2) carbonyldiimidazole (CDI), and 3) CDI plus laminin-5. Dorsal skin containing the implanted biomaterial was harvested at seven days after implantation and processed for light microscopy and immunohistochemical analysis. There was evidence of KC migration into the pores of the pHEMA in the CDI and CDI plus laminin-5 treated samples, while the KCs migrated parallel to the pHEMA in the PBS treated control samples. This *in vivo* model permitted examination of the pHEMA/skin interface in both paraffin and O.C.T. embedded frozen tissue. These preliminary findings are encouraging for future studies to facilitate skin cell attachment where transcutaneous devices penetrate the skin.

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Epidermal keratinocytes utilize edogenous glutathione to modify the advanced glycation end-product intermediate, methylglyoxal, as a mode for cytoprotection

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Proteins exposed to reducing sugars can undergo non-enzymatic glycosylation yielding the formation of Advanced Glycation Endproducts (AGE). These AGE proteins have been implicated in various pathologies including diabetes, retinopathy, aging and photoaging. Intermediates of AGE's such as methylglyoxal are also detrimental to cells. The reaction between methylglyoxal and amino acids, particularly lysine and arginine, leads to the production of free radicals such as superoxide anion, which can damage cells and structural proteins. Glutathione has been reported to act as a cofactor in the detoxification of methylglyoxal in vitro via the glyoxylase pathway. In these experiments, we wanted to determine if epidermal keratinocytes utilize this pathway to provide cytoprotection from these toxic intermediates. Viability assays were performed on normal human keratinocyte cells challenged with methylglyoxal either with or without glutathione, or buthionine sulfoximine (BSO). The addition of exogenous glutathione provides statistically significant increases in cell viability displaying methylglyoxal detoxification by this antioxidant. The addition of BSO exacerbated the effects of methylglyoxal displaying the need for endogenous glutathione to counteract the effects of methylglyoxal. Antioxidants were investigated as well to determine the mode of action of glutathione. Treatment of keratinocyte cells with Resveratrol and Butylated Hydroxytoluene did not significantly protect against methylglyoxal toxicity.

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BP180 (type XVII collagen) triple helix assembly: evidence for multiple nucleation sites

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BP180 is a key constituent of the epidermal anchoring complex and a member of the collagen protein family. Efficient assembly of a collagen triple helix requires a nucleation step involving intersubunit binding that usually occurs within a terminal non-collagen domain. Previously published reports have provided support for the hypothesis that BP180's nucleation site resides within NC16A, the amino-terminal non-collagenous stretch of the extracellular domain (ECD). To further examine this issue, we have generated and structurally analyzed a series of recombinant forms of BP180, which includes the full-length ECD (sec180e) and its counterpart with the NC16A domain removed (sec180-delN16), as well as a pair of truncated proteins comprising the COL15-NC15 stretch expressed either with (designated sec180-trunc) or without the NC16A domain (sec180-trunc-delN16). The two constructs containing NC16A, i.e. sec180e and sec180-trunc, were shown to form stable homotrimers by chemical cross-link and gel filtration analyses. As expected for collagen triple helices, these trimers were resistant to proteolysis and each denaturation curve showed a sharp transitional phase with a melting temp in the range of 42-46°C. Sec180-trunc-delN16 was also able to form a triple helix, but only at a high protein concentration or when incubated at a low temperature for an extended time. In contrast with its counterpart containing NC16A, the denaturation of the sec180-trunc-delN16 trimer occurred over a much broader temperature range (20-40°C), suggestive of structural heterogeneity. Unexpectedly, the construct composed of the entire BP180 ECD minus NC16A formed a stable collagen triple helix with properties quite similar to those of its NC16A-containing counterpart, sec180e. In summary, our findings with the shorter constructs provide strong support for the hypothesis that NC16A functions as a nucleation site for triple helix assembly, while the results from the longer constructs are indicative of a secondary nucleation site located within the C-terminal portion of the BP180 ECD.

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The absence of detectable anti-desmoglein antibodies in patients with myasthenia gravis

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Both pemphigus vulgaris and myasthenia gravis are diseases considered to have an autoimmune basis. While the presence of one autoimmune process is generally believed to correlate with an increased risk of other autoimmune diseases, the association of both pemphigus vulgaris and myasthenia gravis has been reported in patients at a rate greater than that expected by chance alone. In most of the reported cases, myasthenia gravis preceded the development of pemphigus. Pemphigus is known to be associated with antibodies directed against desmogleins 1 (DSG-1) and 3 (DSG-3), two cell surface keratinocyte proteins which are part of the cadherin superfamily of proteins.

We have evaluated the serum of 13 patients with documented myasthenia gravis using a commercially avaliable elisa kit to evaluate for the presence of antibodies to DSG-1 or DSG-3. While there was a trend for some of the patient sera to have non-zero levels of antibody, documented levels consistent with a diagnosis of pemphigus were not observed. In this small sample, serological evidence of anti DSG-1 and DSG-2 antibodies was not detected.

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Complexity of Dsg1-a, -b, and -g expression in diverse mouse tissues and during hair follicle formation

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The four isotypes of desmogleins (Dsg1-4) are expressed in a differentiation- and keratinization-specific manner. Recently, we reported cloning of two novel mouse Dsg1 genes, Dsg1-b and -g, flanking Dsg1-a on chromosome 18. Sequence conservation between the Dsg1 isoforms is extremely high, however diverges significantly at the extracellular anchoring (EA) domains. Here, we generate antibodies using synthetic peptides and recombinant proteins localized within the EA domains. These antibodies were used to demonstrate differential expression of Dsg1 isoforms in specific compartments of embryonic and adult mouse skin. Unexpectedly, expression of Dsg1-b is observed also in some non-epithelial tissues including the testis. Dsg1-b and -g but not Dsg1-a are detected in the sebaceous gland epithelium. In the hair follicle, all Dsg1 isoforms are present throughout the entire process of its development and cycling. In anagen, Dsg1-a and -b are expressed in the outer root sheath while Dsg1-g is also detected in the inner root sheath. During anagen-catagen transition, expression of both Dsg1-a and -b is up-regulated in diminishing hair matrix but later, in mid catagen, dramatically reduced in cells within the epithelial strands that undergo active apoptosis. In late catagen, Dsg1-a and -g are actively expressed in the zone of club hair formation while Dsg1-b is mostly expressed in the outermost cells of permanent hair follicle portion. In telogen, Dsg1-b expression in hair follicle declines but Dsg1-a and -g levels are still high thus suggesting their significant role in club hair formation. Also during telogen, follicular papilla cells express Dsg1-a but not Dsg1-b or -g, however, this expression is abolished at onset of early anagen. Thus, expression of Dsg1 isoforms is a subject of significant hair cycle-dependent changes being up-regulated in stable, differentiating cellular compartments and declining significantly with the onset of proliferation/remodeling associated with elevated cell motility.

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Melanocortin receptors in fibroblastic cell types of the skin - *in vitro* and *in vivo* expression and functional relevance

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In contrast to the well established role of melanocortins on melanocyte function expression and relevance of melanocortin receptors (MC-Rs) in skin fibroblasts is incompletely understood. We recently showed that human dermal fibroblasts derived from foreskin express functional MC-1Rs which mediate an inhibitory action of alpha-melanocyte-stimulating hormone (alpha-MSH) on collagen metabolism (Bohm et al, J. Biol. Chem. 2004, in press). Here we show by RT-PCR, immunofluorescence, immunohistochemistry and immune electron microscopy that MC-1R expression is conserved in vitro and in vivo in additional human fibroblastic cell types of the skin including adult dermal fibroblasts, fibrosarcoma cells, connective tissue sheath fibroblasts and dermal papilla cells of the hair follicle. In vitro expression of MC-1R declines as a matter of cellular senescence in dermal fibroblasts. Interestingly, alpha-MSH inhibits the inductive effect of gamma-interferon, an important proinflammatory mediator in inflammatory and fibrotic skin diseases, on expression of adhesion molecules such as intercellular adhesion molecule-1 in human dermal fibroblasts. These data point towards an additional biological role of alpha-MSH as a modulator of inflammatory reactions in the connective tissue compartment of the skin.

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Chronic UVB exposure inhibits collagen gel contraction by normal human dermal fibroblasts: a model for photoaging and a possible mechanism for chronologic aging

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The formation of a wrinkle in human skin involves multiple mechanisms including environmental factors, chronologic aging, as well as mechanical stresses (e.g. gravitational forces, musculature folding). We investigated these various mechanisms on the ability of Normal Human Dermal Fibroblasts (NHDF) to contract three dimensional collagen gels, and measured concomitant cytokine release (IL1-?α, IL1?α receptor antagonist, IL1-?β) by these cells. NHDF cells grown in three dimensional collagen gels, and exposed to UVB (10, 20, 30, 40, and 50 mJ/cm2), yielded a dose dependent decrease in contraction (35, 50, 65, 69, and 85 % respectively) with a concomitant increase in IL1 alpha and beta. Additionally, when donor aged (45 and 92 year old) fibroblasts were seeded in three dimensional collagen gels, we observed much less contraction, and contraction occurred at a slower rate. Similarly, cytokine production was increased in these aged donors. Lastly, as a measure of mechanical stress, full thickness organotypic cultures were subjected to continuous folding and cytokine levels were determined. In those tissue undergoing mechanical folding, increases were observed in IL1 alpha and beta. Histologically, gross increases in apoptotic bodies were observed. These data suggest that both chronologically aged fibroblasts, and UV exposed fibroblasts produce cytokines which are involved in the decrease in cell contractility, and this decrease may contribute to the development of a wrinkle. Similarly, mechanical stress increases cytokine production providing another means toward the development of wrinkles.

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Characterization of ECM-1 protein interactions by yeast two-hybrid system

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Lipoid proteinosis (LP), an autosomal recessive disorder affecting the skin and mucous membranes, is characterized by deposition of hyaline material in the extracellular spaces, and results from loss-of-function mutations in the extracellular matrix protein 1 (ECM-1), a glycoprotein expressed in the skin and other tissues. Since the function of ECM-1 has not been fully determined, we used the yeast two-hybrid genetic system to explore its interactions with other extracellular matrix components. We cloned two full-length ECM-1 cDNAs into the GAL4 DNA-binding domain vector to be used as bait vectors for two-hybrid library screening. One construct starts at the translation initiation codon and continues through the stop codon, and the other lacks the secretory signal peptide. We also generated five different partial ECM-1 bait constructs corresponding to the N-terminal cysteine-free domain, the central two tandem repeat domain, and the C-terminal region. From the screening of human placental cDNA library in the GAL4 activation domain vector and selection on Trp-, Leu-, His- plates, we found that legumain, matrix metalloproteinase 9 (MMP-9, type IV collagenase), and epidermal growth factor (EGF) interacted with full length ECM-1. Further studies with the partial ECM-1 bait vectors revealed that legumain and MMP-9 interact only with the two tandem repeat domain, while EGF interacts with the cysteine-free and C-terminal regions of ECM-1. These interactions were further confirmed by beta-galatosidase assays. Overall, these findings increase the understanding of the potential mechanisms underlying dermal pathology in lipoid proteinosis as well as the putative role of ECM-1 in normal human skin.

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Pemphigus vulgaris antibody targets antigen that are not limited to desmogleins

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It is currently believed that pemphigus vulgaris is caused by autoantibodies directed to antigen that are localized strictly in the desmosomes. However, some prior immunoelectron microscopic studies have shown that the antibodies also react with keratinocyte cell membrane components other than desmosomes. To further explore the anatomical site targeted by pemphigus antibodies, we reacted the serum of a patient with pemphigus vulgaris and with high titer of pemphigus antibodies against monkey esophageal epithelium using antihuman antibodies and the diaminobenzidine peroxidase system. We then utilized electron microscopy to visualize the anatomical location of the antigen(s) targeted by the pemphigus vulgaris antibody. We found that there was continuous staining along the external cell membrane, including sites other than desmosomes. Control study with normal human serum were negative for cell membrane staining. This observation suggests that in addition to desmogleins, pemphigus antibodies target keratinocyte surface antigens that are not limited to desmosomes.

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BEG4 is a novel actin bundling protein

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While it is well known that Shh controls organ growth by altering cell shape and migration, the molecular basis is unknown. The Shh target gene BEG4 (Basal Cell Carcinoma Enriched Gene 4), which is expressed in basal cell carcinomas of the skin (BCCs), shares a conserved WH2 domain with the human Wiskott-Aldrich Syndrome protein (WASP), suggesting it may regulate actin dynamics and consequently cell shape, adhesion and migration. Here we show that overexpression of BEG4 in the mouse embryonic cell line C3H10T1/2 results in an altered actin cytoskeleton with reduced actin stress fibers and the production of long cytoplasmic extensions. This activity localizes to the conserved 408 amino acids of the protein and does not require the G-actin binding WH2 domain. in vitro assays and electron microscopy techniques demonstrate that this conserved region of BEG4 promotes the creation of actin filament bundles. Deletions of BEG4 that lose bundling activity fail to stimulate cytoplasmic extensions in vivo. These data demonstrate that human BEG4 is a novel actin bundling protein expressed in BCCs that might link this tumor type to alterations in the actin cytoskeleton.

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Compound heterozygosity for mutations in the type VII collagen gene (COL7A1) results in severe dystrophic epidermolysis bullosa

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Dystrophic epidermolysis bullosa (DEB) is a rare hereditary bullous disease caused by mutations in the type VII collagen gene (COL7A1), the main component of anchoring fibrils. Blister formation in DEB occurs at the level of the anchoring fibrils located at the dermal-epidermal junction. DEB is inherited through both autosomal dominant and recessive transmission. The nature of the mutation in the COL7A1 gene, its location, and the presence of either dominant or recessive inheritance determines the clinical phenotype of each patient. In this work, we studied the proband in a small nuclear family of Chinese and Dutch/German descent and identified two novel mutations in COL7A1 leading to severe recessive dystrophic epidermolysis bullosa (RDEB). The maternal mutation is a single base pair deletion of a cytosine nucleotide in exon 26, designated 3472delC, resulting in a frameshift and a premature termination codon (PTC) within the same exon, 7 bp downstream from the site of the mutation. The paternal mutation is a G→A transition located at the 5’ donor splice site within intron 51, designated IVS51+1G→A. This mutation is predicted to lead to activation of a cryptic splice site, 32 bp downstream of the mutation site and to subsequent aberrant out-of-frame splicing, leading to an unstable mRNA and a downstream termination codon. These findings extend the body of evidence for compound heterozygous mutations leading to severe RDEB and provide the basis for prenatal diagnosis in this family.

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Understanding mechanical factors in bullous disease using a novel suction blister instrument

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Bullous pemphigoid (BP) is a blistering disease resulting from auto-immune disruption of hemidesmosome mediated keratinocyte adhesion of to the epidermal basement membrane. BP predominantly affects the elderly. Negative pressure, applied to the skin surface causes suction blisters through successive detachment of hemidesmosomes from the basement membrane. Thus, susceptibility to suction blistering reflects the strength of hemidesmosomal adhesion. In order to understand the role of basement membrane mechanical properties in blistering disease, we have developed a novel device to quantitatively measure basement membrane breaking strength under precisely defined negative pressure. We measured the time needed to induce a suction blister on the ventral forearm of 10 subjects using an average negative pressure of −524mmHg. Volunteers tolerated the procedure without complaints of pain, but did describe a sensation of mild discomfort and pruritis to the area being tested approximately 5 minutes prior to developing a small vesicle at the test site. Subject age showed a strong inverse correlation with time to blistering. Male subjects exhibited longer blistering times than female subjects. Topical application of a high potency topical steroid (clobetasol) 24 hours prior to measurement did not significantly effect blistering time. The decreased mechanical stability of the basement membrane in older subjects may help explain the increased prevalence of bullous pemphigoid in this age group.

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Dysregulation of genes encoding cytoskeletal and signal transduction proteins in desmocollin 1 mutant mice

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The desmocollin 1 (dsc1) gene is expressed in the suprabasal layers of the skin where dsc1 is a component of the desmosomal cell adhesion complex. We have recently generated mice in which the endogenous dsc1 gene is replaced by a mutant gene that encodes a COOH-terminally truncated protein. This adhesion receptor does not bind the desmosomal plaque proteins plakoglobin and plakophilin. Nevertheless, it is sufficient to maintain cell adhesion since mutant mice do not show a gross phenotype. However, we did observe a dramatic increase in dsc2 mRNA synthesis in these mice, although dsc2 protein levels were normal. In the present study, we analyzed the effects of the dsc1 mutation on genes encoding cytoskeletal components using Affymetrix gene chip arrays. Interestingly, the expression levels of several genes that are expressed in the hair follicles were affected in dsc1 mutants; whereas keratins 19 (K19) and K15 were up-regulated, several hair keratin and keratin-associated proteins were down-regulated (high-sulfur and glycine/tyrosine-rich keratins). What triggered these expression changes in dsc1 mutants? As a first step to address this question, we analyzed the expression of the major signal transduction pathways genes that affect the skin. We found that the expression levels of components of the WNT pathway (frizzled 3, Wnt 6, dishevelled 3) and several transcription factors (s-myc, c-rel) were affected. Furthermore, a sequence analysis predicted putative binding sites for TCF/Lef, myc and NFκB in the dsc2 promoter. In summary, we have identified gene regulatory pathways that might affect dsc2 expression in our mice, providing a first glance into the regulation of this desmosomal cadherin.

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Knock-in of the mouse *lamc2* gene defines the functional role of the unprocessed laminin γ2 chain in keratinocyte adhesion, migration and proliferation

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Laminin-5 (α3β3γ2), the primary keratinocyte adhesion ligand, is secreted in the extracellular matrix to provide a specific substrate for adhesion of proliferating and migrating cells. The multifunctional role of extracellular laminin 5 is governed by the proteolytic processing of the α3 and γ2 chains. To elucidate the biological function of the γ2 chain, we have generated knock-in (ki) mice lacking the γ2 amino acid sequence YSGD required for the proteolytic cleavage of the γ2 N-terminal short arm using site-directed mutagenesis of the *lamc2* gene and the CRE-loxP technology. The ki YSGD-/- mice exclusively express laminin 5 molecules harbouring an unprocessed γ2 chain. The animals are viable, fertile, affected by slight growth retardation, and present no evident morphological and histological abnormality. Immunohistochemical analysis disclosed abnormal accumulation of laminin 5 and other basement membrane components in the epithelial basement membranes, which, at the structural level, correlated with an irregular thickening of the lamina densa of the dermal-epidermal junction. Analysis of primary ki mouse keratinocyte cultures confirmed the enhanced deposition of laminin in the extracellular matrix, which induces formation of anchoring structures, an improved cell adhesion capacity that results in the flattened shape and reduced scattering of these keratinocytes. Expression of unprocessed laminin 5 was found to exert an inhibitory effect on cell proliferation both in vitro and in vivo, and to enhance cell migration at the wound edge of scraped confluent cell monolayers. These results for the first time clearly define the functional role of the unprocessed extracellular form laminin 5 in adhesion and migration of keratinocyte in physiological processes like wound healing.

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Crosslinked hyaluronan with PEG nanostents inhibits collagen gel contraction: a potential preventative of burn contracture

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Burns are difficult to treat wounds, with complex local and systemic pathology and high mortality, which often heal slowly with scars and contractures. Fetal wound healing is a remarkable process that occurs in a unique environment that triggers a complex cascade of tightly controlled events culminating in a scarless wound phenotype of fine reticular collagen and abundant Hyaluronic Acid (HA). Contraction of wounds has been modeled in vitro by blending fibroblasts into collagen gels and then adding a stimulus such as serum or platelet-derived growth factor (PDGF). We tested the effect of high molecular weight (HMW) HA, thiolated HA (HA-DTPH) and HA Poly (ethylene glycol) diacrylate (HA-PEGDA) on the contraction of collagen gels. The effect of different crosslinking ratios of PEGDA functional groups to HA thiol groups was also studied. None of these constructs were cytotoxic, nor did they inhibit cell proliferation. As previously reported, high molecular weight (HMW)HA (0.125% - 0.25% w/v) facilitated contraction of gels with the higher concentration being more active. HA-DTPH (0.125% w/v) had no effect on contraction. In contrast, HA-DTPH (0.25% w/v) and HA-PEGDA (0.125% w/v) inhibited and HA-PEGDA (0.25% w/v) strongly inhibited contraction. Inhibition by HA-PEGDA increased as the crosslinking ratio was increased. Biomaterials, which inhibit collagen gel contraction in vitro, may also prevent wound contraction in vivo. Hence, PEGDA may be used as nanostents in patients to prevent collagen contraction, thereby preventing contracture.

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Effect of fibronectin functional domains and substrate crosslinking density on the morphology and dynamics of human dermal fibroblasts

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Tissue development depends largely on how cells sense and respond to extracellular matrix (ECM). Coupling of cell integrins with ECM ligands generates both, a ligation-induced signaling and a state of isometric tension, within the cell cytoskeleton (CSK). The extent to which ECM supports this prestress governs cell shape and function. In this study, we formulated hydrogels by coupling the three recombinant fibronectin functional domains (rFNfs) to cross-linked thiolated HA backbone using a polyethylene glycol diacrylate (PEGDA) crosslinker. We investigated cell stiffness and the corresponding actin CSK arrangement as a function of hydrogel stiffness and adhesiveness. Hydrogel stiffness was controlled by the amount of crosslinker added, while the adhesiveness was controlled by the nature and amount of the rFNfs coupled to the hydrogel backbone. AFM was used to determine the surface modulus of live cells. Confocal microscope was used to obtain images of the actin CSK of fixed and stained cells. It was found that cells seeded on either stiffer or more adhesive surfaces had larger surface moduli and more stretched actin CSK than those seeded on softer or less adhesive substrates. This indicated that the cortical tension was able to sense both, the underlying substrate stiffness and adhesiveness. Diffusion dynamics of cells seeded on these substrates was also monitored and correlated with cell morphology, substrate stiffness and nature of rFNfs.

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Infiltration of CD4+ T cells and dendritic cells is associated with imiquimod-induced regression of actinic keratosis

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Imiquimod 5% cream is a topically applied immune response modifier that has been shown to effectively treat actinic keratosis (AK). The therapeutic effects of imiquimod likely involve the provocation of an immune response against abnormal cells; however no clinical studies have conclusively proved this mechanism. The objective of this study was to investigate the mechanism of action of imiquimod when applied to AK lesions by relating cellular infiltrates to lesion clearance. A total of 18 subjects participated in this phase I, randomized, double-blind, parallel group, vehicle-controlled study. Enrolled subjects were randomized to receive imiquimod cream or vehicle cream in a 2:1 ratio and applied study cream to 5 lesions on the scalp, forearm, or upper trunk once daily, 3 days per week for 16 weeks or until clear. Each subject had two 5 mm punch biopsies of AK lesions: at prestudy to establish baseline biomarker levels and after 2 weeks of treatment to determine which cellular infiltrates were induced by the application of imiquimod. Biopsy specimens were examined using routine and immunohistochemical staining. Complete clearance of all treated AK lesions was achieved by 41.7% (5/12) of imiquimod subjects and 0% (0/6) of vehicle subjects. The imiquimod group showed statistically significant increases in biomarker levels from baseline to week 2 for CD3, CD4, CD8, CD11c, CD86/CD11c, CD68, HLA-DR, and TUNEL. No significant differences were seen for the vehicle group. The increases seen for the imiquimod group suggest that imiquimod stimulated both the immune response and apoptosis. However, no significant correlations between clearance and week 2 biomarker levels were seen for the imiquimod group. This was most likely due to the small sample size and the choice of a single time point for the biopsy. Further studies are needed to elucidate the exact mechanism of imiquimod, however these data provide preliminary evidence that imiquimod stimulates both the innate and adaptive immune responses.

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Carbon dioxide laser treatment of pyogenic granuloma

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Background: Pyogenic granuloma is a frequently encountered benign vascular lesion .The present study was designed to describe the use of CO2 laser(continuous mode) as a treatment method,to compare it with established methods and to assess its results. Patients and methods :prospective observational study between July 2001-Nov. 2003.sixty-three patients with pyogenic granuloma were selected randomly for treatment with CO2 laser (continuous mode),power 15 W in one treatment session .Major outcome measures including complete resolution of treated pyogenic granuloma during the follow up period. follow up visits were done 1 week,1 month,three months and six months. Results: Pyogenic granuloma was removed completely in one treatment session in 61(95.5%) patients without recurrence .In 56(88.8%) patients there were no scar and in 3(4.7%) patients only slight textural changes of the skin were observed. Atrophic scars were found in 2(3.6%) patients. Hypertrophic scar or keloid did not occur. No permanent hpopigmentation, hyperpigmentation or erythema was detected. Conclusion: Continuous mode CO2 laser is a good choice for treating pyogenic granuloma as this kind of laser is widely available with easy manipulation and good haemostasis,low recurrence rate and few adverse effects .

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Marked improvement in staging accuracy in mycosis fungoides/sezary syndrome (MF/SS) using integrated positron emission tomography and computed tomography

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Staging of MF/SS, the most common cutaneous T cell lymphoma (CTCL), is primarily based on type/extent of skin involvement and presence/absence of extracutaneous disease. Traditional staging uses CT to look for visceral or lymph node (LN) disease followed by biopsy of enlarged LN (axillary/inguinal LN > 1.5 cm). PET detects various tumors with hypermetabolic activity but its role in staging MF/SS has not been investigated. We assessed the utility of integrated PET and CT (PET/CT) in staging 10 patients with MF (T2=1,T3=3,T4=1) or SS (T4B2=5) at high-risk for LN disease. Based on anatomic data from the CT component alone, only 3 of 10 had enlarged LNs and would have been referred for biopsy. In comparison, PET showed that all 10 patients had hypermetabolic activity in at least 1 LN region. All patients had excisional LN biopsy and the extent of LN involvement was classified according to NCI criteria. Four patients had dermatopathic lymphadenitis (LN1-2) and 6 had effacement of LN architecture by lymphoma cells (LN4). Of the 6 patients with LN4, 4 had SS and 2 had tumor MF. In all 6 patients, PET/CT helped determine the pathologic LN for biopsy, which led to the accurate stage of IVA. Furthermore, we quantified the intensity of PET activity using standard uptake value (SUV) and correlated this with LN grade. Patients with LN1-2 had a median SUV of 2.2 (2.0-3.0); patients with LN4 had a median SUV of 5.1 (3.2- 11.8). This suggested that the threshold on PET between dermatopathic lymphadenitis and frank neoplasia was around 3 SUV. Also, large cell transformation and higher Ki67 showed a trend toward higher SUVs. Thus, for staging MF/SS, PET/CT was more sensitive and specific in detecting malignant LNs compared to CT alone and thus led to improved patient management. A larger scale study would be essential to confirm the superior staging capability of PET/CT over CT alone.

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Damage and recovery of skin barrier function after diamond microdermabrasion

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Microdermabrasion had become an extremely popular method for superficial resurfacing. But, published studies of skin barrier function changes following diamond microdermabrasion are rare. The aim of this study is to objectively evaluate the degree of skin damage induced by diamond microdermabrasion and time to recovery of skin barrier. For twenty three normal volunteers, the right side of the face was treated with diamond peeling and the left side untreated. Transepidermal water loss(TEWL), stratum corneum hydration and erythema were taken from both sides of the face before peeling and at 24 hour-interval after peeling. The results showed that compared to the controls, the treated area demonstrated a statistically significant increase in TEWL caused by peeling returned to a baseline value after 1 day. In addition, erythema induced by peeling was also normalized after 1 day. Skin hydration values were widely varied among the patients. In conclusion, the skin barrier is recovered in 1 day after diamond microdermabrasion, which is earlier than recovery after other forms of microdermabrasion such as glycolic acid peel or crystal peel. Therefore, clinically, diamond peeling done at one or two weeks'interval may not cause a significant damage to the skin barrier.

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***In vitro* skin penetration/permeation of pimecrolimus and tacrolimus from their marketed formulations (Elidel and Protopic)**

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Recent *in vitro* studies have shown that pimecrolimus permeates less through human skin than tacrolimus when applied in 1% solutions (Billich et al, Int J Pharm, in press). Here, we report on comparative studies with the marketed formulations of pimecrolimus (Elidel cream 1%) and tacrolimus (Protopic 0.1% and 0.03% ointment). The formulations were applied to split-thickness human cadaver skin and the penetration/ permeation of the active compounds was evaluated in vitro. The concentration of the two drugs in the skin was similar when comparing Elidel 1% and Protopic 0.1% (4.4±2.6 and 3.6±0.6 µg/g, respectively), while Protopic 0.03% yielded about two-fold lower levels (1.7±0.4 µg/g). The permeation rate through the skin of pimecrolimus from the 1% cream was about 6-fold lower than from the 0.1% tacrolimus ointment (0.67±0.35 vs. 4.1±0.7 ng/ml/hr). Comparing Elidel 1% with Protopic 0.03% (2.9±0.3 ng/ml/hr), pimecrolimus permeation was still lower by a factor of 4.3. To conclude, permeation of pimecrolimus from the 1% cream through human skin in vitro is considerably lower than that of tacrolimus from 0.1% and 0.03% ointments, despite the higher drug concentration in Elidel cream. Thus, the intrinsically lower permeation of pimecrolimus as compared to tacrolimus is also reflected in its lower permeation from the galenical formulations. These *in vitro* findings are in line with results from clinical studies.

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Pulsed dye laser treatment is very sufficient in recalcitrant viral warts

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Various therapeutical approaches including surgical and Co2 laser excision have been tried to remove verrucae vulgares, but a treatment of choice has not been developed. Therefore, over a period of 12 months, 56 patients (29 female, 27 male)with with recalcitrant verrucae vulgares on their hands or feet (1:2) were treated with a flashlamp pumped dye laser (FPDL) every 2 weeks until completly clearance. We used a laser energy desity of 8J/cm2, spot size 5 mm, pulse duration 450 microseconds. 14% cleared after 1 session and 44% after 2-5 sessions, so that a remission was seen in 58% after 5 treatment and additionally in 94% after 6-9 treatments. Only 3 patients failed and 1 patients stopped the therapy because of pain. There were no relapses in a period of 6 months after treatment. FPDL therapy is very effective and safety in treatment of viral warts.

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***In vivo* mechanism of action of corticosteroids in psoriatic plaques**

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Although topical corticosteroids (CSs) are the mainstay of psoriasis treatment, their mechanism of action is primarily based on in vitro studies. These demonstrate CS-induced, increased IKBα synthesis that leads to decreased NFκB activity. By different mechanisms, decreased activity of a variety of other inflammation-related nuclear transcription factors are also observed in vitro. In order to better understand which mechanisms are clinically relevant, we treated psoriatic plaques with clobetasol propionate 0.05% foam (OLUX®) twice daily for 2 weeks, assessed clinical response (target lesion scoring) and studied skin biopsies by immunocytochemistry and RT-PCR for a panel of inflammatory cytokines and chemokines many of which are overexpressed in plaques. Lab scientists were blinded to clinical outcome. The genes studied included IKBα, IKBκ. iNOS, granzyme B, TNFα, IFNγ, STAT-1, IL-20 and IL-8. CD3+ T cell infiltration, K-16 keratin and keratinocyte I-CAM-1 protein expression were studied by immunocytochemistry. We correlated gene expression and immunocytochemistry data with clinical response, T cell infiltration and decreases in epidermal thickness. Clinical response correlated best with decreased epidermal CD3+ T cell infiltration (r=0.65), increased IKBα (r=0.48) and decreased STAT-1 mRNA expression (r=0.43). Histologic response, (i.e., decreased epidermal thickness), correlated best with decreased K-16 protein and mRNA (r=0.88, r=0.67), ICAM-1 protein (r=0.84), iNOS (r=0.71), granzyme B (r=0.71), IL-20 (r=0.70), IL-8 (r=0.69) and STAT-1 (r=0.57) mRNA. Decreased total CD3+ T cell infiltration correlated best with decreased IKBβ (r=0.77) and STAT-1 (r=0.62) mRNA and ICAM-1 protein (r=0.63). The accumulated data suggest that although increasing IKBα expression is important in vitro, CS-induced effects on a panel of psoriasis-associated lymphocytes, cytokines and chemokines are also clinically relevant mechanisms of action and merit further investigation.

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Segmental hemangiomas are metameric: image analysis and classification

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Hemangiomas of infancy can be classified as "segmental", "indeterminate", and "localized". Some authors postulate that segmental hemangiomas are metameric. Our aim was to identify patterns of facial segmental hemangiomas by pattern analysis, and to define them using anatomic criteria. Digital images of hemangiomas from a cohort from 5 U.S. pediatric dermatology centers were utilized. Lesions were mapped onto a facial template. Frequently observed patterns were defined using anatomic landmarks. Three independent observers were given criteria for the proposed classification scheme with a diagram and text description. A series of 108 digital images (50% segmental and 50% indeterminate) were presented to each observer for site/segment classification. Inter-rater reliability was assessed using a kappa statistic. Four primary segmental patterns were identified. Two of these were easily recognizable as the accepted distribution of the maxillary (M2)and mandibular (M3) metameres. Two additional facial segments were identified: the laterotemporal segment (M1) and the frontonasal segment (M4). M1 (laterotemporal segment) encompassed the lateral forehead, anterior temporal scalp and lateral frontal scalp. M4 resembled the previously accepted frontonasal metamere, but differed significantly in its pattern. It includes the medial forehead, scalp, nasal bridge, nasal tip, ala, and philtrum, but in contrast to classic embryologic teaching, does not extend onto the lateral forehead. A very high degree of agreement was observed between 3 independent observers utilizing this classification schema with a kappa statistic = 0.845 for segmental hemangiomas and 0.863 for indeterminate hemangiomas. Our conclusions are that facial hemangioma segments were easily identified;inter-rater reliability was high; pattern analysis supports the metameric origin of hemangiomas; previous depiction of the frontonasal metamere may be incorrect; indeterminate hemangiomas represent partial (forme fruste) segmental HOI.

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Histone deacetylation and DNA methylation: novel epigenetic therapeutic targets in cutaneous T cell lymphomas

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Histone deacetylase (HDAC) inhibitors suppress the activities of multiple HDACs, leading to an increase in histone acetylation. This histone acetylation modulates the expression of specific genes that cause growth arrest, differentiation and apoptosis. DNA methyltransferase (DNMT) inhibitors reactivate tumor suppressor genes silenced by methylation-mediated mechanisms. Since histones are connected to DNA by both physical and functional interactions, the combination of HDAC inhibitors with demethylating agents has become attractive. Suberoylanilide hydroxamic acid (SAHA), an orally administered inhibitor of class I and II HDACs, is currently in phase II clinical trials for malignancies, including CTCL. 5-aza-2'-deoxycytidine (5-aza-CdR), a DNMT inhibitor, is also in phase II clinical trials for solid and hematological malignancies. In this study, we investigated the anti-tumor effects of SAHA, 5-aza-CdR, and their synergism in CTCL cell lines and/or freshly isolated PBL from patients with circulating atypical T-cells. SAHA treatment (2.5 & 5 µM for 48 hrs) induced dose-dependent apoptosis over vehicle control in all three CTCL cell lines: MJ (9 & 24%), Hut78 (40 & 56%), and HH (61 & 75%). SAHA (0.5-5 µM for 48 hrs) also induced apoptosis up to 59% in 13 of 14 CTCL patients' PBL. SAHA treatment (2.5 µM for 24 hrs) resulted in the accumulation of acetylated histones (H2B, H3, and H4) and an increase in expression of the cyclin-dependent kinase inhibitor p21 protein in Hut78 and HH cells. Gene profiling of mRNAs induced by SAHA in HH cells confirmed the up-regulation of p21. 5-aza-CdR (0.1-10 µM for 48 hrs) also induced dose-dependent apoptosis in three CTCL cell lines and was synergistic with sub-therapeutic concentrations of SAHA (0.5-1 µM) resulting in apoptosis of 46-70% in the HH cell line. In conclusion, both SAHA and 5-aza-CdR induce apoptosis with synergism in CTCL cells. Our findings thus provide a rationale for using SAHA or 5-aza-CdR alone and their combination as novel treatments for CTCL.

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Skin permeability in pigs is less for pimecrolimus than for tacrolimus under normal and diseased conditions

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The barrier function of the skin plays a pivotal role in the percutaneous absorption of epicutaneously applied drugs. Various inflammatory conditions are associated with an impaired skin barrier and can therefore result in increased skin permeability and consequent enhanced systemic exposure to topically applied drugs. In a side-by-side comparison we evaluated the penetration and permeation of pimecrolimus and tacrolimus in normal and inflamed, barrier-disturbed porcine skin. Inflammation was induced locally by a 48-hr occlusive treatment with 5% sodium lauryl sulfate prior to dissection of skin samples. These specimens and and normal pig skin were then used in Franz-type diffusion chambers *in vitro*. Both compounds were applied epicutaneously at 1.0%, prepared in propylene glycol/oleyl alcohol (9:1). While inflammation did not significantly enhance the levels of the two drugs in the skin, impaired barrier function was associated with immediate passing through of the compounds (as opposed to a latency period of approx. 17 hrs in normal skin) and a significantly higher permeation rate. Although the permeation rates were increased for both compounds, the rate for pimecrolimus was 2.5 times lower than for tacrolimus. Thus, at 24 hrs pimecrolimus concentrations in the receptor fluid were 2.8-fold lower than tacrolimus levels, when using inflamed skin. The permeation rate of pimecrolimus through irritated skin was similar to the rate of tacrolimus through normal skin (173 vs. 166 ng/ml/hr). The present *in vitro* data suggest that in diseased skin, permeation of pimecrolimus might be lower than that of tacrolimus, particularly in atopic dermatitis patients, whose skin barrier function is typically impaired.

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***Phaeodactylum tricornutum* extract protection effect on human stratum corneum oxidized proteins level**

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Modification of proteins by reactive oxygen species is implicated in different disorders. The proteasome is a multicatalytic proteinase in charge of intracellular protein turnover and of oxidized proteins degradation. Consequently, proteasome function is very important in controlling the level of altered proteins in eukaryotic cells. Evidence for a decline in proteasome activity during skin photo-aging has been provided by us in Bulteau et al. (2002). We previously described the ability of a lipid algae extract (Phaeodactylum tricornutum) to stimulate 20S proteasome peptidase activities (Nizard et al. 2002). Furthermore, keratinocytes treated with Phaeodactylum tricornutum extract and then UVA and UVB irradiated, exhibited a sustained level of proteasome activitiy comparable to the one of non-irradiated cells. The level of modified proteins can be quantified by measurement of protein carbonyl content (Oxyblot technique), which has been shown to increase with aging and other disorders. Here, we describe that, in the presence of this lipid algae extract, the level of oxidized proteins is reduced as assessed by the Oxyblot technique. These results are obtained both with culture human keratinocytes and stratum corneum skin cells (obtained by stripping) from human volunteers. Altogether, these results argue for the presence of compounds in this algae extract that have a stimulating and/or protective effect on proteasome activity resulting in a decrease level of protein oxidation.

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Presence of "inflammatory" dendritic cells in psoriasis vulgaris lesions and modulation by Efalizumab (anti-CD11a)

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Dendritic antigen presenting cells (DCs) may be key inflammation-inducing cells in psoriasis vulgaris. In this study, we quantified the number of CD1a+, CD11c+, and CD83+ DCs in normal vs. psoriatic skin and also determined the impact of efalizumab on DCs. In lesional skin we identified a 54-fold increase in CD83+ DCs ($p<10^{-10}$) and a 5-fold increase in CD11c+ DCs ($p<10^{-10}$). The overall number of infiltrating DCs in psoriasis lesions is similar to T-cell infiltrates, with 407 CD11c+ cells as compared to 335 CD3+ cells per analysis field. "Inflammatory" DCs were located in both epidermis and dermis of psoriasis plaques. Patients who responded well to treatment had an 88% reduction in CD83+ cells ($p<10^{-3}$) and 56% decrease in CD11c+ cells ($p<10^{-3}$) within skin lesions after 8 weeks of efalizumab therapy. Reduction in CD11c+ and CD83+ cells correlate well with a response score composed of difference in epidermal acanthosis and keratin 16 immunohistochemistry at the end of treatment (r=0.75 and 0.53, respectively). Surprisingly, disease improvement is more correlated with changes in "activated" dendritic cell subsets than overall changes in lesion-infiltrating T-cells (r=0.40), with the exception of the CD8+ T-cell subset, the reduction of which correlated well with disease improvement (r=0.52). These results suggest that dendritic cells may have important direct effects in psoriasis disease pathogenesis and may be significantly impacted even with presumptive "T-cell-targeted" therapeutics.

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Sub-cellular high resolution optical coherence tomography: a novel non-invasive diagnostic tool in dermatology

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Using low coherent superluminescent diodes, conventional optical coherence tomography (OCT) non-invasively generates two dimensional microscopical tomograms of human skin *in vivo*. Tomograms are available almost in real time with an axial resolution of 15 µm and a lateral one of 12 µm. Here, we used the OCT technique for the preoperative characterization of different skin tumors, e.g. basal cell carcinoma and bowen's disease. We could demonstrate the lateral tumor borders and pathological changes in tumor vascularization. However, currently available conventional OCT can not visualize the basement membrane zone (BMZ) nor single cells. Demonstration of the BMZ, however, is crucial to differ between precarcinomatous and invasive skin tumors, the latter ones usually showing penetration of malignant cells through the BMZ. Thus, the success of modern non-invasive therapeutic strategies, e.g. immunomodulators or photodynamic therapy, to treat precarcinomatous skin tumors could not be visualized non-invasively to date. Further, we are currently not able to show if these therapies indeed lead to complete tumor eradication without taking a skin biopsy. To overcome the aforementioned problems, we have developed a refined high resolution OCT system (HR-OCT) using a broadband femtosecond Ti sapphire laser. Hereby, we accomplished a sub-cellular resolution of 3.1 µm when studying human three dimensional skin equivalents in different states of differentiation. Results were confirmed by histological examination and immunohistochemical analysis, indicating that HR-OCT is a powerful tool to visualize and characterize the BMZ as well as a single epidermal keratinocyte layer. Consequently, HR-OCT is expected to provide us with *in vivo*-insights into the skin to reliably diagnose malignant tumors non-invasively and to confirm the success of non-invasive treatment strategies.

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Treatment of superficial basal cell carcinoma with 595 nm pulsed-dye laser: a clinical and histological study

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Basal cell carcinoma (BCC) is the most common skin malignancy in Caucasians. The pulsed-dye laser selectively destroys vessels using hemoglobin as a chromophore. The objective of this study is to determine the therapeutic response of 595nm pulsed-dye laser (V-Beam laser, Candela Corp., Wayland, MA) on BCC. Clinical and histological responses after laser exposure were studied in 24 superficial BCCs (in 14 patients who had previous BCCs) using 4 sets of laser parameters varying in energy (J/cm2) and cooling mode (DCD on/off): A) 3 J/cm2, DCD on, B) 7 J/cm2, DCD on, C) 15 J/cm2, DCD on, and D) 15 J/cm2, DCD off. Pulse duration 3 msec, spot size 7 mm, one pass of laser, 10% overlapping pulses, and 4 mm treatment margins were used in all groups. At 3-8 weeks after laser exposure, the treated BCCs were clinically evaluated, excised, and examined histologically. Clinical response was evaluated as: no change, flattening, less erythema, or disappearance of tumor. The clinical response was: group A no change (100%), group B no change (50%), and slight flattening and less erythema (50%), group C marked flattening and less erythema (28.6%), and slight flattening and less erythema (71.4%), and group D disappearance of tumor (100%). Therapeutic response was determined by histological findings and graded as: failure (tumor seen) or success (no tumor seen). The therapeutic response was success in 0% of group A and B, 28.6% of group C, and 100% of group D. Pulsed-dye laser, 595 nm, 15 J/cm2 without cooling mode, provides a significant therapeutic response with histological proof of absence of tumor for superficial BCC.

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Low-energy helium-neon laser irradiation induces repigmentation in segmental-type vitiligo lesions and has some biostimulatory effects on nerve repair and control

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Segmental-type vitiligo (SV) results from the dysfunction of sympathetic nerves in the affected areas. Its recalcitrant treatment response is indeed an ardous challenge for all dermatologist. Low-energy helium-neon laser (He-Ne laser) has been employed in a variety of clinical treatments including vitiligo management and repair of nerve injury. The purpose of this study is to determine the effectiveness of He-Ne laser in treating SV and its biostimulatory effect on nerve repair and control. Forty patients with SV on the head and/or neck were enrolled in this study. He-Ne laser light was administered locally at 3.0 Joules/cm^2 with point stimulation once or twice weekly. Laser Doppler flowmetry and iontophoresis with sympathomimetic drugs were used for evaluating the cutaneous blood flow and adrenoceptor function. After an average of 17 treatment sessions, initial repigmentation was noticed. Marked repigmentation (>50%) was observed in 60% of patients with successive treatments. Tendency of gradually normalizing cutaneous blood flow and adrenoceptor responses were found in 6 patients after regular He-Ne laser treatment. So we concluded that He-Ne laser can effectively treat SV and have some repairing effect on damaged sympathetic nerve.

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Perilesional versus lesional skin changes in senile lentigo

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Senile lentigo (SL) (lentigo senilis, aging spot, liver spot, solar lentigine) is a common component of photoaged skin. It is characterized by hyperpigmented macules which affects chronically irradiated skin mostly after age 50. This study was undertaken to assess the basic morphology of SL on dorsum of hands. A systematic comparison between lesional vs perilesional skin using immunohistochemistry and electron microscopy was done to detect precursor lesions of SL and to determine whether melanocytes or keratinocytes were first affected in the evolution of lesions. In 12 cases studied, the main findings show that clusters of perilesional keratinocytes accumulate melanin in large melanosomial complexes, and that melanocytes counts are increased respective to total length of section in lesional skin, but the increment is probably due to the development of characteristic epidermal rete ridges. Melanocytes had overall a normal ultrastructure, with mostly quiescent features in perilesional skin, and melanosomial transport seeming more active in lesional skin. Dermal changes were comparable between lesional and perilesional skin. Our data indicate that SL may represent a loss of epidermal melanin unit homeostasia due to chronic irradiation, where keratinocytic changes predominate over melanocytic changes. Non -UV related similar histopathologic changes are noted in monogenic Dowling Degos disease, suggesting common pathogenetic pathways. We hypothesize that abnormal pigment retention in keratinocytes is the primary defect in SL, which may partly explain the therapeutic effect of retinoids.

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SCORTEN accurately predicts mortality in toxic epidermal necrolysis patients in the United States

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Toxic epidermal necrolysis (TEN) is a severe hypersensitivity reaction to certain medications, with associated mortality upwards of 40%. SCORTEN, a TEN-specific illness severity score, which predicts mortality based on 7 criteria: blood urea nitrogen, glucose, carbon dioxide, age, malignancy, body surface area involved, and pulse, has been reported in Europe to accurately predict mortality, but has not been studied in the United State. Therefore, we retrospectively analyzed our population of patients with TEN prior to determine if SCORTEN would accurately predict the mortality in these patients and determine whether SCORTEN is applicable to patients in the United States.

Twenty four patients were treated in our intensive care unit with supportive care, including wound care, nutritional support, fluid and electrolyte monitoring, and prevention of infection. There were 16 women and 8 men with an average age of 52.7 years. Eight were Hispanic, 9 were Black and 7 were White. There were 5 SJS/TEN overlap patients, 11 TEN with spots, and 8 TEN. For each patient, the 7 SCORTEN risk factors, the causes of TEN and other medical problems were documented. Based on SCORTEN, 8.77 patients were expected to die. Of the 24 patients, 8 patients died. The expected and actual mortalities were compared using Standardized Mortality Ratio (SMR) (SMR=(?observed deaths/?expected deaths) x 100) to determine the applicability of SCORTEN to a United States population of TEN patients. Using SMR, we determined that our TEN patients had a mortality risk of 9% less than the original SCORTEN patients, which was not found to be statistically significant (SMR 0.912, 95%CI 0.393-1.8). Based on the comparison of expected and actual mortalities using SMR, We have found that SCORTEN is applicable to a United States population of TEN patients.

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Collagen-like peptide exhibits a remarkable anti-wrinkle effect on the skin when topically applied: *in vivo* study

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Collagen is an essential molecule that plays a crucial role in skin regeneration, wound healing, and in maintaining the structural integrity of the skin. Moreover, collagen synthesis and degradation play a central role in skin appearance and in wrinkle formation caused by aging and photoaging. Consequently, in the following

double-blind clinical study, we were interested in developing a synthetic oligo collagen-like peptide and evaluating its effect on wrinkles. 20 healthy women volunteers, age 40 to 62, participated in the study. Volunteers applied either a gel formula containing 3% of this collagen-peptide and 1% of a booster molecule that stimulates general cell metabolism with no specific effect on wrinkles, or a placebo gel, on the eye zone area twice a day for 4 weeks. Control visits were performed at the beginning and end of the study. Skin wrinkles were evaluated clinically and by silicon replica analysis followed by statistical treatment using the matched paired student test. Silicon replica results showed that application of the collagen-peptide on the skin significantly reduced the total surface of wrinkles, and this effect was observed in 75% of the replicas. Similarly, according to student test, the decrease in number and average depth of wrinkles was significant, observed in 65% and 70% of the replicas, respectively. The effect of collagen-peptide on reducing the total length of wrinkles was also remarkable. This effect was estimated highly significant by student test, and was observed in 75% of the replicas. Moreover, these results were supported by volunteer questionnaires and clinical observation. This study reveals that the rapid effect of collagen-like peptide on reducing skin wrinkles, within only four weeks of application, is remarkable and very significant. The results also demonstrate that collagen-like peptide acts deeply and intensely on wrinkles, properties that are of great interest in the field of anti-aging skin care products.

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Synergistic inhibition of T-cell proliferation in human peripheral blood mononuclear cells by combinations of pimecrolimus with corticosteroids

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T-cell activation plays a key role in inflammatory skin diseases, such as atopic dermatitis and psoriasis, as well as in other chronic inflammatory conditions. Although corticosteroids have been the mainstay of therapy of these indications, corticosteroid resistance has been reported frequently. Pimecrolimus is an ascomycin macrolactam derivative specifically designed and developed to treat inflammatory skin diseases. In this study we examined the effects of corticosteroids and pimecrolimus on T-cell proliferation in strongly stimulated human peripheral blood mononuclear cells (PBMC). PBMC (50,000—200,000 cells/well in a 96-well plate) were stimulated with the superantigen staphylococcal enterotoxin B (SEB) or with the combination of anti-CD3 plus anti-CD28 monoclonal antibodies for 72 hr, and proliferation was measured via incorporation of 5-bromo-2′-deoxyuridine (BrdU). SEB- and anti-CD3/CD28-stimulated T-cell systems have been documented to exhibit resistance to corticosteroids and/or calcineurin inhibitors. Indeed, when used as single agents in the present study, the corticosteroids dexamethasone (Dex) at 300 nM, betamethasone 17-valerate (Beta) at 300 nM and hydrocortisone (HC) at 10,000 nM, as well as pimecrolimus (PI) at 30 nM exerted, at most, only partial inhibitory effects on T-cell proliferation. However, combinations of the corticosteroids with pimecrolimus (at the concentrations indicated above) exhibited strong and synergistic inhibition of proliferation. The following ranges of inhibition (stimulated control = 0%) were observed: SEB-induced proliferation: Dex (≤14%), Beta (≤15%), HC (≤11%), PI (4—33%), Dex+PI (94—98%), Beta+PI (93—97%), HC+PI (93—94%). Anti-CD3+anti-CD28-induced proliferation: Dex (13—14%), Beta (≤25%), HC (≤8%), PI (≤21%), Dex+PI (82—95%); Beta+PI (70—95%), HC+PI (61—87%). These results suggest that combination therapy may be effective for the case of corticosteroid resistance and/or indications, where corticosteroid or pimecrolimus monotherapy is insufficient.

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Quercus suber cork extract displays tensor and smoothing effect on human skin: *in vivo* study

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Recently, it has become indispensable for anti-aging active ingredients to give a lifted and renewed tension to the skin in order to offer a visible and immediate smoothing anti-wrinkle effect. Quercus suber is the aliphatic part of suberin, the most important structural component of cork cell walls. Studies have shown that Quercus suber is made up mostly of hydroxycarbonylic acids and is endowed with many special mechanical and chemical properties which evoke a possible smoothing effect on the surface of the skin. Therefore, we were interested in investigating the effect of cork extract on the skin surface in the following double blind clinical study. The study was conducted on 15 healthy women, age 22 to 52. The volunteers applied a gel formula with 3% of cork extract, or placebo gel, on a delimited zone of 30cm2 on each forearm. Skin surface roughness was evaluated visually by pictures, and by silicon replicas one and two hours after application, followed by statistical treatment using matched paired McNemar statistical test. McNemar treatment of the pictures revealed that application of cork extract on the skin resulted in a very significant reduction of roughness after one hour of application. This effect was observed in 73.3% of volunteers. After two hours of cork extract application, a highly significant improvement of skin roughness was found in 78.6% of volunteers. Moreover, silicon replica treatment confirmed the significant improvement of the average of roughness at two hours. These results demonstrate that cork extract provides for a remarkable and very significant tensor and smoothing effect on the skin, which can be of great use in anti-aging skin care products.

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The efficacy of etanercept in psoriasis patients with varying treatment histories

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Psoriasis patients with a history of systemic therapy or phototherapy may represent a cohort with unique baseline demographics and disease characteristics. The purpose of this analysis was to determine if such patients respond differently to etanercept therapy than those without a history of systemic therapy or phototherapy. Patients from 3 placebo-controlled, randomized studies were pooled. The 415 psoriasis patients included in this analysis initiated etanercept 25 mg twice weekly after a washout of systemic therapy and phototherapy. Patients with a history of systemic therapy or phototherapy had more severe psoriasis at baseline than those without such a history, as indicated by greater body surface area involved (30% vs 21%, p<0.01), longer duration of disease (21 vs 17 yrs, p<0.01), higher Psoriasis Area and Severity Index [PASI] score (19.2 vs 16.0, p<0.01), higher Dermatology Life Quality Index [DLQI] score (11.9 vs 10.4, p<0.01), and higher proportion of patients with psoriatic arthritis (28% vs 16%, p<0.05). Despite different baseline characteristics, comparable proportions of patients with and without a history of systemic therapy or phototherapy achieved a PASI 50 response with etanercept therapy at week 12 (62% vs 64%, not significant [ns]) and week 24 (72% vs 83%, ns). PASI 75 response rates also were comparable at week 12 (33% vs 34%, ns) and week 24 (45% vs 50%, ns), as were mean percent improvements in DLQI at week 12 (60% vs 54%, ns) and week 24 (64% vs 61%, ns). Patients with prior use of methotrexate had more severe disease at baseline than those without such a history, but all efficacy endpoints indicated the magnitude of improvement with etanercept therapy was similar. Despite having more severe disease at baseline, psoriasis patients with a history of systemic therapy or phototherapy were able to achieve a response to etanercept therapy comparable with patients without such a history.

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A novel therapy for tumors of epithelial origin

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Mammalian cells are protected from malignant transformation via adaptive DNA damage responses resulting in senescence or apoptosis. We hypothesize that in normal cells, DNA damage disrupts the telomere loop, exposing the single-stranded TTAGGG repeat sequence of the 3' telomere overhang, otherwise concealed in the telomere loop structure. We further hypothesize that the overhang then interacts with a nuclear sensor protein to initiate senescence or apoptosis, responses abrogated in malignant cells. To examine our hypothesis, 40uM of GTTAGGGTTAG, an oligonucleotide homologous to the 3' overhang sequence (T-oligo), or its complementary sequence or diluent alone as controls were provided to well differentiated (MCF-7) or poorly differentiated (BT-20) epithelial carcinoma lines. Within 3-7 days, T-oligo decreased MCF-7 yield by 64±17% (p<0.008) and BT-20 yield by 56±18% (p<0.03), an effect comparable to cisplatin (10uM). T-oligo induced apoptosis of cells and senescence of the remaining cells, as determined respectively by TUNEL and induction of the senescence-associated β galactosidase activity (MCF-7) and inability to phosphorylate the retinoblastoma protein despite fresh medium supplementation (BT-20). By western blot analysis, within 24-48 hrs T-oligo induced ATM (480%), p53 (2451%), p95/Nbs1 (557%) and BRCA1 phosphorylation (243%), as well as p53 (545%) and p21 (205%) level, suggesting that T-oligo acts through the ATM kinase and its effector proteins, a known DNA damage response pathway. Furthermore, T-oligo decreased yields of adriamycin resistant, multidrug resistant gene-1 (MDR1)-overexpressing MCF-7 cells by 53%, decreased MDR1 expression by 47% and induced differentiation-associated gene products in BT-20 cells by 356% to 1563%. Moreover, T-oligo administered intralesionally to MCF-7 tumors implanted into the flanks of athymic nude mice reduced tumor volume by 88% (p<0.003) without detectable toxicity. We propose that T-oligo activates physiologic DNA damage signaling pathways ordinarily blocked in malignant cells and is thus a promising novel cancer therapy.

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Ultra pure medical grade lanolin enhances wound healing

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Ultra pure medical grade lanolin has been designed for the treatment of superficial wounds and a range of dermatological disorders. The purpose of this clinical research study was to evaluate ultra pure medical grade lanolin versus white petrolatum (USP), the "gold standard" for promoting healing of minor wounds, on the healing rate of cantharidin induced blisters. Cantharidin blisters are intra-epidermal clefts, which form above the basement membrane and are a prime example of common superficial wounds. Twenty-four hours after blister induction on the forearm, the blisters were de-roofed, no treatment (control), ultra pure medical grade lanolin, and white petrolatum (USP) were inoculated directly onto the blisters b.i.d. and covered until day 4. The wounds were treated b.i.d. and left uncovered through day 10. Photographs were taken on days 3, 6, 7, 8, 9, and 10 using a Hi-scope 20x prior to morning treatment. There were no adverse events and all subjects completed the study. As early as day 2 the ultra pure medical grade lanolin treated blisters were less swollen and less inflamed as compared to the white petrolatum (USP) treated blisters or the controls. By day 8, glyphic lines were becoming visible in some subjects treated with the ultra pure medical grade lanolin. Glyphic lines are a measure of the stratum corneum barrier restoration. It was evident by day 10 that glyphic lines were more developed with the ultra pure medical grade lanolin treatment as compared to the white petrolatum (USP) or the untreated control. It can be concluded from this data that ultra pure medical grade lanolin is far superior at promoting superficial wound healing than white petrolatum (USP). Future experiments will include a full stratum corneum restoration (3 week) study evidenced by return of TEWL to baseline and original geographic pattern of intersecting furrows.

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Study of skin penetration of vitamin E submicron emulsion

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Alpha-tocopherol is a lipophilic vitamin that exhibits an antiaging, antioxidative activity and antiphotodamage.The particle diameter of tocopherol submicron emulsion is within 200 nanometer. In our study, we focus on the penetrating capacity of vitamin E submicron emulsion through rabbit skin.Tocopherol-loaded submicron emulsion was prepared by high pressure homogenization and the concentration was measured by HPLC method. The penetrating capacity test was conducted on the rabbit skin with the experimental device. The absorbance of collected samples at different intervals was measured by spectrophotometry. The sample concentration was determined according to vitamin E standard curve. The penetrating accumulation was calculated by the specific formula and the penetrating coefficient was evaluated by regression equation between the accumulation and the different intervals.The regression equation of vitamin E standard curve was $y = 0.0241x + 0.0128$, $R2 = 0.9994$. The absorbance of samples at different intervals was 0.113 at 0.5h and increased gradually,up to 0.386 at 24 h. The corresponding concentration and accumulation was also increasing with experimental duration. The regression equation between the accumulation and the time was $y=30.059x + 231.64$, $R2 = 0.9907$; the penetrating coefficient was 30.059.Tocopherol-loaded submicron-emulsion is of better skin penetrating capacity because of its perfect vehicle approach and its small particle diameter. The sample concentration and the accumulation penetrating through rabbit skin is time-dependent. The penetrating efficiency of vitamin E submicron emulsion is stable.

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Isolation of quinolone resistant ureaplasma urealyticum and identification of gene mutations in quinolone resistant determining regions

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Quinolones are often used for clinical therapy of UU (Ureaplasma Urealyticum) infection, but are not all effective because of drug-resistant isolates. This research was designed to isolate quinolone-resistant UU from clinical isolates and further to determine the gene mutations in QRDRs (quinolone-resistant determining regions) in such resistant isolates. Drug susceptibility test kits and broth dilution method were used to determine the quinolone-resistant UU isolates from 66 clinical isolates. PCR was performed by two pairs of specific primers to amplify gyrA and parC, two major genes in QRDRs, and the expected amplicons for gyrA parC were 336bp and 309bp respectively. The positive amplicons were sequenced .Of 34 tested isolates, 10 were resistant to both OFX (ofloxacin) and SPX (sparfloxacin), 15 only resistant to OFX and 1 resistant to SPX. Of another 10 tested isolates, 6 were resistant to CFX (ciprofloxin), and 3 resistant to SPX. For 4 kinds of quinolones tested, MICs of UU3 reference strain were 1μg/ml for LFX (levofloxacin), 4μg/ml for CFX, 8μg/ml for NFX and 2μg/ml for SPX. MICs of 2 out of 22 UU isolates were found four times higher than those of the reference strain and were considered as resistant ones. After PCR the amplified fragments with 336bp (gyrA) and 309bp (parC) were observed .The sequenced results revealed a T to C change at 165nt of gyrA in 2 isolates. It can be implied that resistance of UU to quinolones may have some relation to the gene mutations in QRDRs such as gyrA and/or parC. The results of drug susceptibility tests are important for clinical therapy of UU infection because of drug-resistant isolates. And drug susceptibility test kits are easier and more convenient to apply than broth dilution method for further and wider usage.

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Contact sensitivity in patients with leg ulcerations: a North American study

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Over the last two decades, there have been a number of studies in Europe on contact sensitivity in patients with chronic leg ulcerations. The frequency of positive patch test results has ranged from 40 to 82.5%. Although several groups have studied this in Europe, the prevalence of sensitization has not been studied in North America. Furthermore, many of the newer dressings and wound care products in the market have not been studied for contact sensitivity in patients with chronic wounds. Our objectives were to 1) determine the prevalence of allergen sensitivity in patients with past or present leg ulcers in two North American study centers, 2) compare our results to the European studies and the North American Contact Dermatitis Group (NACDG) database and 3) help delineate a standard battery of allergens for patch testing in North American leg ulcer patients. Fifty-four patients with a history of or an active leg ulcer were prospectively entered in the study. The patients were patch tested to both the NACDG Standard series, as well as, a comprehensive supplemental series of 48 allergens. 63% (34) of patients had one or multiple positive patch tests and 37% (20) had no positive patch tests. The most common allergens were Balsam of Peru (29.6%), bacitracin (24.1%), fragrance mix (20.4%), wood tar mix (20.4%), propylene glycol (13.5%), neomycin sulfate (13%), benzalkonium chloride (13%), carba mix (11.1%), nickel sulfate (11.1%) and Duoderm CGF (11.1%). Duoderm CGF was the most allergenic dressing in our study group. There is a high incidence of positive patch tests in patients with past or current leg ulcerations. Our results were comparable to similar studies done in Europe. The incidence of the most common allergens in our patient population was higher than those seen in the NACDG, with the exception of nickel.

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Photoaging and expression of retinoic acid receptor α

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Cutaneous aging results from the passage of time and from chronic sun exposure (photoaging). We have previously shown that retinoic acid receptor α (RARα) is significantly increased in intrinsically aged skin. We investigated whether photoaging was associated with alterations in RARα and if topical all-*trans* retinoic acid (t-RA), a treatment for photoaged skin, modulates RARα expression.

Subjects with clinically moderate photoaging were recruited. In cohort 1 (n=10; age range: 33-65 yrs), 4mm punch biopsies were taken under local anesthesia from: photoaged extensor forearm, photoprotected hip and upper inner arm. In cohort 2 (n=6; age range: 40-51 yrs) Finn chambers containing either 0.025% t-RA (in vehicle: 70% ethanol/30% propylene glycol) or vehicle alone were applied to photoaged extensor forearm. At day 4, 3mm biopsies were taken from the 2 treated sites and from a third untreated area. Biopsies were processed and 10μm frozen sections prepared for either *in situ* hybridization or immunohistochemistry. RARα mRNA was identified using a biotin-labeled oligo probe. Protein was identified using standard immunoperoxidase techniques (rabbit anti-human RARα; Santa Cruz Biotechnology Inc). Sections were randomized, blinded and quantified (3 high power fields/section; 3 sections/site). RARα mRNA was not identified in photoprotected sites but expressed in photoaged forearms in all volunteers studied (n=10; p<0.01). Photoaged skin contained significantly higher numbers of RARα-positive keratinocytes than photoprotected sites (hip: 45.9±5.5; upper inner arm: 45.6±5.4; forearm: 93.3±9.3; p<0.001). Topical application of t-RA significantly reduced RARα protein levels as compared to control and vehicle-treated sites (control: 77.6±8.4; vehicle: 66.9±11.9; t-RA: 35.1±6.5; p<0.01).

These results demonstrate that RARα is over-expressed in the epidermis of photoaged as well as intrinsically aged skin. Furthermore, topical application of t-RA significantly reduces RARα expression. We hypothesise that alterations in RARα expression may be a common pathogenic mechanism in intrinsic and extrinsic aging.

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Proliferation and apoptosis are inversely present in acute chronic wounds

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The edges of chronic wounds typically show acanthosis with the absence of epidermal migration, and there are questions about the migratory and proliferative activity of keratinocytes in chronic wounds. In order to determine the status and contribution of keratinocyte proliferation and apoptosis to the wound healing process, we performed immunohistochemistry for a proliferation marker (Ki-67) and apoptosis (TUNEL) on specimens obtained from the edges of acute wounds and chronic ulcers. Eighteen patients with chronic venous leg ulcers underwent biopsies of normal thigh skin and chronic ulcers, and the initial thigh biopsy sites were re-biopsied after 48 hours to create an acute wound. In eight patients the ulcers were re-biopsied after 3 weeks. The specimens were routinely processed for histology and immunohistochemistry. Immunostaining for Ki-67 was uniformly increased after wounding, with marked sparing of the advancing epidermal edge of acute wounds. However, Ki-67 immunostaining was dramatically increased in non-healing ulcers. Apoptosis staining was very high at baseline in normal skin, and slightly increased after wounding. It was lower at baseline in chronic ulcers but increased at the advancing epidermal edge. With wounding, there was increased keratinocyte migration. The migrating keratinocytes closest to the edge of the wound, however, showed decreased proliferation as demonstrated by Ki-67 immunostaining. Contrary to what is commonly thought, chronic wound keratinocytes are actively proliferating. Keratinocyte apoptosis continues and in fact increases during healing, suggesting that it is part of the normal mechanism for wound repair. We propose a model for epithelialization of chronic wounds, whereby the increased keratinocyte proliferation and the decreased apoptosis of the wound edges have to become inverse, so as to approximate acute wounds.

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Bexarotene induces apoptosis of malignant T-cells purified from Sezary syndrome patients and inhibits IL-4 production: variable susceptibility correlates with clinical response

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The RXR specific retinoid bexarotene, which is efficacious in the treatment of cutaneous T-cell lymphoma (CTCL), has been shown to induce apoptosis in long term transformed cell lines. In an effort to determine if Sezary T-cells (CD4+/CD26-) exhibit the same sensitivity to bexarotene, purified malignant T-cells isolated from the peripheral blood of Sezary syndrome (SS) patients were cultured with bexarotene followed by the performance of a TUNEL assay using flow cytometric analysis. Dose-dependent apoptosis of malignant T-cells peaked following 72 hours of culture with bexarotene. Interferon alpha, another active agent for CTCL, although inducing apoptosis of malignant T-cells itself, failed to increase levels of apoptosis when co-cultured with bexarotene. T-cells from 66% of SS patients (N=9) repeatedly manifested high levels of apoptosis after culture with bexarotene while cells from 33% of patients consistently failed to demonstrate apoptosis on repeat assay. Similarly, significant inhibition of in vitro production of IL-4 in response to mitogens, which correlated with susceptibility to apoptosis, was observed following 48 hours of culture with bexarotene. Clinical response of patients to systemic bexarotene therapy appeared to correlate with in vitro susceptibility to apoptosis. Thus, bexarotene exhibits the ability to suppress IL-4 production and to induce apoptosis in freshly isolated malignant T-cells from SS patients, which correlates with response to therapy.

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Double-blind, vehicle-controlled study to evaluate the clinical, histological and confocal microscopy effects during 4 weeks of treatment for actinic keratoses with imiquimod 5% cream

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The primary objective of this study is to assess the clinical, histological and confocal microscopy effects of actinic keratoses (AK) lesions treated with imiquimod 5% cream or vehicle cream once daily 3 times per week for 4 weeks. We studied 16 male subjects with histologically confirmed AK. Subjects determined to be eligible were randomized to imiquimod or vehicle cream in a 3:1 ratio. Both creams were supplied in a single-use sachet each with 250mg of cream. Each imiquimod sachet contained 12.5mg of active drug. Subjects applied 1 sachet of study cream once daily 3 times per week, for 4 weeks to a defined contiguous 25cm^2 treatment area on the balding scalp. Each subject was seen weekly during treatment period and then 4 weeks after treatment. During the treatment period each subject underwent clinical, histological and confocal microscopy exams. At the 4 week post-treatment visit the subjects underwent clinical and confocal microscopy evaluations. For those subjects using imiquimod, a significant clinical, histological and confocal microscopy improvement was noted. Results indicate that imiquimod 5% cream is an effective treatment option for AK.

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Embryonic mouse stem cell migration in the presence of stem cell factor and bioengineered skin

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Bioengineered skin substitutes have been widely used in the treatment of chronic wounds, but their mechanisms of action are not completely understood. These constructs are able to synthesize and release growth factors and cytokines, and are responsible for their stimulation of wound repair. Chemotaxis is thought to be involved in many biological processes, including healing, inflammation, immune responses, and embryonic morphogenesis. In this study we used a modified Boyden chamber assay to assess murine embryonic stem cell migration and how it might be affected by a living bilayered skin substitute (BSC), consisting of neonatal foreskin keratinocytes and fibroblasts. The lower well of the modified Boyden chamber contained the BSC with and without stem cell factor (SCF), and was separated from the upper chamber by a polycarbonate membrane with a pore size of 8-$_{\mu}$m. To evaluate the migration potential of embryonic stem cells (ESC) they were cultured on a STO feeder layer, separated, and then plated as 10^5 cells per membrane. After overnight incubation, cells on top of the membranes were scraped and removed, and the remaining cells on the bottom side of the membranes were fixed, stained, and then counted using light microscopy. We found up to a 5-fold increase in ESC migration from the upper to the lower chamber where BSC was placed. The addition of SCF (10,50 and 100ng/ml) prevented the migration of ESC in a dose dependent manner. We conclude that another mechanism of action of skin substitutes may be recruitment of primordial cells to the site of injury.

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Uncovering histological criteria with prognostic significance in toxic epidermal necrolysis

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Toxic epidermal necrolysis (TEN), a frequently lethal disease, usually represents an adverse drug reaction. While extensive epidermal cell death is always present, a role for immunocytes is unclear. A search of burn unit database (1992-present) yielded 34 patients with at least 30% surface area sloughing who underwent punch biopsy of skin immediately following admission. Records and histology slides were reviewed and the following clinical data extracted: age, %TBSA slough at admission, serum glucose, bicarbonate and BUN values. Each biopsy included a portion of denuded skin as well as adjacent non-denuded skin. Routinely stained sections were reviewed by two investigators blinded to clinical data. Extent of dermal inflammation was evaluated at least 2 high power fields (HPF) away from the perimeter of epidermal detachment, and 5 HPFs were graded as either: sparse (164+/-34 cells/HPF, n=14), moderate (273 +/-76 cells/HPF, n=15), or extensive (392 +/-141 cells/HPF, n=5). SCORTEN values were calculated and patients categorized as alive or dead during hospitalization. All patients were treated in a uniform fashion by an experienced team of physicians and nurses. There was good concordance between observers in rating the extent of inflammation (p=0.0005). While only 22% of patients with sparse inflammation died, 53% with moderate inflammation died, and 80% with extensive inflammation died. Optimal data analysis used to predict patient outcome with the leave-one-out method revealed similar classification accuracy for the current gold standard SCORTEN (71%), and histological grade of inflammation (65%). We conclude: a) there is a histological spectrum of TEN ranging from a pauci-cellular to extensive dermal inflammatory infiltrate, and b) the degree of cutaneous inflammation in peri-lesional skin predicts clinical outcome as well as SCORTEN. Future clinical trials should consider the possibility that various patient subsets exist within TEN, and a role for immunocytes in TEN needs to be critically re-evaluated in this devastating disease.

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Topical treatment with collagenase accelerates healing in a new model of full thickness tail wounds in mice

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The purpose of this study was to determine the effect of topically applied collagenase and the other debriding agents on the healing process in full thickness wounds in mice. C57BL/6 6 to 8 weeks old mice were used, with 25 animals for each experimental group. Under general anesthesia full thickness wounds down to fascia and corresponding to a template (15 x 4 mm) were created on the dorsal surface of mouse tails 5 mm distal to the base of the tail. Starting on the first post-operative day, wounds from all mice were treated topically for the next seven days with the following agents: 1) no treatment (control air-exposed); 2) collagenase ointment; 3) papain-urea ointment; 4) papain-urea chlorophyllin copper complex sodium ointment. Starting on the day of surgery and every 5 days, five mice for each group were euthanized by carbon dioxide inhalation. Samples of tails containing the wounded site were fixed in 10% neutral buffered formalin for 24 hours, decalcified, and processed for routine histology. The percentage of wound closure was measured by computerized planimetry of the tissue sections, and was analysed using one way analysis of variance (Anova). Treatment with collagenase ointment led to faster healing than the control and other debriding agents, and this was most evident by Day 5 (45.9% and 28.3% closure for collagenase and control, respectively; p<0.005). There was no difference between control treated wounds and those treated with the other debriding agents. All wounds healed by 25 days. In conclusion, we report a new model of full thickeness wounds on the dorsal surface of the mouse tail. This model displays delayed wound closure and shows that topically applied collagenase ointment is an effective agent for accelerating healing.

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PUVA therapy for early stage cutaneous T cell lymphoma: long-term follow-up, curability, and carcinogenesis in 67 patients

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PUVA is probably the preferred treatment option of mycosis fungoides stage IA to IIA with several long-term studies confirming the efficacy of this treatment. Despite the documented benefits of PUVA therapy, little data exist on potential long-term remissions or on indications of disease cure. The objective of our retrospective, nonrandomized, single center study was to investigate the long-term effects of PUVA therapy, impact of maintenance therapy, and potential curability in early stage CTCL. We present the follow-up data of 67 patients with clinical stage IA to IIA disease who achieved complete remission (CR) after treatment with PUVA between 1979 and 1995. Fifty-one percent maintained a CR for 81 months (range 0-238 months). Among these non-relapse group 50% of patients with stage IA, 41% of patients with stage IB, and 9% of patients with stage IIA remained free of disease. In patients who experienced relapses, the mean disease-free interval was 45 months (range 5-127 months). There were no significant differences in baseline characteristics, PUVA and maintenance treatment between the non-relapse and relapse patients. However, PUVA duration and tumor stage are significant predictors of recurrence when adjusted for age, cumulative dosage, and maintenance duration. 27 percent of patients developed secondary cutaneous malignancy. Although beneficial as monotherapy for early stages of the disease, PUVA is also a useful adjunct to other regimens such as interferons, and retinoids to minimize adverse effects and decrease PUVA exposure.

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Correlation between single and multiple serological markers of melanoma (CYT-MAA, HMW-MAA, S100) and the extent of melanoma

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Our goal was to examine whether the presence and/or level of several serological markers of melanoma were correlated with the stage of disease, and whether the sensitivity of these assays can be increased by multiple marker analysis. We measured the levels of three melanoma-associated antigens (CYT-MAA, HMW-MAA, and S100) using double-sandwich ELISA in 117 melanoma patients and in 59 age and sex-matched unrelated disease controls. Melanoma patients were randomized into four categories: 30 with stages IIb/ IIIa, 30 with stages IIc/IIIb/IIIc, 30 with resected stage IV and 27 with measurable stage IV disease. All three antigens were found more frequently in melanoma patients than in controls and the differences were statistically significant. Both CYT-MAA and HMW-MAA were detected more frequently than S100. Among positive patients, there was a correlation between increasing serum levels of CYT-MAA and HMW-MAA, but not of S100, and more advanced stages of melanoma. For example, mean levels of CYT-MAA were 6.0 and 7.3 units in patients with stage II or III disease but 13.1 units in patients with resected and measurable stage IV disease. There were no correlations between detection or levels of positive responses and sex or age. Concurrent measurement of multiple antigens increased the sensitivity of detection by approximately a third over using the single most prevalent antigen (CYT-MAA). These results suggest that the CYT-MAA and HMW-MAA have the potential to serve as molecular markers for the presence and extent of melanoma and that measurement of multiple markers improves the sensitivity of this approach.

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Bexarotene blunts trafficking of malignant T cells in Sezary syndrome: reduction of CCR4-positive lymphocytes and decreased chemotaxis to TARC (Thymus and activation-regulated chemokine)

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Malignant cells in Sezary syndrome (SS) preferentially express the chemokine receptor CCR4 which is involved in mediating lymphocyte trafficking to the skin. High levels of its ligand, TARC, are associated with more advanced disease activity. The RXR specific retinoid bexarotene has been shown to be efficacious among SS patients. To evaluate the effects of bexarotene on CCR4 expression and chemotaxis to its ligand, peripheral blood mononuclear cells from SS patients and normal controls were isolated and treated in culture with bexarotene (10uM). Flow cytometric analysis was performed to determine the percentage of CCR4+ lymphocytes at different post-treatment time-points and transwell migration assays were performed to evaluate lymphocyte chemotaxis to TARC. At 24hrs, no change in CCR4 expression was noted among patient (~70% CCR4+) and normal control lymphocytes (~8% CCR4+). Approximately 40% of patient lymphocytes, and 4% of normal control lymphocytes exhibited chemotaxis to TARC, irrespective of treatment. At 36hrs, an 8% reduction in CCR4+ lymphocytes was noted among the bexarotene treated patient cells (54% to 46%), with a significant reduction in chemotaxis to TARC ligand (from 33% to 23%). The normal controls exhibited no change in chemotaxis to TARC, maintaining a baseline of 2.8%, and no change in CCR4 expression. At 96hrs, a 28% reduction in CCR4+ lymphocytes was noted among the bexarotene treated patients' cells (73% to 45%) with a significant reduction in chemotaxis to TARC. Our results show that the percentage of CCR4+ cells correlates closely with chemotaxis to its ligand. Whether bexarotene downregulates CCR4 expression among malignant cells, or induces their selective elimination, remains to be determined. In addition to its apoptotic ability, bexarotene may, in part, mediate disease activity in SS through its ability to regulate lymphocyte trafficking patterns.

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Tazarotene 0.1% cream is an effective treatment for correcting the multiple morphologic changes associated with the photoaged face

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Tazorac 0.1% cream was applied twice daily for 3 months to the photoaged faces of ten women who showed wrinkling, dyspigmentation, laxity, sallowness and dry scaling. A variety of sophisticated techniques were used at baseline and after the end of the treatment to obtain objective data relating to improvement of the effects of excessive exposure to sunlight in childhood. We were able to quantify the beneficial effects by our techniques. After three months treatment, substantial global improvement of the clinical stigmata of photodamage was observed by clinical assessments and digital photography. In addition, UVA photographs showed a decrease in dyspigmentation, including solar lentigines. The improvement of fine wrinkles and microtopographic abnormalities was observed by using fringe projection technique that showed a smoother surface. Dryness, evaluated by hydration of the horny layer, based on measurements of conductance and capitance, was considerably relieved. The use of Tazarotene 0.1% cream is an efficient treatment for photoaging skin and twice daily regimen was well tolerated.

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Evaluation of a semi-quantitative MRI scoring method in psoriatic arthritis

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Plain radiographs are the standard technique for assessing disease progression in psoriatic arthritis (PsA). Yet, MRI can detect early changes in bone and visualize synovium, tendons and entheses. To date, no scoring method has been formally evaluated in PsA. In this study, we developed an MRI scoring system and used it to analyze PsA joints before and 6 months after etanercept therapy. 20 patients with active disease and ≥ 1 erosion on x-rays were selected. Gadolinium enhanced, fat suppressed MRIs (T1 and T2 weighted FSE and GRE sequences) were performed at baseline and after 6 months of etanercept. The images were assessed semi-quantitatively for - bone marrow edema (BME), cortical erosions, synovial proliferation, joint effusion, bony proliferation and soft tissue changes. Each feature was graded for contrast enhancement, intensity of T2W signal and size. The total MRI score is the sum of these scores. Three radiologists scored each MRI independently. The sum of the tenderness and swelling is the individual joint score (IJS). The MRI score was compared to the IJS. 13 images were available for analyses. Baseline MRI revealed synovial proliferation and erosions (100%), marrow edema (81%), soft-tissue swelling and bony proliferation (69%) and intra-articular effusions (56%). At baseline, the IJS and the MRI scores had a positive correlation (r=0.41). Mean total MRI score improved from 23.9 ± 8.0 to 19.8 ± 8.5 (p=0.04), after treatment. Mean IJS improved from 3.54 ± 1.4 to 0.77 ± 1.23(p=0.0001). Improvement in BME and synovial proliferation scores was significant (p=0.04). Erosion and effusion scores also improved but did not reach significance. Interobserver reliability between radiologists was 72%. These results show that synovial proliferation, erosions, marrow edema and soft tissue swelling are frequent findings in PsA. Bone marrow edema improves with etanercept therapy. This scoring system has good reproducibility and interobserver reliability. Additional studies are required to validate this scoring system.

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Skin blanching response to topical corticosteroids reflects ADH allelic haplotype associated with fast alcohol metabolizing isoforms

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We have previously shown that highly variable human skin blanching responses to topical corticosteroids is independent of skin drug content, but correlates with single nucleotide polymorphisms (SNPs) in alcohol dehydrogenase genes (ADH). The current study determined the allelic haplotypes of SNPs in three ADH genes: ADH2 exon 3 G143A, ADH3 exon 6 A815G, ADH7 exon 3 G239C, and evaluated their association with skin blanching responses (SBR). Seventy-four male (23) and female (51) Caucasian (60) and Asian (14) subjects, age 18-65 yrs, were evaluated with four commercial 0.05% betamethasone dipropionate products containing either no alcohol, or containing propylene glycol (PG), or isopropyl alcohol (IPA), or PG+IPA. Subjects were dosed with 5 mg of all products for 0, 10, 30, 60, 120 and 240 min. SBR was measured by a reflectance colorimeter 1 hr before drug application, at drug removal and 2, 4, 6, 19 and 24 hrs later. Area-under-the-SBR-time curves were calculated for each dose and individual maximal SBR determined. ADH allelic haplotype was determined from DNA extracted from peripheral blood using PCR and nucleotide sequencing. Data demonstrate eleven ADH haplotypes composed of ADH2*2, ADH3*1 and ADH7*1 alleles associated with fast alcohol metabolizing isoforms. Increasing the number of alleles associated with fast alcohol metabolizing isoforms from 0 to 6 was associated with a 6X increase in SBR ($p < 0.05$) to ointment without alcohol in gene order: ADH2 > ADH7 > AHD3. PG, IPA and PG+IPA increased SBR 2-5X ($p < 0.05$) in haplotypes containing 0-3 alleles associated with fast alcohol metabolism in ADH3 and ADH7, but had little effect on haplotypes containing the ADH2*2 allele. Thus, SBR is dramatically increased with alcohols in slow alcohol metabolizing haplotypes, but unaffected, or diminished, in high alcohol metabolizing haplotypes. These data demonstrate the importance of alcohol metabolism haplotype on local cutaneous SBR phenotype and may be useful in enhancing individualized therapy with these drug products.

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Trapping of bacteria by cadexomer beads

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Slow-release antiseptics are increasingly being used in the treatment of exudative wounds with a high bacterial load. One such agent, cadexomer iodine, carries iodine (0.9% weight/weight) immobilized in beads of dextrin and epichlorhydrin and has been demonstrated to be highly effective in promoting healing of exudative wounds. We reported previously that cadexomer iodine lacks toxicity in vivo and in vitro, at least within a certain concentration range. Additionally, we also reported that, in vivo, bacteria can be found in the cadexomer beads. In this study, to test the hypothesis that cadexomer beads are capable of trapping bacteria, we used the vehicle cadexomer beads (without iodine) and exposed it to varying amounts of E. coli in vitro. A number of experiments were performed, testing the optimal exposure time of bacteria to beads (up to 144 hours) and bacterial load in DMEM plus 10% FBS. In addition, we also incubated cadexomer beads overnight with bacterial suspensions on glass slides. Both histology with hematoxylin and eosin and gram staining showed bacteria inside the cadexomer beads and surrounding them. In summary, it appears that cadexomer beads, in addition to their property of absorbing fluid, can also act as a trap, at least temporary, for bacterial organisms. These findings may have important clinical implications for treatment of infected wounds and for the technical development of better agents capable of trapping bacteria and other microorganisms.

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Dectin-2 is a pattern recognition receptor for Der p 1, the major allergen in house dust mites

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Having shown that dectin-2 on dendritic cells (DC) is a pattern recognition receptor (PRR) recognizing mannan-like carbohydrates on hyphae, that induces DC expression of Th2 cytokines, we posited that dectin-2 may also serve as PRR for other Th2-stimulatory agents. To address this hypothesis, we used affinity chromatography to evaluate the ability of soluble dectin-2 to bind whole extract of house dust mite. SDS-PAGE analysis of the dectin-2-bound/mannan-eluted fraction revealed 6 separate molecules within the extract. Buoyed by these findings, we specifically determined whether dectin-2 recognizes Der p 1 (the major allergen). By ELISA, we found dectin-2 (but not controls) to bind purified native Der p 1 dose-dependently (2.5-20 μg/ml). We also found this binding to be inhibited completely by mannan (but not other carbohydrates), indicating that dectin-2 recognizes Der p 1 (like hyphae) via mannan-like carbohydrates. This selective binding was supported by 4-fold greater binding of dectin-2 to native (than to recombinant) Der p 1, since the two forms contain identical peptide sequences but disparate carbohydrates. We next employed ^{125}I- Der p 1 and COS-1 cells altered to express full-length dectin-2 tagged with V5 epitope. COS-1 cells bound Der p 1 (4°C) and also internalized it (37°C). Both processes were blocked completely by preincubation of COS-1 cells with anti-V5 (but not control) Ab. Finally, we examined binding of bone marrow-derived DC (constitutively expressing dectin-2) to bind Der p 1 and its effect on DC function. DC bound and internalized ^{125}I-Der p 1 at appropriate doses and temperatures. Preincubation of DC with mannan reduced uptake to 40%. Importantly, Der p 1 stimulated a 20-fold rise in DC expression of IL-10 mRNA, as measured by real-time PCR. These findings support the concept that dectin-2 acts as a PRR for pro-Th2 agents (hyphae and house dust mites) by recognizing mannan-like carbohydrates on these microbes.

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Bioengineered skin preservation using epiboly as a measure of construct functionality

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A prototypic bioengineered bilayered skin construct (BSC) consisting of human keratinocytes and dermal fibroblasts is used in the treatment of chronic cutaneous wounds. One disadvantage of this and other similar products is their limited shelf life, which in the case of BSC is 5 days based on FDA-approved morphological criteria. The functional viability of the product beyond the expiration date has not been well studied, and there is a need to determine whether BSC could be used clinically beyond its present expiration criteria. Here we report on the contructs viability by evaluating for the capacity for epiboly. Six-mm punch biopsies were made in BSC before and after its morphologically-based expiration date and these samples were floated in multiple culture media and placed at various temperatures. We found that epiboly, as determined 3 days after harvesting of the biopsy samples, was consistently present up to 5 days after the expiration date. Epiboly could be demonstrated at room temperature but not at 4°C and was best in serum-free media (AIM-V). These results point to the potential for BSC and similar constructs to be functional well after their morphologically-based expiration date and to epiboly as a phenomenon that could be used to determine construct functionality.

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Effects of a novel compound, Seletinoid G, on keratinocyte proliferation and differentiation in aged human skin *in vivo*

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Retinoids are natural and synthetic derivatives of vitamin A. It possesses the broad biological activities such as photoaging prevention, anticancer, acne and psoriasis interference. Although its potent efficacy has been useful for many skin conditions, the side effect (skin irritation) has been an obstacle for the plentiful application of RA. New pyranone derivative, 2-((3E)-4(2H,3H,-benzo[3,4-d]1,3-dioxolan-5-yl)-2-oxo-but-3-enyloxy)-5-hydroxy-4H-pyran-4-one(Seletinoid G), was designed as a novel retinoid on the assumption that the pyranone ring may mimic the carboxylic acid moiety in retinoid structure. Seletinoid G, retaining similar molecular length and shape to RA, showed exclusive RAR? specific binding affinity. In this study, we investigated the effects of topical Seletinoid G on the epidermal proliferation and differentiation in aged human skin in vivo. Keratinocyte proliferation was assessed by Ki67 staining and epidermal differentiation was assessed by immunostaining of involucrin, filaggrin, loricrin, K1 proteins. We demonstrated that Seletinoid G cause little skin irritation and its effects on epidermal proliferation and differentiation were similar to those of retinoic acid in aged human skin *in vivo*. Therefore, our data suggests that Seletinoid G is a new generation of receptor-selective retinoid with low skin irritation and may be a candidate for treatment of intrinsic aging and photoaging like a retinoic acid.

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Characterization of the chronic wound fibroblast abnormal phenotype

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Dermal fibroblasts cultured from chronic wounds exhibit an abnormal phenotype characterized by altered morphology, senescence, and poor response to growth factors, including TGF-beta. In these studies, we have further characterized the morphological, proliferative, and synthetic abnormalities of ulcer fibroblasts. We compared fibroblasts from the edge of non-healing venous ulcers with those from acute wound thighs created in the same patients. We show that ulcer fibroblasts have a bizarre epithelioid morphology with large nuclei and vacuolar changes. An identical and irreversible morphology is obtained when exposing normal control fibroblasts to low (0.5%) serum and TGF-beta for as short as seven days. This induced phenotype of normal cells also shows decreased expression of TGF-beta Type II receptors, similar to what is seen with ulcer fibroblasts, which also show decreased Smad3 and MAPK p42/44 phosphorylation. Failure to increase collagen synthesis in response to TGF-beta is not due to DNA methylation, which was found to be identical for COL1A1 in normal and ulcer cells. The poor proliferative response of ulcer fibroblasts could be corrected by transduction with a constitutively phosphorylated form of the TGF-beta Type I receptor or by simple exposure of ulcer cells to a living bioengineered skin construct. We conclude that the abnormal phenotype of chronic wound cells can be reproduced by exposure to low serum and TGF-beta and in an irreversible manner, and that the decreased Type II receptor expression and transduction abnormalities are critical to the poor response of ulcer fibroblasts to TGF-beta.

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Phase II randomized bilateral comparison of bexarotene 1% topical gel in alopecia areata

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Alopecia areata (AA) is a T-cell mediated autoimmune disease directed against hair follicles. It can result in loss of all scalp hair (alopecia totalis, AT) or body hair (alopecia universalis, AU), and is often refractory to therapy. Bexarotene (bex) is an RXR-selective retinoid (rexinoid) that induces T-cell apoptosis [CCR 8: 1234-1240, 2002] and is approved for cutaneous T-cell lymphoma. Following our observation that bexarotene reversed alopecia in CTCL patients [Br. J. Derm. 148: 1-4, 2003], we tested the hypothesis that benign perifollicular T-cell infiltrates could be similarly targeted. We have conducted Stage I of a Phase II study of AA in 13 of 42 total patients. Thirteen patients include 4 Alopecia Totalis/universalis and 9 patchy AA and 4 males and 9 females. Consenting patients were randomized to apply topical bex gel to to one half of their scalp once daily x 2 wks then twice/daily for up to 24 weeks. No other form of oral or topical therapy was allowed for one month prior to and during the study. Assessment of index lesions, hair growth and density, labs, and adverse events were evaluated monthly. Four patients had >50% partial hair regrowth, two pts had minor hair regrowth, two had stable disease, and three progressed while on therapy. One AT pt with 99% alopecia (AT) for 4 years grew back 88% of her hair by week 8 and one with 78% alopecia achieved 50% hair regrowth by week 16, beginning with the treated side. No grade 3 toxicity occurred but erythema, scaling, and/or itching due to local irritation at the application site were experienced by 11 pts, and required decreased application frequency in 4 pts. These data suggest that topical bexarotene 1% may be effective in treating some patients with alopecia areata with a response rate of 31%, even in patients with more persistent and severe AA phenotypes.

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Targeted inhibition of CCR5 with PSC-RANTES is sufficient to block intravaginal transmission of SHIV in rhesus macaques

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An effective topical microbicide applied intravaginally could protect women from initial HIV infection and save millions of lives. We are developing microbicides that block HIV infection by binding to CCR5, a critical HIV co-receptor, on susceptible cells and interfering with HIV attachment and virus-cell fusion. Since RANTES is a natural ligand for CCR5, we have developed a synthetic RANTES analogue, called PSC-RANTES, that causes prolonged intracellular sequestration of CCR5 and protection from HIV infection *in vitro*. To assess the efficacy of PSC-RANTES as a microbicide *in vivo*, female rhesus macaques were atraumatically and intravaginally treated with 4 mL of PSC-RANTES diluted in saline at concentrations of 1 mM (n=5), 330 uM (n=5), 100 uM (n=5), or 1-10 uM (n=5), or 4 mL of saline alone (n=5). Fifteen minutes after dosing, animals were intravaginally inoculated with 300 TCID50 of SHIV162P3, a CCR5-utilizing virus that has high relevance to human HIV transmission. Viral loads in plasma were then monitored weekly by a bDNA assay. All 5 macaques pretreated with the highest dose (1 mM) and 4 of 5 treated with the 330 uM dose of PSC-RANTES were completely protected from intravaginal SHIV infection as evidenced by undetectable virus in plasma. By contrast, only 3 of 10 macaques in the low dose groups were protected, and 4 of 5 in the control group became infected, indicating that protection from vaginal challenge with virus was dose-dependent. These results clearly demonstrate that topical PSC-RANTES inhibits vaginal transmission of a CCR5-utilizing SHIV in a dose-related manner in macaques. Since CCR5-utilizing strains predominate in early mucosal transmission, these results suggest that topically blocking the binding of virus to CCR5 may be sufficient to block vaginal transmission of HIV in humans.

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The sensitivity and specificity of confocal microscopy in the diagnosis of allergic contact dermatitis: a preliminary study

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The reproducibility and efficiency of patch testing in the diagnosis of allergic contact dermatitis (ACD) has been questioned in recent years. Recently, reflectance-mode confocal microscopy (RCM) has been used for non-invasive evaluation of the histopathological features of ACD. This work was designed to determine the sensitivity and specificity of RCM as compared to patch testing. 16 subjects with a prior ACD diagnosis were patch-tested with the allergens and appropriate controls. Clinical scoring, digital photography and RCM evaluation were performed at 72 hours following patch removal. RCM and clinical images were evaluated and scored by three dermatologists blindly and independently. The following RCM parameters were assessed: stratum corneum (SC) disruption, parakeratosis; stratum spinosum (SS) and stratum granulosum (SG) spongiosis, exocytosis and vesicle formation. Logistic regression analysis was performed on all significant variables, p-values were determined using Chi-square.

Overall, there is high specificity for all features, ranging from 95.8% - 100%. Sensitivity ranges from 51.9% - 96.3%. Significant parameters with high sensitivity and specificity include spongiosis at the level of SS (sensitivity: 100%, specificity 92.6%, $p<0.05$), spongiosis at the level of SG (95.8%, 96.3%, $p < 0.05$), and exocytosis (100%, 74.1%, $p< 0.05$). All other parameters are also significant, with the exception of SC disruption. Furthermore, logistic regression analysis indicates that stratum spinosum spongiosis is the best predictor for diagnosing allergic contact dermatitis. The comparable sensitivity and specificity of RCM to clinical diagnosis of ACD makes RCM a promising tool in diagnosing allergic contact dermatitis. In particular, spongiosis at the level of the spinous layer shows excellent correlation and can be used as a criterion in the detection of ACD using RCM.

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Quantitative investigation on the progression of acne vulgaris

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Acne vulgaris affects males and females of all ages around the world. The condition manifests itself in the form of one or more lesions that typically take several days to weeks to resolve if left untreated. The purpose of this study is to use objective instrumental methods coupled to self and dermatologist evaluations for quantification of inflammation-related parameters relevant to acne vulgaris in order to develop fundamental understanding of the progression of the disease. Seventeen subjects participated with at least one inflammatory acne lesion that was no more than 24 hrs old and had not been treated with any product. Self-assessment questionnaires were collected and high-resolution digital images were used for expert grading. Lesion height and volume were measured using 3D surface profilometry. Erythema intensity, area involved, and interstitial fluid accumulation in the cases of edematous lesions, were evaluated by hyper-spectral imaging. Study results show that lesion diameter, elevation, erythema, and peeling increase at 24 and 48 hours after baseline. At 7 days after baseline erythema and elevation persist. Lesion oxyhemoglobin levels (a component of erythema) are highest during the first 4 days after baseline. After this point, erythema levels steadily decrease toward healthy skin levels over 2 weeks. Lesion diameter peaks at 24-48 hours after baseline and subsequently starts to decrease at day 4. The lesion is still evident at day 14 although elevation and interstitial fluid accumulation under the lesion may not be present. This indicates that edema and erythema dynamics follow different patterns. In conclusion, self and dermatologist evaluations coupled with instrumental methods that quantify acne-relevant parameters, such as lesion volume and diameter, interstitial fluid accumulation, and erythema expressed as increased oxy-hemoglobin concentration, have revealed new findings in acne lesion dynamics. Fundamental understanding of the progression of acne lesions from formation to resolution helps to develop safe and effective treatments for patients and consumers.

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Juvenile polyposis coli, juvenile polyps and cafe-au-lait macules: is there a relationship?

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Cafe-au-lait macules (CALMs) and lentigines are associated with the PTEN (CR 10q23) mutation polyposis syndromes including Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRR). CALMs have also been associated with a subset of hereditary non-polyposis colorectal cancer (HNPCC) with early onset colorectal neoplasia. The extra-colonic phenotypic abnormalities, in particular cutaneous findings, of two other polyposis syndromes, juvenile polyposis coli (JPC) and juvenile polyps (JP) have not been well characterized. (JPC is defined as 10 or more juvenile polyps and often associated with heritable cancer syndromes. In contrast JP is defined as isolated hamartomas not associated with an increased cancer risk and found in 1-2% of children.) Children with polyps were identified through the clinical practice database of the section of pediatric gastroenterology at a single institution. These children and their first-degree relatives were invited to participate in this case series. Subjects underwent a comprehensive skin examination by dermatologists and retinal examinations if pigmented lesions were identified. 9 families consisting of 11 children (range 10-23 years old) and 17 first-degree family members (range 3-49 years old) participated in this case series. 4 of 10 subjects with JPC or JP had multiple (>3), large (> 15mm) CALMs identified, primarily on the trunk. 3 of 17 first-degree relatives of JPC or JP subjects had CALMs identified. All patients were Caucasian except 1 Hispanic JP patient who did not have a CALM. Patients with identified CALMs underwent ophthalmologic examinations without identification of retinal pigmented lesions. In this case series, CALMs were identified at a greater rate in JPC and JP subjects. This data may support a common pathway for hamartomatous polyps (JP/JPC/CS/BRR) as all may have polyps and CALMs. Perhaps CALMs serve as a cutaneous marker for these syndromes and could assist in the identification of asymptomatic carriers.

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A comparison of keratin gene expression between inflamed and control skin obtained by tape harvest

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The purpose of this study was to determine if tape-harvested RNA samples could be used to monitor changes in gene expression induced by 1% sodium lauryl sulphate or water compared to normal skin. An additional objective was to compare keratin expression profiles in biopsy skin samples with tape-harvested samples. 10 subjects were occlusively patched on the upper back with two 1% aqueous SLS and two water patches. After 24 hours, patches were removed and one replicate site was shave biopsied and the other site was tape harvested by application and removal of 4 custom adhesive tapes (circular 17 mm diameter). Normal, adjacent skin was similarly sampled. mRNAs for β-actin, keratin-10, keratin-16 and keratin-17 were assayed by semi-quantitative RT-PCR. The keratin mRNAs were normalized to actin mRNA in each sample. The keratin mRNA / βactin mRNA ratio in SLS samples was calibrated to that same ratio in water or normal skin samples. Similarly, the keratin / actin mRNA ratio in water samples was calibrated to normal skin. The results showed that Krt-10 mRNA decreased relative to b-actin mRNA in both tape and biopsy samples of SLS treated skin relative to water treated and normal skin (95% confidence interval). Analysis of Krt-16 mRNA expression in SLS relative to normal skin showed the expected increase in biopsy samples. However, tape-harvested samples showed the opposite results with the Krt-16 / actin mRNA ratio decreasing. In order to investigate this result further, Krt-17 mRNA (co-regulated with Krt-16) was analyzed in all samples. The expression of Krt-17 mRNA in biopsies of SLS-treated skin increased relative to actin while expression of Krt-17 mRNA in tape-harvested samples decreased. Previous comparison of RNA profiles of tape and biopsy skin samples had suggested that the two methods were recovering different skin cell populations. This work confirms that hypotheses. This work continues our efforts to develop tape harvesting as a non-invasive substitute for biopsy, thus allowing the routine direct qualitative and quantitative assessment of physiologic and pathologic biomarkers in the skin.

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Failure of physiologic doses of pure UVB or UVA to induce lesions in photosensitive cutaneous lupus erythematosus: implications for phototesting and sunblocking strategies

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Subacute cutaneous lupus erythematosus (SCLE) is a photosensitive disease. Several phototesting studies have identified UVB and UVA as the wavelengths that induce skin lesions. There is a large difference of results between these studies, which is attributed to non-standardized light testing protocols. Most of these studies have been performed with doses of UVA and UVB that are above physiologic levels, and some UVB sources may be contaminated with non-physiologic UVC, while UVA sources often emit substantial amounts of visible light and heat. We phototested SCLE patients with type 2 skin (n=9) and skin type-matched healthy controls (n=12) with physiological doses of UV light from controlled irradiation devices. Our UVB source incorporated Kodacel filters to block all UVC. Our UVA source had a UG11 filter to block the majority of infrared and visible light and a 3-mm thick 335-nm Schott filter to block all wavelengths shorter than UVA. We correlated the minimum erythema doses (MEDs) with the −308 TNF-α promoter genotype (GG wildtype vs GA TNF overproducer) and examined the induction of LE lesions. Patients had their MED determined, and were then irradiated with either UVB (1 MED) or UVA (20 J/cm^2) daily for 5 days, and then followed weekly for 3 weeks. We found that the MED of control GG vs GA patients was 95±6.4 mJ/cm^2 (mean±SEM, n=8) vs. 108±9 mJ/cm^2 (n=4; NS). In the SCLE patients, the MED was 107±11 mJ/cm^2 (n=3) vs 87±7 mJ/cm^2 (n=5; NS; the ninth patient was AA). Including one patient with type 3 skin, we tested a total of 10 SCLE patients with both UVB and UVA. No patient showed induction of skin lesions with UVB. Only the single AA patient showed UVA-induced skin lesions. We conclude that pure, physiologically relevant doses of UVB and UVA do not consistently induce lesions under our conditions. Thus, there must be other factors that contribute to clinical photosensitivity.

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The biological effects of a novel retinoid, Seletinoid G, in human skin *in vivo*

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Retinoids are vitamin A, its naturally occurring and synthetic derivatives. They exert a wide variety of profound effects in acne, psoriasis, photoaging, and skin cancers. When retinoids are used topically, they are associated with the development of a local irritative response, referred to as a "retinoid dermatitis". New pyranone derivative, 2-((3E)-4(2H,3H,-benzo[3,4-d]1,3-dioxolan-5-yl)-2-oxo-but-3-enyloxy)-5-hydroxy-4H-pyran-4-one (Seletinoid GTM), was designed as a new retinoid analogue on the assumption that the pyranone ring may mimic the carboxylic acid moiety in retinoid structure. In gene transactivation assays, Seletinoid G bound with high affinity to RARg than RARa. Seletinoid G induced the mRNA expression for specific celluar markers of retinoid action, i.e. tazarotene-induced gene 1/2, and keratin 4, but not cellular retinoic acid binding protein (CRABP)-II. Seletinoid G showed little skin irritation at the human primary skin irritation test. We investigated the effects of topical Seletinoid G treatment on the expression of extracellular matrix proteins and MMP-1 in aged human skin. Seletinoid G increased the expression of type I procollagen, tropoelastin, and fibrillin-1, and decreased the expression of MMP-1. We investigated the inhibitory effects of Seletinoid G on UV-induced changes of type I procollagen, MMP-1, ERK, and c-Jun expression in young skin. Seletinoid G not only blocked the UV-induced decrease of type I procollagen but also UV-induced increase of MMP-1, phospho-ERK, and c-Jun. Therefore, our data suggests that Seletinoid G is a new generation of receptor-selective retinoid with low skin irritation and may be a candidate for treatment of intrinsic aging and photoaging like a retinoic acid.

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Antigen-specific peripheral tolerance induced by topical application of NF-kB decoy oligodeoxynucleotides

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Activation and maturation of dendritic cell (DC) are crucial for the establishment of delayed-type hypersensitivity (DTH). However, antigen presentation by immature dendritic cells (iDC) might lead peripheral tolerance. iDC are characterized by low surface expression of co-stimulatory molecules, i.e., B7-1 and B7-2. Recently, nuclear factor kappa B (NF-kB) decoy oligodeoxynucleotides (ODN) has been used for heart graft transplantation by inhibiting NF-kB-related gene expression. NF-kB plays significant roles in upregulation of co-stimulatory molecules and immunostimulatory cytokines. Here we investigated whether NF-kB decoy oligodeoxynucleotide (ODN) could induce tolerance in DTH. NF-kB decoy ODN ointment (2%) was applied and sensitized by OVA on shaved abdominal skin of BALB/c mice, and elicited by intradermal injection into footpads 7 days after sensitization. NF-kB decoy ODN suppressed induction of DTH. Adoptive transfer of the cells from draining lymph nodes from the tolerant mice suppressed the induction of DTH. Deletion of CD25+ T cell population by PC61 mAb abrogated the suppression of DTH. Adoptive transfer of CD4+CD25+ T cell (Treg) from tolerant mice induced tolerance in the sensitized mice. Furthermore, topical NF-kB decoy ODN inhibited DC migration and upregulation of co-stimulatory molecules on DC. Antigen crossing assay revealed that the tolerance to OVA is not suppressed by adoptive transfer of lymph node cells from mice which are tolerant to hen egg lysozyme (HEL). These findings indicate that topical NF-kB decoy ODN induces antigen-specific peripheral tolerance in DTH in vivo, and this tolerance is mediated by modulating DC activation and inducing Treg. The topical application of NF-kB decoy ODN is a new strategy for the induction of antigen specific peripheral tolerance and gives us a new format for the treatment of allergic diseases.

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Unique formulation dramatically improves wound closure and healing, by accelerating critical healing stages

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Effective wound healing requires a highly organized series of events that comprise inflammation, re-epithelialization, proliferation, angiogenesis and wound contraction. Together, these processes result in the restoration of tissue integrity and functional healing. The present study investigates the various stages of acute wound healing and presents a new formulation accelerating this process. Protein Kinase C is a family of serine/threonine kinases that play a role in skin physiology as well as in various skin pathologies. We developed effective PKC based formulation consisting of nPKC activator and cPKC inhibitor which augment wound healing. We have completed preclinical studies in rodents and pigs. Moreover we developed a unique strategy for wound healing assessment enabling quantification of critical healing stages. Longitudinal wound incisions (2 or 3 cm) were performed on upper backs of animals. Healing processes were analyzed at critical time points: 1-30 days post wounding. Assessment was made utilizing antibodies to keratin 14 (basal epidermal layer formation), keratin 1 (spinous layer differentiation), keratin 6 (migration), PCNA (mitosis), H&E (dermal contraction and remodeling). Results clearly demonstrate that 100% of HO/03/03 treated mice exhibit formation of a new epidermal layer at the gap of the wound 7 days post wounding. Moreover the treated animals display full spinous differentiation 9 days post wounding and contraction of the wound by day 12. In contrast PBS treated control animals display epidermal closure by day 12 and spinous differentiation as well as dermal contraction only by day 18 post wounding. Similar results were obtained in pig model system. All critical healing stages in pigs are significantly accelerated by HealOr's PKC based formulation. We have completed pre-clinical studies demonstrating the efficacy of an innovating formulation that accelerates the healing of wounds.

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Imiquimod treatment of basal cell carcinoma induces expression of a novel tumor antigen, opioid growth factor receptor: direct or IFN-α-mediated effect?

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Imiquimod is a synthetic local immune response modifier that has demonstrated efficacy in clearing superficial basal cell carcinoma (sBCC). Via interaction with Toll-like receptor 7 on immune cells, imiquimod induces local production of cytokines, such as interferon (IFN)-α. To more closely define and elucidate mechanisms leading to BCC clearance in vivo, we examined gene expression profiles of skin sBCC before and after treatment with 5% imiquimod cream (Aldara, 3M Pharmaceuticals, St. Paul, MN) by using high-density oligonucleotide arrays. We show that imiquimod predominantly induces genes involved in different aspects of immune response. In addition to effects on immunity, imiquimod treatment modulates the expression of genes involved in the control of apoptosis (e.g. bcl-2, bax, various caspases, TRAF1,2) and oncogenesis (e.g. Hedgehog pathway - PTCH, GLI1,3; Wnt pathway - FZD7, DVL3). Array data indicated that imiquimod treatment induces expression of opioid growth factor receptor (OGFr), a molecule with anti-proliferative properties recently reported to be a target for anti-tumor antibody responses. Quantitative PCR confirmed OGFr up-regulation in Aldara treated sBCC skin samples. Immunohistochemistry revealed in vivo up-regulation of OGFr protein on tumor and on infiltrating cells after Aldara treatment. By using BCC cell lines treated with IFN-α or imiquimod, we show that up-regulation of OGFr mRNA as well as OGFr protein is IFN-α rather then directly imiquimod mediated. Through IFN-α-mediated up-regulation of OGFr, imiquimod might not be only suppressing tumor growth but it might as well increase tumor immunogenicity.

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Retinoid-induced epidermal hyperplasia is mediated by epidermal growth factor receptor activation via specific induction of its ligands heparin binding-EGF and amphiregulin in human skin *in vivo*

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Retinoids (RA) are widely used in the treatment of photoaging to stimulate dermal repair. However, retinoids also induce epidermal hyperplasia, which can lead to excessive scale. Scaling is the major deterrent to topical RA use. Keratinocyte growth is strongly stimulated via activation of epidermal growth factor receptor (EGFR). Therefore, we examined the role of EGFR in RA-induced hyperplasia. Topical treatment of human skin *in vivo* with EGFR inhibitor genistein (1%) reduced RA-induced KC proliferation 55% (n=12, p<0.05), as determined by quantitation of KI67 (proliferation marker) positive immunostaining. In addition, specific EGFR inhibitor PD169540 completely prevented the stimulation of keratinocyte growth by RA in human skin organ culture. In normal skin *in vivo*, RA specifically induced expression of EGFR ligands heparin binding-EGF-like growth factor (HB-EGF) and amphiregulin (AR). RA had no effect on expression EGFR ligands transforming growth factor-alpha, epidermal growth factor, or epiregulin, and reduced expression of betacellulin (80%. p<0.05). HB-EGF and AR mRNAs were increased within 16 hours post-treatment, were maximal (9.6-fold and 9.4-fold respectively) 24 hours post-treatment (p<0.05), and remained elevated for at least 4 days. Laser capture microdissection coupled real-time RT/PCR revealed that HB-EGF and AR were primarily expressed in suprabasal keratinocytes in normal, untreated human skin. RA treatment induced HB-EGF in both basal and supra-basal layers, whereas AR induction was limited to basal KC. These data demonstrate that RA-induced epidermal hyperplasia is mediated by EGFR activation via induction of its ligands HB-EGF and AR, in both basal and suprabasal epidermal layers. Inhibition of EGFR activation by compounds such as genistein may mitigate unwanted scaling caused by topical RA treatment, and thereby improve compliance and resultant efficacy.

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Innovative PKC modulating formulation dramatically improves the healing of diabetic wounds

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One of the well known complications associated with the diabetic state is the diabetic foot ulcer. The impairment of wound healing in diabetes results in high rates of morbidity and mortality. Protein Kinase C (PKC), a family of serine/threonin kinases affect various physiological and biochemical properties of skin. Keratinocytes express at least five different PKC isoforms which have distinct roles in skin physiology and pathology. We have developed a novel formulation, we refer to as HO/03/03, combining nPKC activator and cPKC inhibitor which can specifically regulate distinct stages of the wound healing process. We demonstrate the effect of our formulation on wounds of diabetic NOD and STZ injected C57BL mice, models of type 1 diabetes. These animal models closely imitate the wound healing impairment as seen in diabetic patients. A longitudinal, 2cm, incision was made on the upper backs of the diabetic mice. Healing processes were analyzed at critical time points: 6-15 days post wounding. Assessment was made utilizing antibodies to keratin 14 (basal epidermal layer formation), keratin 1 (spinous layer differentiation), keratin 6 (migration), PCNA (mitosis) and H&E (dermal contraction and remodeling). Control (PBS treated) diabetic wounds exhibit impaired differentiation, proliferation and migration properties. Within 9 days, only 16% of control wounds exhibited epidermal closure and contraction of the dermis. Conversely, following topical treatment with HO/03/03, 83% of STZ diabetic mice displayed complete closure of the wound gap by a newly formed basal epidermal layer. Furthermore 45% of the HO/03/03 treated wounds displayed significant contraction and remodeling of the dermis similarly to results obtained in non diabetic mice. Analogous results were obtained in the NOD diabetic model. In conclusion our unique formulation augments wound healing progression in type 1 diabetic animals, affecting all fundamental wound healing parameters.

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Universal mechanism of IVIG action in pemphigus and pemphigoid

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Numerous mechanisms of action have been proposed for IVIG. In this study, we used IgG passive transfer murine models of bullous pemphigoid (BP), pemphigus foliaceus (PF), and pemphigus vulgaris (PV) to test the hypothesis that the effect of IVIG in autoantibody-mediated skin bullous diseases is to accelerate the degradation of pathogenic IgG through FcRn, the receptor protecting circulating IgG from catabolism. BP, PF, and PV are organ-specific antibody-mediated autoimmune diseases in which autoantibodies target BP180, desmoglein-1, and desmoglein-3, respectively. The serum titers of anti-BP180, anti-dgs1, and anti-Dsg3 IgG autoantibodies correlate with disease activity. The experimental models of BP, PF, and PV were induced by passive transfer of rabbit anti-murine BP180, human anti-dsg1, and anti-Dsg3 IgG into neonatal mice, respectively. Pathogenic IgG levels in circulation were determined by antigen-specific ELISA. We found that FcRn-deficient mice were resistant to experimental BP, PF, and PV. Circulating levels of pathogenic IgG in FcRn-deficient mice were significantly reduced compared to wild-type (WT) C57BL/6J mice. Administration of IVIG to WT mice prevented blistering triggered by pathogenic IgG. This protection is associated with a significant reduction of circulating pathogenic IgG. The protective effect of IVIG depends on FcRn activity because pathogenic IgG levels at 24 and 48 h post-injection were similar in FcRn-deficient mice with and without IVIG treatment. These data demonstrate that IVIG treatment leads to increased FcRn-dependent clearance of pathogenic IgG. This mechanism may account for beneficial effects of IVIG in many autoantibody-mediated inflammatory and autoimmune diseases.

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Induction of matrix metalloproteinases and inhibition of procollagen synthesis in human skin *in vivo* by ultraviolet A1 irradiation is profoundly diminished by skin pigmentation

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The ability of UVA1 (340-400nm) to induce matrix metalloproteinases (MMPs) and simultaneously inhibit procollagen synthesis without causing sunburn in human skin has been the basis for its application as an antifibrotic phototherapy. Extent to which skin pigmentation affects UVA1 responses is largely unknown. Thus, we investigated the magnitude of MMP induction and procollagen inhibition (mRNAs quantified via real time RT-PCR) as a function of skin pigmentation in 28 normal subjects. Subjects were stratified into three groups based on surface skin color (determined by colorimeter): Light (L value >65, ~skin phototypes [SPT] I/II); Medium (L 65-55; ~SPT III/IV); and Dark (L<55; ~SPT V/VI). In the Light group, MMP-1, MMP-3, and MMP-9 mRNAs all demonstrated significant dose-dependent induction with UVA1 (maximum inductions: 1100-fold, 1900-fold, 6.5-fold, respectively; p<0.05; n=8). In the Dark group, all three MMPs mRNAs were not induced even at the highest UVA1 dose (150 J/cm^2) (n=8). Although MMP-1 mRNA was induced by 150 J/cm^2 UVA1 in the Medium group (p<0.05; n=8), the magnitude (10-fold; p<0.05; n=8) was less than 1% of that in the Light group. Reduction of type I and III procollagen mRNAs by UVA1 were similarly affected according to skin color. In the Light group, there was a dose-dependent inhibition in both type I and III procollagen mRNA expression (~50% with 150 J/cm^2; p<0.05; n=8). In the Dark group, 150 J/cm^2 UVA1 had no effect on procollagen mRNAs. The exposure time for 150 J/cm^2 of UVA1, using a high output device takes close to one hour. Therefore, an attempt to deliver even higher doses of UVA1 for therapeutic purposes is not practical. In conclusion, skin pigmentation effectively attenuates UVA1-induced responses that lead to increased turnover of dermal collagen. Usefulness of UVA1 as an antifibrotic device is limited to patients with light skin pigmentation, which likely explains varied clinical responses of scleroderma to UVA1.

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Strategies for enhancement of the host immune response in cutaneous T cell lymphoma: CpG Oligonucleotides and IL-15

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Patients with advanced cutaneous T-cell lymphoma (CTCL) exhibit profound defects in cell mediated immunity partially resulting from marked deficiencies in the numbers of peripheral blood dendritic cells (DCs) and in their capacity to make DC derived cytokines (IL-12, IL-15 and IFNα). Because host immune function appears to play an integral role in mediating disease-controlling responses in CTCL, we investigated the effects of synthetic oligodeoxynucleotides which contain CpG motifs (CpG-ODN) and which have been recognized as immune stimulatory by virtue of activation of DCs following binding to Toll like receptor (TLR) 9. CpG-ODN were cultured with freshly isolated peripheral blood mononuclear cells (PBMC) from patients with advanced CTCL (erythroderma with circulating malignant T-cells) and normal volunteers. Patient PBMC exhibited marked induction of IFNα release by CpG-ODN. Similarly significant activation of NK cells and CD8+ T-cells occurred as assessed by up-regulation of CD69 expression and by natural killer cell lytic activity. Nevertheless, patients with a high burden of circulating malignant T-cells ($\propto$20‰ Sezary cells) exhibited blunted responses to CpG-ODN compared to normal volunteers. In such cases, IL-15 was capable of producing levels of activation of NK and CD8+ T-cells that were superior to CpG-ODN, while the combined effects of CpG-ODN plus IL-15 may synergistically activate NK and CD8+ T-cells. These findings have important implications for the potential enhancement of anti-tumor immunity among patients with advanced CTCL.

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A variable response to pregnancy in psoriasis patients

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Pregnancy has been shown to have varying effects, either detrimental or beneficial, on a variety of inflammatory diseases including rheumatoid arthritis, SLE, and psoriasis. We explored the variable effects of pregnancy on psoriasis severity through analysis of phenotypic information obtained from patients enrolled in the Utah Psoriasis Initiative (UPI). From the first 351 patients enrolled in the UPI, we were able to identify 89 women with an onset of psoriasis during the childbearing years of 18-40. These women accounted for a total of 283 children. Of this population of women, 21.1% reported an improvement in the severity of psoriasis with pregnancy, 12.8% reported a worsening, with the remainder stating no change. We predicted that a variable response to the pregnancy state would also correlate with other phenotypic differences between these three groups of patients. Those women reporting an improvement of psoriasis with pregnancy had a significantly lower age of onset (p<.001) and a higher incidence of koebnerizing psoriasis (p<.025). There was no significant difference in the incidence of smoking, obesity, psoriatic nail changes, or palmar/plantar disease between the three groups. We also evaluated these groups for disease response to stress and infection and found no significant differences between them. This initial analysis suggests a unique subset of patients with psoriasis who seem to exhibit pregnancy responsive disease. Follow up questionnaires have been created and sent out which will validate these findings and further explore the potential effects of the post partum state, menopause, and hormone replacement therapy on psoriasis severity. Further characterization of these groups will be followed by genotyping. The expected emergence of unique genotypes will be followed by linkage to the Utah Population Database (UPDB) for pedigree analysis.

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Induction of HIV gag-specific cytotoxic T cells by percutaneous peptide immunization in humans

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Dendritic cell (DC)-based strategies for treatment of cancer and infection have been shown to be promising in humans. However, intricate *in vitro* processes are currently used for the generation of mature DCs. Percutaneous peptide immunization (PPI) is a simple and noninvasive way of delivering all classes of peptide to skin from which the stratum corneum (SC) is removed by a physical means. Removal of SC not only enhances permeability of peptide but also mature Langerhans cells (LCs) to be potent for antigen presentation. We have already demonstrated that this procedure is highly effective in prophylaxis and therapy to tumor development in mice. The present study was designed to validate the feasibility of PPI as clinical interventions in humans. Histological examination of healthy subjects revealed that LC migrated from the epidermis to the upper dermis as early as at 24 h after stripping of SC. HLA-DR$^+$ cells were found larger and stained more brightly at 6 and 24 h as compared with those in intact skin. HLA-DR, CD80, CD86 and HLA-class I on CD1a$^+$ cells in epidermal cell suspensions was also up-regulated at 24 h as assessed by flow cytometric analyses. Two volunteers received PPI with HIV gag peptide, and appearance of HIV gag tetramer binding cells among CD8$^+$ cells was assessed in the peripheral blood. HIV gag-specific CD8$^+$ cells were successfully induced when assayed 7 days after the fourth PPI and their levels were maintained by repeated immunizations. HIV gag-specific CD8$^+$ cells were markedly expanded by the *in vitro* stimulation in an antigen specific manner. In addition, CD8$^+$ T cells positive for intracellular IFN-γ were expanded after stimulation *in vitro* with HIV gag peptide. The present study demonstrated that removal of SC induced LC maturation. Functional HIV gag-specific CTL seemed to be generated and increase in number by repeated PPI. Based on these results, we propose PPI as new breakthrough in preventive and therapeutic interventions to cancer and microbial infection in clinical settings.

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Human dectin-2 resembles its mouse counterpart in cell expression profile and pattern recognition receptor function

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Having identified dectin-2 in mice to be expressed constitutively by Langerhans cells (LC) and dendritic cells (DC), to be inducible in macrophages and T cells following activation, to function as a pattern recognition receptor (PRR) for hyphae (but not yeasts) by binding to mannan-like carbohydrates on these fungi, and to mediate Th2 cytokine expression by DC, we identified its human homolog using lowly stringent RT-PCR cloning. Our work yielded DECTIN-2, a gene encoding a type II-configured receptor of 209 amino acids, with 66.5% homology (highest in GenBank) and domain structures similar to mouse dectin-2. We next used RT-PCR and Southern blotting to examine DECTIN-2 mRNA in various human tissues, and found it expressed most abundantly in peripheral blood leukocytes (PBL) and spleen, and less in lymph nodes, bone marrow, and lung. Among epidermal cells, DECTIN-2 expression was restricted to CD1a$^+$ LC, which were sorted by anti-CD1a magnetic beads. Among PBL, DECTIN-2 was expressed constitutively (in decreasing order) by: CD14$^+$ monocytes > LC-like DC (generated by culture of CD14$^+$ cells with GM-CSF, IL-4, and TGFβ) > CD8$^+$ T cells > CD19$^+$ B cells. Interestingly, close to background DECTIN-2 expression in CD4$^+$ T cells was upregulated markedly after activation with concanavalin A, whereas expression in CD8$^+$ T cells and CD19$^+$ B cells was abrogated by activation with phytohemagglutinin and pokeweed mitogen, respectively. Finally, we examined the ability of DECTIN-2 to bind hyphae using ^{125}I-binding assays, and showed that soluble DECTIN-2 (V5-tagged extracellular domain) bound *C. albicans* hyphae (but not yeasts) and that this binding was inhibited completely by mannan, indicating that the PRR function of mouse dectin-2 is conserved in humans. These findings form the basis for studying a role for DECTIN-2 in the causation of Th2-dominant diseases and/or treatment of Th1-dominant disorders.

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The Utah psoriasis initiative: a tool to investigate phenotypic and genotypic relationships

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The Utah Psoriasis Initiative (UPI), "a psoriasis registry" was founded on the belief that the phenotypic characteristics of psoriasis are a reflection of specific genotypic variants of the disease. The UPI is designed to facilitate the correlation of phenotypic and genotypic data via the identification of unique phenotypes with presumed specific genotypes. The UPI collects, through questionnaires and physical examination, a variety of phenotypic information, including anatomic location, co-morbid conditions, age of onset, response to therapy, body surface area, clinical appearance, etc. on each patient. DNA from blood is also collected. This process is aided by electronic linkage with the Utah Population Database (UPDB), a data warehouse that provides access to ≈ 85% of the Utah population. Over 50% of the UPDB are members of informative kindreds, multigenerational pedigrees some with over 20,000 living relatives. In a preliminary analysis it was determined that the > 1000 psoriatics recently seen in the University of Utah clinics distribute in the UPDB in a manner similar to that of the general population. To date over 450 patients have undergone extensive phenotyping. Analysis of the first 351 patients has demonstrated several unique groups of patients including smokers, obese/overweight individuals, those exhibiting inverse disease, and women showing a change in their disease with pregnancy. Smoking has been shown to associate with an increased incidence of koebnerization (p<.01), psoriatic nail changes (p<.01), and palmar-plantar disease (p<.025). The average body mass index of UPI patients is significantly higher than that of age matched controls, and these overweight/obese patients show a higher incidence of inverse disease. Women report a variable response to pregnancy, with 21.1% reporting improvement in their disease and 12.8% reporting worsening. These findings have prompted further evaluation of the apparent association between obesity and psoriasis and the effect of pregnancy on psoriasis.

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Novel germline mutation of c-KIT in familial mastocytosis and gastrointestinal stromal tumors with positive response to imatinib *in vitro*

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Mastocytosis is characterized by accumulation of mast cells in tissues. Sporadic mastocytosis is usually associated with somatic mutations in the intracellular exon 17 of c-KIT, affecting the enzymatic site and causing constitutive activation of the KIT tyrosine kinase. In contrast, the cause of familial mastocytosis is unknown. Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors usually caused by somatic c-KIT mutations that activate KIT by affecting regulatory portions of the molecule such as the intracellular juxtamembrane exons 11 or 13, leaving the enzymatic site unchanged. Most GISTs respond to tyrosine kinase inhibitors that bind to the enzymatic site of KIT, unlike most mastocytosis cases whose modified enzymatic site is not affected by these drugs. In the present study, three members of a kindred with familial mastocytosis and GISTs were investigated. We identified a novel germline c-KIT mutation in exon 8, resulting in deletion of codon 419 and affecting the extracellular portion of KIT. In vitro studies showed that this mutation produces constitutively activated KIT, which is inhibited by the tyrosine kinase inhibitor imatinib. This is the first report of mastocytosis or GISTs associated with an exon 8 mutation and the first demonstration of KIT activation by a mutation affecting the juxtamembrane extracellular region. In addition, our data strongly suggest that imatinib is effective in patients whose tumors carry this mutation.

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Increased reactivity to *Candida albicans* elicited by atopy patch testing to commensal skin organisms in patients with symptoms of vulvar itch

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Allergic response may contribute to idiopathic vulvodynia. In this IRB approved study, 13 patients with a history of vulvar itch and/or burning were patch tested to a standard series of chemical allergens, a customized vulvar series and commensal organisms. Commensal organisms included UV-killed *Candida albicans (Ca), Malassezia sympodialis (Ms)* and dust mite, and were applied topically under occlusion as in the atopy patch test (APT). The APT sites were examined for reactivity at 6 hr, 48 hr, and 7days. Comparison was made to a group of 15 female Atopic Dermatitis (AD) patients. Patients with vulvar symptoms (n=13) were significantly (p=0.0046) more likely to be patch test positive to *Ca* than those patients in the comparison group (n=15). Reactivity in vulvodynia patients was not due to nonspecific reactions to commensals, because Ms and dust mite APT's did not show elevated reactivity relative to AD controls (p=0.4577 and p=0.4917). The vulvar series of chemical allergens demonstrated more relevant patient responses than did a standard series; the vulvar chemical series included acrylates, clothing dyes and azole antifungals. Interestingly, the *Ca* patch tests were inhibited at the highest concentrations even when positive at lower concentrations. Patients with vulvar symptoms may benefit from patch testing to a customized vulvar series as well as to low concentration *Ca*. Six patients who were APT + to *Ca* completed a 10 week course of fluconazole. Of these patients, 2 showed a marked improvement following treatment. Patients with symptoms of vulvar itch and burning who are *Ca* APT+ may be a special subgroup of patients who are allergic to baseline flora of yeast, possibly due to a reversal of tolerance to normal flora burden.

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Hyperthermia treatment of cancer cells *in vitro*

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Hyperthermia, the procedure of exposing cells to a temperature between 42 and 49°C, has been shown in clinical trials to be a promising approach for cancer treatment. However, to understand the underlying mechanisms of hyperthermic killing of cancer cells, it is critical to have an accurate temperature measurement technique and a reproducible heating method. To this end, we have developed a measurement method using thermocouples to monitor continuously the temperatures in multiple wells of a 96-well plate. Such a capability gives a complete record of time and temperature for the treatment procedure, and helps us in defining an accurate thermal dose. We have also compared several methods for heating 96-well plates, and found that use of copper blocks in the incubator provides a highly reproducible heating method. The commonly used method of using water-baths to heat cells *in vitro* resulted in a decrease of cell viability even at the control temperature of 37^{0}C and a decrease in the reproducibility of certain biological assays. Using our newly developed temperature measurement technique and heating method, we then examined the relationship of cell survival and hyperthermic thermal dose in several cancer cell lines, including melanoma and epithelial cancer cells. As expected, the cytotoxicity of hyperthermia was found to depend on the temperature and time of treatment. The results also clearly establish that hyperthermia kills various cancer cells including some that appear to be resistant to other apoptotic triggers, demonstrating the therapeutic potential for hyperthermia.

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Expression and potential pathological role of cathelicidin expression in rosacea

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The human cathelicidin antimicrobial peptide gene (CAMP) is expressed in skin by keratinocytes, mast cells, eccrine glands and infiltrating neutrophils. Expression correlates with resistance to infection, and increases following injury, infection or in psoriasis. One peptide product of CAMP, LL-37, has been shown in some systems to be chemotactic and angiogenic. To determine if this molecule is involved in rosacea, affected and normal facial skin was compared. Immunohistochemical staining showed that LL-37 is abundant in the epidermis of rosacea patients but barely detectable in normal skin. Dot-blot analysis showed that lesional skin from rosacea patients has 0.02 to 0.04 pmol/ug LL-37 and normal skin less than 0.001 pmol/ug. In situ hybridization revealed that CAMP mRNA is broadly detected in affected epidermis and neutrophils of rosacea patients. Preliminary quantitative RT-PCR data show that CAMP is expressed 20 to 30 times more in skin of rosacea patients compared to normals. The increase in CAMP may be stimulated by bacteria, as extracts from P. acnes, S. aureus, Group A Streptococcus, S. Epidermis, and Helicobacter pylori increased CAMP mRNA expression 2 to 4 fold in normal human keratinocytes in vitro. The cathelicidin peptide found in neutrophils, LL-37, increased IL-8 secretion from normal human keratinocytes 102.8 - 6.7 pg/ml and stimulated human dermal microvascular endothelial cell proliferation (1.1 nM, optimally). Conversely, cathelicidin peptides found on normal skin (RK-31, KS-30) increase antimicrobial activity but loose the ability to stimulate IL-8. These data suggest that cathelicidin is persistent in the skin of rosacea, and an excess of cathelicidin in the form of LL-37 can induce a proinflammatory chemokines and increase angiogenesis. Proper regulation of cathelicidin expression and processing may therefore be critical for the development of rosacea.

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An engineered autologous dendritic cell therapy induces potent tumor specific t-cell activity and clinical response in a patient with end-stage cutaneous t-cell lymphoma

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Sezary syndrome (SzS) is an end-stage variant of CTCL with no effective therapeutic options. DC-based immunization represents a promising new adjuvant immunotherapy for cancer and has been shown to elicit immune and clinical responses in patients with a variety of malignancies. The development of immunotherapy for CTCL has been limited by the lack of identified tumor-specific antigens and tumor induced immune deviation. To develop a tumor-specific DC based autologous immunotherapy for SzS we derived DCs from peripheral blood precursors of SzS patients, loaded DCs with antigens derived from whole circulating autologous tumor cells, and engineered the antigen-loaded DCs to express potent Th1-skewing T-cell activation function. In preclinical studies engineered DCs from several SzS patients demonstrated phenotypic characteristics and cytokine production characteristic of DC1 function, including upregulation of CD86 expression and secretion of high levels of IL-12p70. Engineered DCs induced potent Th1-skewed T-cell stimulation in mixed lymphocyte reactions. Immunization of an end stage SzS patient who had failed all other therapies with the autologous tumor-DC vaccine correlated with the development of a positive tumor specific DTH reaction, induction of a tumor specific IFNg producing CD8+ T-cell response (ELISpot assay), normalization of CD4:CD8 T-cell ratios and reduction of the clonal tumor population in peripheral blood to below levels of detection, and a dramatic clinical response. These results suggest that DCs loaded with antigens derived from autologous whole tumor cells and engineered to express potent Th1 skewing T-cell stimulatory function, can induce potent tumor specific T-cell responses in vitro and may induce effective anti-tumor immunity in vivo even in the setting of advanced disease. These preclinical studies and this single clinical application provide important data for the development of DC-tumor conjugate vaccines for the immunotherapy of CTCL.

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Protein delivery to cells and skin tissue via reversible decoration with transporter peptides

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In spite of numerous attractive intracellular targets, current therapeutic proteins target only the extracellular space due to a lack of a straightforward delivery method to reach cell interiors. Recent efforts to overcome this problem demonstrated that specific arginine-rich protein transduction (PTD) sequences from Tat, antennapedia, VP22 and others deliver proteins into cells and tissues. Current PTD limitations include the need to generate and denature PTD-protein fusions. This labor-intensive process, customized for each protein, often abrogates biologic activity. To address this, we developed a new approach to protein transfer that relies on a rapid, single-step decoration of any soluble protein by cysteine-flanked internally spaced arginine-rich (CFIS-R) transporter peptides. Native globular proteins were reversibly decorated with CFIS-R PTDs via charge interactions and disulfide bonding. Decoration dramatically enhanced cell and injection-based skin tissue delivery of a variety of functional proteins, including antibodies and enzymes (beta-galactosidase, PLAP, caspase-3). Protein transfer approached 100% efficiency within 30 minutes in a variety of cell types in vitro. Decorated proteins enter cells at low temperature in an energy-independent manner, suggesting minimal input from classical endocytic mechanisms. PTD-mediated cellular entry was not a mere fixation artifact as cellular internalization was clearly observed with fluorescein-conjugated antibodies to actin, tubulin and the GM130 golgi protein. These antibodies were detected in their appropriate corresponding subcellular locations in unfixed, living cells in contrast to undecorated antibody which failed entry. Injection of decorated caspase-3 into subcutaneous tumors in vivo produced a >184-fold increased apoptotic index over untreated tumors and a >5-fold increase over undecorated caspase-3 alone, confirming retention of decorated protein function in vivo. Decoration with transporter peptides thus provides an attractive general means of intracellular delivery of functional proteins in vitro and in tissue.

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The efficacy of denileukin diftitox in patients with low expression of CD25: a retrospective chart review of 16 patients with CTCL

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Denileukin diftitox is thought to selectively target and kill cells with surface expression of CD25, a component of the high affinity IL-2 receptor. Denileukin diftitox is thought to act in CTCL by inducing apoptosis of malignant T cells expressing CD25+. Use of this drug has therefore only been recommended in patients expressing CD25 on >20% of malignant cells. We recently observed complete clearance of recalcitrant stage IVA Sezary syndrome in a patient with <5% CD25+ T cell after the first two of six cycles of denileukin diftitox, suggesting that levels of lesional CD25 expression does not accurately predict treatment response. We therefore undertook a retrospective chart review of 16 CTCL patients treated with denileukin diftitox at our facility with the goal of comparing CD25 expression and therapeutic response. Patient response was classified into the following four groups based on changes in tumor burden six weeks after initiation of therapy: Complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). 100% of patients with CD25 expression greater than 20% experienced a partial or complete clinical response to denileukin diftitox. Surprisingly, 77% of patients with less than or equal to 20% CD25+ cells also experienced a positive treatment response. The patient numbers in this retrospective analysis are small, and definitive conclusions cannot be made. Nevertheless, our study suggests that denileukin diftitox may be useful in the treatment of patients with CTCL regardless of their levels of CD25 expression. We hypothesize that denileukin diftitox may be acting to kill CD4+CD25+ regulatory T cells, a subset of cells recently shown to interfere with the successful development of tumor immunity. Removal of these immunosuppressive cells may allow the development of an effective innate tumor response. Confirmation of this concept could lead to successful use of this drug in patients with a variety of malignancies.

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NLite pulsed dye laser therapy for acne vulgaris: a randomized clinical trial

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The NLite laser was recently reported to have substantial efficacy in the treatment of acne (*The Lancet*, Vol 362, Oct., 2003). We also sought to evaluate this pulsed dye laser therapy for acne. Ours was a randomized, controlled, split-face clinical trial. Forty subjects with facial acne received 1 or 2 pulsed dye laser treatments to half of the face using the NLite laser (585nm wavelength, 3J/cm^2, 350µsec pulse duration). Subjects were serially assessed for 12 weeks. The evaluating physician was blinded to treatment assignment and did not perform the laser procedures. Subjects were evaluated for total counts of papules, pustules, cysts, comedones, and red macules. As no difference in efficacy between subjects receiving 1 vs 2 treatments was found, the data were pooled for analysis. Counts of each type of lesion failed to reveal any statistically significant differences between treated and untreated skin at any time point. For example, week 12 mean papule counts were increased by 4.3 and 2.0 in treated and untreated skin ($p = 0.13$), respectively. Other lesion counts similarly revealed no significant differences as they tended to ebb and flow bilaterally with statistically insignificant improvements and periods of slight worsening. Three dermatologists, who were blinded to treatment assignment and who did not participate in either the treatment or assessment of subjects, independently evaluated standardized bilateral serial subject photographs obtained during the study. Photos from baseline and weeks 4 and 12 were scored for overall acne severity using the Leeds scale. This confirmed the clinical findings with no statistically significant differences in acne severity found between treated and untreated skin. Several lasers have been reported to improve acne; however, few well-designed clinical trials in this area have been available. While laser therapy may prove useful, our study does not corroborate the recent report of efficacy of NLite laser therapy for acne, perhaps due to the presence of differences in study design between the two trials.

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Durability of treatment response following withdrawal from etanercept in psoriasis patients

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Dermatologists may choose to withdraw psoriasis therapies intermittently following a satisfactory response. Herein we describe disease characteristics and the durability of treatment response in psoriasis patients in a phase 3 trial of etanercept, which had a 24-week double-blind period followed by a study drug withdrawal period. Patients were randomized to receive etanercept (50 mg twice weekly [BIW], 25 mg BIW, or 25 mg once weekly [QW]), or placebo BIW. Placebo patients began etanercept (25 mg BIW) at week 12 in a blinded fashion. At week 24, all patients who achieved a treatment response (had ≥50% decrease from baseline PASI score) were discontinued from etanercept and followed until their disease relapsed. Durability of treatment response was defined as the time period from the discontinuation of etanercept (week 24) until the first visit at which at least 50% of PASI improvement that occurred between baseline and week 24 was lost. Of 652 patients initiating this trial, 573 (87.9%) completed 24 weeks of treatment, and 409 (71.4%) achieved a treatment response (at least PASI 50) at week 24. Upon drug withdrawal, the median time to disease relapse was 85 days, and 25% of the responders did not relapse until at least 145 days. During the withdrawal period, only 1 patient (from the 25 mg QW group) met the National Psoriasis Foundation definition of rebound (return to more than 125% of baseline PASI score within 3 months). No patients rebounded to 150% or more of their baseline PASI score. Importantly, no serious adverse events or hospitalizations occurred that were related to worsening psoriasis, and no conversion of psoriasis morphology (eg, guttate, erythrodermic, or pustular) occurred during the withdrawal period. When psoriasis patients are withdrawn from etanercept, the return of signs and symptoms is gradual and without untoward events.

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Merkel cell carcinoma (MCC): a novel treatment for stage III disease and a proposed algorithm for initial management

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MCC is a rare (~500 cases/year in US), but lethal skin cancer (~25% mortality) with high rates of local recurrence and metastasis. Given the rarity of this neuroendocrine malignancy, there is little data on outcome, and no uniformly accepted treatment recommendations. Based on similarities to small cell lung cancer (SCLC), another neuroendocrine tumor, MCC has been treated with SCLC chemotherapy regimens with limited success and significant toxicity. A recent trial with thalidomide (putative antiangiogenic agent) and temozolomide (analog of alkylating agent DTIC) demonstrated efficacy and decreased toxicity in metastatic neuroendocrine tumors. We report two patients with stage III MCC treated with this combination: 1) 81 year old man who presented with scalp MCC that metastasized following surgery and during radiotherapy and 2) 51 year old woman with retroperitoneal MCC that progressed on irinotecan, cisplatin and radiotherapy. Significant disease regression was noted in both patients on thalidomide (200 mg po qd) and temozolomide (150 mg/m2 po qd, every other week). While this combination appears to have activity in patients with metastatic MCC refractory to more standard treatments, further trials are planned to confirm these observations. Through our experience with >40 patients and analysis of available literature (>300 patients), we propose the following working treatment algorithm for MCC: 1) surgery: a margin of >/=2cm, when possible, 2) sentinel lymph node biopsy: indicated due to high rate of clinical or microscopic lymph node involvement at presentation (45% in our series), 3) radiotherapy: >/=50Gy to primary site and draining lymph nodes is associated with a statistically significant decrease of >50% in local recurrence, and 4) chemotherapy: consider for high-risk patients with good functional status or as palliative therapy. Especially in the case of adjuvant chemotherapy, multi-institutional randomized controlled trials must be organized.

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Aluminum oxide microdermabrasion induces transcription factors, cytokines, and matrix metalloproteinases that function in dermal extracellular matrix remodeling in human skin *in vivo*

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Microdermabrasion is reported to improve fine wrinkles in photodamaged skin through stimulation of dermal collagen remodeling. However, molecular mechanisms by which microdermabrasion acts in skin have not been elucidated. Therefore, we examined biochemical responses, associated with dermal collagen remodeling, following microdermabrasion in human skin *in vivo*. A single, standard microdermabrasion procedure with aluminum oxide crystals was performed at 15 mm Hg for three passes on the hip of volunteers. Skin samples were obtained 15 min to 16 days post treatment, and genes of interest were analyzed for alterations of mRNA and protein expression by reverse-transcriptase real time PCR, and immunohistochemistry, respectively. Transcription factors NF-κB and c-Jun were rapidly activated, within 1 hour of treatment (p<0.05, n=6). Activation was localized to superficial epidermis or the stratum granulosum. NF-κB and c-Jun-regulated genes include primary cytokines, IL-1β and TNF-α. Expression of both primary cytokines was elevated within 2 hours post treatment, and was maximal (9-fold and 4-fold, respectively, n=6, p<0.05) at that time point. Both IL-1β and TNF-α induce matrix-degrading metalloproteinases (MMP), which were elevated within four hours post treatment. MMP-1 (collagenase), MMP-3 (stromelysin), and MMP-9 (92kDa gelatinase) were maximally elevated 300-fold, 350-fold, and 2.5-fold, respectively, within 8 hours of treatment (n=12, p<0.05). MMP expression occurred focally in epidermis and dermis near the dermal-epidermal junction. In summary, a single microdermabrasion treatment causes a cascade of acute molecular alterations that result in increased MMP expression in the upper dermis in human skin *in vivo*. Increased MMP expression may degrade damaged collagen, and thereby initiate collagen remodeling in photodamaged skin.

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Foot ulcers in people with diabetes: signs of infection are the major determinant for use of cultures, antibiotics and x-rays and are associated with amputation

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This study describes patients with diabetes and moderately severe foot ulcers, their signs of infection, patterns of utilization for bacterial cultures, antibiotics, x-rays, and their subsequent association with amputation. The diagnostic tests and treatment of patients with new onset diabetic foot ulcers were prospectively monitored in six hospitals and outpatient settings until ulcer healing, amputation, or death. Patients were divided into 5 groups (0,1,2,3,4) based on the maximum number of signs of infection at any given visit during the ulcer episode. The signs of infections were erythema > 1cm, serosanguineous or purulent drainage, abscess and local peri-ulcer warmth. Percentage of patients who had cultures, antibiotics, and x-rays in each group were calculated. Amputation rates were calculated by group. There were 128 patients with foot ulcers of intermediate severity across groups 0-4. The percentage across groups for bacterial cultures during the episode ranged from 20-85% and averaged 53% overall. Use of IV and/or oral antibiotics ranged from 75-100% and averaged 84% overall. X-Ray utilization ranged from 60% to 100% and averaged 67% overall. The overall amputation rate was 23% and showed an increase as number of infection signs increased. Prognosis for limb survival decreased with increasing signs of infection. Increasing numbers of signs of infection resulted in an increased utilization of cultures, X-rays and antibiotics. Additional research is needed to understand why in individuals with new onset foot ulcers and no symptoms of infection, the use of antibiotics reached 75%.

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Topical application of a formulation containing the osmoprotectant sorbitol, increases barrier strength and skin moisturization

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Cells undergoing hyperosmotic stress accumulate sorbitol, a compatible organic osmolyte. The renal medulla uses sorbitol, and a host of other organic osmolytes to help cope with a routine exposure to high extracellular osmolality. Epidermal keratinocytes experience similar high osmolality under dry environmental conditions, due to increased transepidermal water loss, and a concomitant drying of the skin. We initially wanted to determine if epidermal keratinocytes, in vitro, could be protected from high osmotic stress, with the exogenous addition of sorbitol. Additionally, we evaluated the effects of formulation of topical sorbitol on skin barrier and moisturization. In our in-vitro experiments, 50 mM sorbitol protected epidermal keratinocytes from osmotic toxicity induced by sodium chloride. In-vivo studies displayed significant increases in both barrier repair and moisturization, in individuals subjected to arid environmental conditions.

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Tacrolimus ointment enhances Th1 and Th2 sensitization through the skin

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Topical calcineurin inhibitors, such as FK506 (tacrolimus), are highly effective in the treatment of atopic dermatitis, presumably due to their ability to inhibit lymphocyte activation. Several groups have shown that systemic administration of FK506 suppresses both the sensitization and elicitation phases of Th1-mediated contact hypersensitivity (CHS) responses in mice. However, the effect of systemic FK506 on Th2 responses induced in the cutaneous environment, such as by epicutaneous (e.c.) exposure to protein antigen, has not been evaluated. Furthermore, the effect of topical FK506 on generation of either Th1 or Th2 responses to antigens encountered through treated skin has not been determined. In this study, FK506 ointment, or vaseline as a control, was applied to shaved back skin of mice on days -4 to -2. Treated areas were covered to prevent ingestion until day -1, when the ointment was removed. For generation of Th2 responses, mice were then exposed on day 0 to e.c. ovalbumin (OVA) (100 μg) in PBS at the same site. Skin draining lymph node (LN) cells were harvested on day 4 and restimulated in vitro with OVA for evaluation of cytokine production. LN cells from mice pretreated with topical FK506, compared with vaseline controls, produced significantly more IL-5 (p=.002) and IL-13 (p<.001) following e.c. exposure to OVA. IFN-γ production by LN cells was also enhanced (p=.03) in FK506 pretreated mice. Consistent with these results, topical FK506 pretreated mice had increased OVA-specific IgG1 (p=.006) in serum. For generation of Th1 type responses, topical FK506 or vaseline pretreated mice were exposed to e.c. DNFB on day 0. CHS to DNFB was also enhanced by pretreatment with topical FK506, as shown by increased ear swelling responses following challenge on day 5 (p<.001). Of note, systemic treatment with FK506 (5 mg/kg i.p.; day -2 to 6) did suppress CHS responses (p<.001), as reported by others. The data indicate that topical exposure to tacrolimus ointment can enhance both Th1 and Th2 type immune responses following subsequent encounter with antigen at the same site.

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The efficacy and safety of etanercept in the retreatment of psoriasis after relapse

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Dermatologists are sometimes required to use intermittent and/or rotational paradigms when treating chronic psoriasis patients. Because the safety and efficacy profiles of etanercept used intermittently are unknown, we studied the effects of study drug withdrawal and retreatment in a large phase 3 trial, in which patients were initially treated with etanercept over a 24-week double-blind period followed by a study drug withdrawal and retreatment period. Patients were randomized to receive etanercept (50 mg twice weekly [BIW], 25 mg BIW, or 25 mg once weekly [QW]), or placebo BIW. Placebo patients began etanercept (25 mg BIW) at week 12 in a blinded fashion. At week 24, patients classified as responders (≥ 50% decrease from baseline PASI) were discontinued from study drug and followed until their disease relapsed (loss of ≥ 50% of PASI improvement obtained between baseline and week 24), when they resumed blinded etanercept for up to 24 weeks. Of 652 patients initiating the trial, 573 (87.9%) completed 24 weeks of treatment, and 409 (71.4%) achieved a treatment response (at least PASI 50) at week 24 (responders) and entered the study drug withdrawal and retreatment period. Of the 409 patients, 342 relapsed and received at least 1 dose of retreatment, and 297 completed 12 weeks of retreatment. The overall effect of etanercept retreatment was similar to the initial treatment effect: the mean PASI score was 5.8 at week 12 of initial active treatment and 6.4 at week 12 of retreatment. PASI 75 and PASI 50 response rates also were similar during initial treatment and retreatment with etanercept. The rates of adverse events, infections, and antibody formation in the 12-week retreatment period were similar to the first 12 weeks of treatment. No neutralizing antibodies to etanercept were noted. These data suggest that etanercept can be used safely and effectively in an intermittent or rotational paradigm in the treatment of psoriasis.

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Sustained anti-fibrotic responses to a single UVA1 exposure are attenuated by subsequent exposures in human skin *in vivo*-implications for phototherapy for fibrotic skin diseases

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UVA1 phototherapy for fibrotic skin diseases such as scleroderma has been reported to show promise. The ability of UVA1 to soften fibrotic skin is believed to be related to induction of matrix-degrading metalloproteinases (MMP) and suppression of type I procollagen (COL-I) production. However, optimal UVA1 dosage and exposure frequency have not been systematically studied. Therefore, we determined effects of UVA1 dose and exposure frequency on MMP-1 and COL-I expression in human skin. Pure UVA1 (340-400nm) light source was used to irradiate sun-protected buttock skin of 36 healthy subjects. All subjects were skin-phototype I or II. MMP-1 and COL-I mRNAs were measured by quantitative real time RT-PCR. UVA1 induction of MMP-1 and inhibition of COL-I were dose-dependent, reaching maximum at 130J/cm^2 (1149-fold; p<0.05; n=8) and at 150J/cm^2 (42%, p<0.05; n=8), respectively, 24 hours post-irradiation. MMP-1 (246-fold induced, p<0.05; n=8), and COL-I (35% inhibited, p<0.05; n=8) mRNA levels remained significantly altered 7 days after a single UVA1 exposure (130J/cm^2). Surprisingly, intervening UVA1 exposures, given on day 2 and day 4 after the initial exposure, substantially lessened responses. MMP-1 induction was decreased from 246-fold to 3-fold, and no inhibition of COL-I was observed, 7 days post the initial exposure. Thus, within the first week following a single UVA1 exposure, subsequent exposures reduce MMP-1 and increase COL-I expression. Furthermore, UVA1 (130J/cm^2) exposures at weekly intervals caused successively less responses. MMP-1 induction was reduced from 1149-fold to 59-fold (p<0.05; n=8), and COL-I reduction was no longer observed, 24 hours post the third weekly exposure. Attenuation of responses to successive UVA1 exposures was associated with increased tanning. These data indicate that UVA1 phototherapy at weekly or greater intervals may be most effective for treating fibrotic skin diseases.

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Achieving target lipid levels in patients with peripheral vascular disease

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Peripheral vascular disease (PVD) is an important factor in the development of leg ulcers and is associated with a significant increase in mortality, therefore risk factor control is paramount. Current guidelines recommend that patients with PVD achieve the same low density lipoprotein (LDL) cholesterol goal as patients with coronary artery disease or diabetes mellitus, but often this is not met. The purpose of this study is to determine if these guidelines are being met and if severity of PVD is predictive of adherence to target LDL goals.

We conducted a retrospective chart rev

nosed with PVD in 2001. The population consisted of patients who had undergone non-invasive vascular studies that year and who had received an ankle-brachial index (ABI) score of less than one in either extremity. Via a detailed electronic medical record review, data was extracted on patient demographics, weight, comorbid conditions, vascular procedures, medications, smoking history, and laboratory results for a follow-up period of 12-18 months. The primary outcome was LDL, with a target level of less than 100 mg/dl. Student's t-tests, Fisher Exact tests, and chi-square tests were performed to associate baseline characteristics with being at goal LDL level. Multivariate logistic regression modeling also was performed.

A total of 175 patients met the inclus

whom were white, older men. The majority of these patients achieved the recommended goal LDL cholesterol. We conclude that veterans with PVD and a specific comorbidity profile are likely to have an LDL cholesterol at goal level. The translation of these findings into improved clinical outcomes is yet unknown.

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Maintenance of efficacy in patients with psoriasis following dose reduction: results of a global phase 3 study

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In this global phase 3 study, 583 patients were randomized to receive etanercept 50 mg twice weekly (BIW, n=194) or 25 mg BIW (n=196) or placebo (n=193) by subcutaneous injection for 12 weeks. Patients from all 3 groups then received etanercept 25 mg BIW for the next 12 weeks. The primary endpoint was the proportion of patients who achieved at least a 75% improvement in the Psoriasis Area and Severity Index (PASI 75) at 12 weeks. At baseline, the groups were balanced for age, duration of psoriasis, and disease severity. At week 12, 49% of patients in the etanercept 50-mg BIW group achieved the PASI 75, compared with 34% in the 25-mg BIW group and 3% in the placebo group (p<0.0001 for each dose group vs. placebo). Statistically significant improvements in physician's and patient's static global assessments and Dermatology Life Quality Index confirmed the efficacy of etanercept. In a last-observation-carried-forward analysis at week 24, the PASI 75 was achieved by 54% of patients in the original 50-mg BIW group, 45% in the original 25-mg BIW group, and 28% in the original placebo group. In the group that received 50 mg BIW for the first 12 weeks and a reduced dose of 25 mg BIW for the second 12 weeks (n=179 with PASI assessments observed at both time points), 77% of PASI 75 responders at week 12 maintained the response through week 24, and 32% who had not responded at week 12 achieved a PASI 75 response by week 24. Etanercept was well tolerated. The proportions of patients reporting adverse events and infections were similar between etanercept and placebo groups throughout the study. In conclusion, etanercept provided rapid, clinically meaningful benefit to patients with psoriasis. The majority of patients treated successfully at 50 mg BIW can have their dose reduced to 25 mg BIW after 12 weeks without loss of treatment response through 24 weeks.

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Methotrexate reduces incidence of vascular diseases in veterans with psoriasis or rheumatoid arthritis

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Methotrexate (MTX) is used in the treatment of moderate to severe psoriasis and rheumatoid arthritis (RA). As MTX oppositely affects inflammation and hyperhomocysteinemia - two independent risk factors for vascular disease - it is unclear how it influences the incidence of vascular disease in these patients. The objective of this study is to determine if long term MTX therapy affects the incidence of vascular disease in patients with psoriasis and rheumatoid arthritis. We conducted a retrospective, case-control study of computerized patient records of 7,615 outpatients with psoriasis from October 1st, 1998 through April 30th, 2003, and 6,707 with RA from October 1st, 1999 through April 30th, 2003. A total of 14,322 with psoriasis or RA were included in the analysis. After adjusting for age, gender, co-morbidities and confounding medications, patients on MTX had significantly reduced risk of vascular disease (psoriasis: RR=0.73, 95%CI=0.55-0.98; RA:0.83; 0.71-0.96). MTX was more beneficial in prevention of vascular disease among patients who took a low to moderate cumulative dose (psoriasis: 0.50, 0.31-0.79; RA:0.65, 0.52-0.80). We also analyzed effects of combination of MTX and folic acid therapy on the incidence of vascular disease. Multivariate logistic regression analysis of this subgroup of patients demonstrated a 44% reduction in vascular diseases for psoriasis patients who were also prescribed MTX (0.56; 0.39-0.80) and a 23% reduction for RA patients with combined therapy (0.77; 0.38-1.56). MTX reduced the incidence of vascular disease in veterans with psoriasis and rheumatoid arthritis, especially in low to moderate doses and/or when used concomitantly with folic acid supplements. Treatment of inflammation in these patients may have benefit beyond the control of primary disease alone.

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Pharmacokinetics of etanercept are unaltered by concurrent administration of methotrexate in rheumatoid arthritis patients

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Our purpose was to evaluate the potential impact of concurrent administration of methotrexate (MTX) on the pharmacokinetics (PK) of etanercept in rheumatoid arthritis (RA) patients. As part of a double-blind randomized trial of 682 RA patients receiving MTX, etanercept (25 mg twice weekly [BIW]), or etanercept (25 mg BIW) plus MTX (median: 20 mg once weekly), 50 patients were randomly selected from each of the etanercept-treated groups for analysis of serum samples. A single blood sample was drawn from each patient at any time after etanercept dose at week 24. A final covariate population PK model was built based on etanercept data from both healthy subjects (n=53) and RA patients (n=212) from 10 prior clinical studies. The influence of demographic characteristics on the PK of etanercept was evaluated. The stability of the final model was evaluated using both internal (bootstrapping) and external (data-splitting) validation approaches. PK parameters were compared using statistical (bioequivalence) criteria. Data from the prior studies were optimally fitted to a two-compartment model with first-order elimination from the central compartment. Interindividual variability of the PK parameters was quantified for CL (25.1%), V_c (41.7%), k_a (53.1%), and F (24.2%). Residual variability consisted of a combined additive (11.4 ng/mL) and proportional error (49.9%). Both age (<17 yrs) and body weight (<60 kg) were found to be important covariates on clearance (CL). The results of both internal and external validation indicated the validity of the population model. Based on the final population PK model, the individual CL values (mean ± SD, in L/h) by Bayesian approach were comparable for patients receiving etanercept alone (0.070 ± 0.007) and those receiving etanercept plus MTX (0.067 ± 0.006). The GLS mean ratio (etanercept plus MTX / etanercept alone, mean [90% CI]) was 0.97 (0.94, 1.00). The PK of etanercept are unaltered by concurrent MTX administration in RA patients. Thus, no etanercept dose adjustment is needed for patients taking MTX.

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Phase II trial of oral suberoylanilide hydroxamic acid (SAHA) for cutaneous T-cell lymphomas

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Acetylation of core nucleosomal histones by opposing histone acetyltransferases and histone deacetylases (HDACs), regulates chromatin structure and gene transcription. Altered HDAC activity is implicated in malignancy. SAHA, a novel inhibitor of class I &II HDACs, induces growth arrest, differentiation, and apoptosis of transformed cells in vitro and inhibits tumors in xenograft models. To determine safety and efficacy of SAHA, we enrolled 25 CTCL patients in a phase 2 open-label trial of oral SAHA, sequentially assigned to either once a day or an intermittent dosing schedule. Patients had refractory/relapsed disease following prior systemic therapies: Ontak (n=12), Targretin (n=21), and/or systemic chemotherapy (n=19). Thirteen patients of median age 64 (range 38-83) received SAHA 400 mg once daily. Their stages were IB (n=1), IIA (n=1), IIB (n=2), IVA (n= 5) or IVB (n = 4); median treatment duration was 12 weeks (range 4-32 weeks). By Physician Global Assessment (PGA), 5 achieved PR (4 Sezary, 1 transformed MF), five had SD, 3 PD for RR of 38%[95% CI: 14%, 68%]. Mean duration of response was 15 weeks (range 8-24 weeks); mean time to response was 10 weeks (range 4-16 weeks). Adenopathy regressed in 7 of 9 in 4 weeks. Two required dose reductions due to dehydration. Twelve patients received 300 mg bid x 3 consecutive days per week with a median age of 71 (range 26-80) and stage IB (n=1), IIB (n=1), IVA (n=7), IVB (n=3). Three patients withdrew at 2-4 weeks: 2 for emboli due to DVT at sub-therapeutic INRs and one for drug rash. Two SS patients achieved PRs (17%) with duration of 8 & 24 weeks, 3 had SD, & 5 progressed. A majority, at either dose, reported >50%-100% reduction in pruritus. Adverse side effects included fatigue, change in taste, GI complaints, shortness of breath, anemia, hypokalemia, increased creatinine, non-specific EKG changes, and thrombocytopenia. SAHA is a well tolerated, orally administered HDAC inhibitor that appears to have significant activity in heavily treated advanced refractory CTCL patients.

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DNA oligonucleotides therapy for UV-induced squamous cell carcinomas

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We have previously reported that DNA oligonucleotides homologous to the telomere sequence (T-oligos) induce multiple cancer prevention responses in normal and malignant cells including transient growth arrest, enhanced DNA repair capacity, senescence and apoptosis. To determine if T-oligos might be effective in treating human squamous cell carcinomas (SCC) the human SCC12F cell line was treated with T-oligos (pGTTAGGGTTAG 40 µm) or diluent alone and determined the expression of selected cell cycle and apoptosis regulating genes (bclx, p21, bax and Mcl-1) using the ribonuclease protection assay. Within 24 hr, the mRNA level of bclx was unaffected, but compared to controls (100%), T-oligos up-regulated mRNA levels of p21 (170%), and the proapoptotic genes bax (190%) and Mcl-1 (260%). To examine the effect of T-oligos on UV-induced SCC in vivo, we UV irradiated hairless mice using a known carcinogenic protocol. Resulting SCC with diameters <3mm and 3-7mm were injected (inj) with T-oligos (0.6 nMoles) intralesionally 3 times a week until disappearance or for up to 4 weeks. T-oligos reduced ($p<0.005$) small SCC volume by 83% on average, with 4 of 9 disappearing completely, whereas control vehicle (saline) inj SCC doubled in size. T-oligos reduced larger SCC volume by 30% ($p<0.07$, n=6), whereas controls enlarged 9-fold. One day after the last inj all SCC were bisected and stained with TUNEL and Ki67 to evaluate T-oligo effect on apoptosis and rate of proliferation, respectively. T-oligo led to ~6-fold increase in the number of TUNEL + presumably apoptotic cells (2216±326 vs 406±135 per mm2 SCC, $p<0.0001$). There was also a 20% decrease in the number of Ki67 + cells in the T-oligo vs. diluent-inj SCC. We propose that T-oligo activation of DNA damage signaling pathways in UV-induced SCC may provide a new non-surgical skin cancer therapy.

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Topical immunomodulators and topical steroids usage in the U.S. for inflammatory dermatoses

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Topical steroids have been the mainstay of therapy for inflammatory dermatoses. In January 2001, the first of two topical immunomodulators (TIMs), tacrolimus, was introduced into the market for the treatment of atopic dermatitis and eczema, followed by the distribution of pimecrolimus in January 2002. To determine the impact of these new medications in the treatment of inflammatory dermatoses, semiannual data from National Data Corporation, an independent data collection company that gathers information on prescribing trends from participating pharmacies, were analyzed. About 6 million prescriptions for topical steroids are issued semiannually; these numbers have not changed appreciably over the past 3 years. Interestingly, utilization of TIMs has risen dramatically, with over 1.7 million prescriptions in the first half of 2003, or a 3.5:1 steroid:TIM ratio currently. Dermatologists utilized TIMs immediately upon availability; whereas other physicians (internists, family practitioners, and pediatricians) lagged behind initially. Non-dermatologists prescribe pimecrolimus more frequently than tacrolimus, at a ratio exceeding 8:1. In contrast, dermatologists, prescribe at a 3:2 ratio of pimecrolimus to tacrolimus. Moreover, dermatologists account for over 73% of all tacrolimus prescriptions. The data confirm that topical steroids continue to be the mainstay of treatment of inflammatory dermatoses in the U.S. Moreover, the data suggests that physicians have embraced co-utilization of TIMS and topical steroids. Finally, the predominance of pimecrolimus usage may be attributable to marketing influences, since pimecrolimus is marketed to all physicians, whereas tacrolimus is marketed primarily to dermatologists.

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Efficacy of etanercept in an integrated multistudy database of patients with psoriasis

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Etanercept has been approved in patients with rheumatoid arthritis (RA), juvenile RA, and psoriatic arthritis at a subcutaneous dose of 25 mg twice weekly (BIW) and has been studied at doses of 25 mg BIW and 50 mg BIW in patients with psoriasis. Here we report the efficacy of etanercept in 1187 psoriasis patients across one phase 2 and two phase 3 studies. Comparisons presented between the 25-mg BIW dose (N=415) and placebo (N=414) used data pooled across all 3 studies. Comparisons between the 50-mg BIW dose (N=358) and placebo (N=359) used data pooled across the phase 3 studies. Pooled data were analyzed using a 2-sided Cochran-Mantel-Haenszel test stratified by study. The primary endpoint was ≥75% improvement from baseline in the Psoriasis Area and Severity Index (PASI) at 12 weeks. Three percent of patients receiving placebo and 33% receiving 25 mg BIW etanercept achieved a PASI 75 ($p<0.0001$). In the 2 studies that included the highest dosage, 3% of patients receiving placebo and 49% receiving 50 mg BIW etanercept achieved a PASI 75 ($p<0.0001$). Highly significant efficacy was also demonstrated for all secondary endpoints at week 12 for both etanercept doses, including PASI 50 and 90 responses, percent improvement from baseline in PASI score, physician's static global assessment (clear/almost clear), Dermatology Life Quality Index, and patient's global assessment. Patients treated with 50 mg etanercept BIW in the phase 3 studies showed a high degree of clearing at week 12, with PASI 90 response rates of 21% vs 1% for placebo. In studies where double-blind treatment continued through 24 weeks, responses to etanercept continued to improve with longer treatment. In conclusion, etanercept 25 mg BIW and 50 mg BIW provided consistent, robust efficacy in the treatment of chronic plaque psoriasis. Etanercept provided a high degree of psoriasis clearing, improvement in both physician- and patient-reported outcomes, and significant impact on quality of life.

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Minoxidil promotes hair growth in androgenetic alopecia by increasing the ratio of terminal/vellus hairs and the number of proliferating Ki67-positive hair follicle cells in horizontal skin sections

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Minoxidil has been shown to promote hair growth. The short-term effects of a 5% solution of minoxidil on the ratio of terminal/vellus hair and the proliferation marker Ki67 have not been examined before. We tested the effects of a 5% solution of minoxidil applied twice daily for 12 weeks on the scalp of 10 women and 9 men with androgenetic alopecia. Hair regrowth was evaluated by histologic analysis of paired 5 mm punch biopsy specimens for horizontal sectioning taken from the parietal region of the scalp from the right side on week 0 and the contra lateral side on week 12. In the histologic sections, the diameter of the hair shafts was determined in blinded fashion employing a micrometer grid; a hair shaft with a diameter of <30 micrometer was defined as vellus hair, a diameter of > 30 micrometers as a terminal hair; data are presented as the ratio of terminal/vellus hair. Cellular proliferation in hair follicles was ascertained by Ki67 staining using the alkaline phosphatase method by counting the stained nuclei of 5 hair follicles in blinded fashion (Dako, Denmark). In addition, visual analysis was performed by scalp photography. We found the following ratios of terminal/vellus hair in women: 2.99 +/- 0.49 (week 0, mean +/- SE) vs. 4.74 +/- 0.54 (week 12, $p<0.05$, Wilcoxon test); in men: 2.89 +/- 0.50 vs. 3.59 +/- 0.54 ($p<0.05$). Ki67 staining in hair follicles in women was: 8.04 +/- 1.45 (week 0) vs. 9.59 +/- 1.62 (week 12, $p<0.05$); in men: 10.98 +/- 1.53 vs. 11.18 +/- 1.18 ($p>0.05$, resp.); the combined values of men and women for Ki67 at week 0 were 9.35 +/- 1.08 vs. 11.3 +/- 1.1 at week 12 ($p = 0.02$). Scalp photography corresponded to increased hair growth after 12 weeks. These findings show that a twice daily, 12-week treatment with 5% topical solution of minoxidil enhances hair growth by increasing the ratio of terminal/vellus hairs in both men and women and by increasing Ki67 in hair follicles.

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Epidermal Genetic Information Retrieval is a non-invasive method of evaluating message (mRNA) profiles of lesional versus non-lesional skin of psoriatic subjects before and after initiations of therapy

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The purpose of this study is to determine the genomic expression in the stratum corneum of lesional and non-lesional skin and the changes with treatment. A non-invasive retrieval method known as the Epidermal Genetic Informational Retrieval (EGIR) utilizes a tape to collect the stratum corneum. Target sites are cleaned with alcohol and tape is applied with repeated pressure for 30 seconds to standardize collection. Four tape strips are performed at each site. In the current trial, we have 4 cohorts of 7 patients treated with alefacept, etanercept, clobetasol or narrow band UVB. Patient visits are at week 0, 1, 2, 4, 8, 12 and 24 in all cohorts except the clobetasol group which are at week 0, 2, 5, 8, and 11. Patients are evaluated for body surface area involvement, erythema and thickness using the National Psoriasis Foundation Score. mRNA is collected from one target psoriatic lesion and three standard sites. The mRNA is extracted and RT-PCR performed using specific primers. A minimum of 200 pcg is needed to accurately quantify mRNA relative to b-actin. Results demonstrate significant differences in message between involved and uninvolved skin. On average, non-lesional skin yields around 1 ng of mRNA whereas yields in lesional skin are ten-fold higher. Keratin 16, GAPDH and TNF are elevated in psoriatic skin compared to uninvolved skin while other markers including CD2 and INF were variable. Data after initiation of therapy are preliminary but do show a trend in normalization of message in TNF and keratin 16. We expect the changes in message to correlate and precede the clinical improvement of psoriasis. We predict EGIR will allow us to better understand the pharmacodynamics of psoriasis therapy and provide us with a prognostic tool in evaluating treatment outcomes.

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Dietary vitamin E: delayed bioavailability in human skin and preferential accumulation of α-tocopherol in human sebum

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While topical vitamin E has been studied extensively, little is known on the oral bioavailability of this antioxidant in skin. We have demonstrated that the highest physiological concentrations of vitamin E are found in facial sebum.[1] We hypothesized that the oral bioavailability of vitamin E in human skin is largely dependent on sebaceous gland secretion and is thus site- and compartment-specific. To test this, 24 healthy volunteers (30±9 years; means±SD) were subjected to a randomized daily supplementation with either 400mg RRR-α-tocopheryl acetate (RRR-α-toc) or 400mg all-rac-α-tocopheryl acetate (all-rac-α-toc) for 14 days. Serum, facial sebum, and lower arm skin surface lipids (SSL) were collected at 0h, 12h, 1d, 2d, 3d, 7d, 14d and 21d after starting supplementation. Sebum and SSL were collected using sebutapes and ethanol extraction, respectively, and analyzed by HPLC using electrochemical detection for α-tocopherol(toc) and UV-detection for squalene.2 Serum α-toc levels were increased as early as 12h after supplementation of RRR-α-toc or all-rac-α-toc and peaked on day 7 with an average increase of 76% and 79%, respectively. No significant changes were observed in lower arm SSL at any time point. Remarkably, while sebum levels remained unchanged during the first 14 days of supplementation, both the RRR-α-toc and the all-rac-α-toc group showed increased α-toc levels in sebum of 87% and 92%, respectively. In conclusion, with respect to dietary supplementation of vitamin E and its bioavailability in human skin these results suggest that a) sebaceous gland secretion is a major mechanism leading to site-specific differences; b) the bioavailability of RRR-α-toc and the all-rac-α-toc is comparable; c) possible protective effects in the skin will not be achieved before a supplementation period of 2-3 weeks.

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Safety of etanercept in an integrated multistudy database of patients with psoriasis

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Etanercept has been approved for the treatment of rheumatoid arthritis (RA), juvenile RA, and psoriatic arthritis at a subcutaneous (SC) dose of 25 mg twice weekly (BIW). Here we report the pooled safety results of 3 randomized, placebo-controlled studies (one phase 2 and two phase 3) of etanercept administered for 12 weeks to 1347 patients with chronic plaque psoriasis. Patients received SC injections of placebo or etanercept 25 mg once weekly (QW), 25 mg BIW, or 50 mg BIW. Across the 3 studies, 1261 patients received at least 1 dose of etanercept for a total of 933 patient-years of exposure. Etanercept was well tolerated at all doses, with an adverse event profile similar to that seen with placebo. Low-grade injection site reactions were more common with etanercept use (6% placebo, 14% etanercept); however, the rate in the pooled etanercept group was lower than that observed in RA trials (37%). No clear dose-related trends were observed in rates of adverse events, infections, serious infections, or laboratory abnormalities. The safety profile in patients receiving etanercept 50 mg BIW was comparable with that of patients receiving 25 mg BIW (approved regimen in RA). At week 12, 2% of patients in the pooled placebo group (N=414) and 1.8% in the pooled etanercept group (N=933) had discontinued treatment because of adverse events. Serious adverse events were experienced by a similar proportion of patients in both groups (1.2% placebo, 1.7% etanercept). Rates of serious infections (requiring hospitalization) were low (1.0% placebo, 0.3% etanercept), and no opportunistic infections or tuberculosis were reported. In conclusion, the safety profile of etanercept in patients with psoriasis was similar to the safety profile seen with placebo and was consistent with experience from RA populations. No new or unanticipated patterns of adverse events were observed.

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Combination therapy with retinoid X receptor and peroxisome proliferator-activated receptor gamma agonists for cutaneous T cell lymphoma

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Treatment options for the lymphoproliferative disorder cutaneous T cell lymphoma (CTCL) remain limited. Recently, the selective retinoid X receptor (RXR) agonist bexarotene was approved for treatment of the cutaneous manifestations of CTCL. One heterodimeric partner of RXR is the nuclear hormone receptor peroxisome proliferator-activated receptor γ(PPARγ). Activation of PPARγ inhibits cell growth and induces differentiation of a broad spectrum of epithelial and mesenchymal cell derived maligancies and in some cases combination therapy with RXR and PPARγ agonists results in synergistic efficacy. Whether the PPARγ signaling pathway is intact in CTCL and the therapeutic potential of PPARγ agonists alone or in combination with RXR agonists in patients with CTCL is not known. PPARγ was expressed at the protein level and was transcriptionally responsive to a high-affinity, subtype-selective PPARγ agonist in two different CTCL cell lines. Treatment of either cell line with a PPARγ or RXR agonist resulted in a decrease in cell viability and an increase in apoptosis. Treatment with activating ligands for both RXR and PPARγ resulted in a synergisitic decrease in cell viability. Combination therapy with PPARγ and RXR activators may be a superior treatment option to bexarotene alone for the cutaneous manifestations of CTCL.

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Clinically meaningful improvements in patient-reported outcomes for patients with moderate to severe psoriasis receiving etanercept

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Our objective was to determine if etanercept therapy leads to clinically meaningful improvements in patient-reported outcomes (PROs) for patients with moderate to severe psoriasis. In a 12-week, double-blind, multicenter clinical trial, 611 patients with stable moderate to severe psoriasis were randomized to receive either etanercept (25 mg BIW or 50 mg BIW) or placebo. PROs assessed included the Dermatology Life Quality Index (DLQI). Two responder analyses were prospectively identified in the trial. For the first, clinically important improvement was defined as a 5-point improvement (Khilji et al, 2002) or a score of 0 in the DLQI. A second analysis evaluated those who achieved a 0 score, the best score possible, where patients report that they are “not at all” affected for any of the domains of the DLQI. All patients who received at least one dose of study drug and provided baseline PRO assessments (n=581) were included in both analyses. Compared with those on placebo, a significantly greater proportion of patients on etanercept reported clinically important improvements in DLQI scores. Response rates were superior to placebo at week 2 ($p<0.01$) for both etanercept treatment groups, and by week 12 response rates were 77% for 50 mg BIW, and 72% for 25 mg BIW, both of which were statistically significantly ($p<0.0001$) superior to placebo (26%). Analyses using the more stringent “not at all affected by their psoriasis” responder definition indicated that patients on both doses of etanercept were significantly more likely to respond to therapy than those on placebo ($p<0.0001$). By this measure, response rates were 25% for 50 mg BIW, 20% for 25 mg BIW, and 1% for placebo. Patients receiving etanercept therapy were significantly more likely than placebo-treated patients to achieve a clinically meaningful improvement in the DLQI. These results imply that in patients with moderate to severe psoriasis, treatment with etanercept leads to meaningful improvements in patient-reported outcomes.

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Oral tazarotene does not affect the pharmacokinetics or efficacy of oral contraceptives

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Purpose: Determine pharmacokinetic (PK) and pharmacodynamic (PD) interactions between tazarotene and commonly prescribed oral contraceptives (OCs). Methods: Three clinical studies evaluated PK and PD interactions of oral tazarotene and OCs in healthy volunteers. Two studies evaluated daily norethindrone (NE)/ethinyl estradiol(EE)with tazarotene 1.1mg (N=27) or 6 mg (N=29) and a third study assessed combination norgestimate (NGM)/EE and tazarotene 6 mg (N=26). OCs were administered for three consecutive menstrual cycles. Tazarotene dosing started in the 2nd cycle and continued through study ending. PK parameters for EE, NE, levonorgestrel, and deacetyl NGM (AUC0-24 and Cmax) were determined before and after tazarotene dosing (on day 6 in cycles 2 and 3). Serum concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were evaluated before and after tazarotene dosing (days 2, 4, 6 in cycles 2 and 3). In two studies, serum progesterone levels were assessed on days 18 and 20 in cycles 2 and 3. Results: The 90% confidence intervals of AUC0-24 and Cmax for EE, NE, levonorgestrel, and deacetyl NGM were completely within the 80-125% boundary, indicating tazarotenic acid did not affect the OC PK components. Similarly, the 90% confidence intervals of progesterone concentrations were totally within the 80-125% boundary. The 90% confidence intervals of FSH and LH were generally within the 80-125% boundary except at some timepoints when they were marginally/partially outside this boundary due to data variability. Mean concentrations of FSH and LH were lower in the 3rd cycle than the 2nd cycle on some days, indicating OC efficacy was not compromised. Serum FSH and LH levels remained within normal ranges for healthy women during the follicular phase. Conclusion: Oral retinoids require female patients of childbearing potential to use reliable birth control measures during treatment and for varying periods of time after treatment. Oral tazarotene, up to 6 mg once daily, does not affect the PK or efficacy of NE/EE and NGM/EE oral contraceptives.

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Etanercept treatment leads to rapid and sustained improvements in the quality of life of patients with moderate to severe psoriasis

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Our objective was to determine if etanercept therapy improves health-related quality of life (HRQOL) of subjects with moderate to severe psoriasis. 652 patients with stable moderate to severe psoriasis participated in a 24-week, double-blind, multicenter clinical trial of etanercept therapy. Patients were randomized to receive either one of three doses of etanercept: 25 mg QW, 25 mg BIW, or 50 mg BIW; or placebo. At week 12, patients on placebo were switched to etanercept 25 mg BIW through week 24, while other patients continued their original doses. HRQOL was assessed using the Dermatology Life Quality Index (DLQI). All patients who received at least one dose of study drug and provided baseline DLQI data (n=649) were included in these analyses and missing data were imputed using the last observation carried forward. Compared with those receiving placebo, patients receiving etanercept reported greater improvements in DLQI scores. For each dose of etanercept, improvement in the DLQI was apparent by week 2 ($p < 0.01$ for comparison with placebo) and continued to improve through week 24. At week 12, the mean percent improvement in the DLQI was 10.9, 47.2, 50.8, and 61.0 percent for patients on placebo, 25 mg QW, 25 mg BIW, and 50 mg BIW, respectively (for each dose $p < 0.001$ for comparison with placebo). At week 24 the mean percent improvement in the DLQI was 54.0, 59.4, and 73.8 percent for patients on 25 mg QW, 25 mg BIW, and 50 mg BIW, respectively (there was no placebo group at week 24). Psoriasis patients receiving etanercept therapy have large, rapid and sustained improvements in their quality of life.

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Pharmacokinetics of etanercept in patients with psoriasis

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Our objective was to characterize the concentration time profiles of etanercept, a TNF antagonist, in subjects with psoriasis. Pharmacokinetic analyses were conducted in 838 patients with psoriasis participating in two phase 3 trials with etanercept. Study 1 was a placebo-controlled, randomized clinical trial of three etanercept dosing regimens: 25 mg once weekly (QW), 25 mg twice weekly (BIW), or 50 mg BIW, administered by subcutaneous (SC) injection for 24 weeks. Study 2 was a placebo-controlled, randomized evaluation of two etanercept dosing regimens: 25 mg BIW and 50 mg BIW SC for 12 weeks. Serum samples for analysis of etanercept concentration were taken at weeks 1, 2, 4, 8, 12, and 24 in study 1 (N = 376) and at weeks 2, 4, 8, and 12 in study 2 (N = 462). A subset of patients in study 1 (N=41) had additional serum samples for characterization of the weekly profiles during weeks 1, 12, and 24. In study 1, the mean ± SD steady-state predose serum concentrations of etanercept at 12 weeks were 768 ± 475 ng/mL for the 25 mg QW regimen, 1990 ± 1030 ng/mL for the 25 mg BIW regimen, and 4020 ± 2100 ng/mL for the 50 mg BIW regimen. In study 2, mean etanercept concentrations at 12 weeks were 1900 ± 1110 ng/mL in the 25 mg BIW group and 3830 ± 1870 ng/mL in the 50 mg BIW group. All three doses produce modest accumulation after multiple doses with accumulation indices around 2. The mean peak-to-trough ratios at steady state did not exceed 2. Pharmacokinetic results were highly consistent across the two phase 3 trials and were in good agreement with the results seen in a previous phase 2 psoriasis trial. In both studies, the concentration time profiles displayed dose proportionality. All doses produce stable and smooth weekly profiles at steady state.

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Differential response of normal melanocytes and melanoma cells to proteasome inhibition: impact on tumor control *in vivo*

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Melanoma is notorious for an extreme multi-drug resistance. The molecular bases for treatment failure are largely unknown, but they are generally attributed to defective targeting of the melanoma cells and/or an excessive toxicity to normal compartments. Here we show that dysfunctional cell cycle checkpoints and differential responses to stress signals can be exploited by the proteasome inhibitor bortezomib (Velcade, previously known as PS-341) for a selective killing of melanoma cells. Comparative analysis of 25 melanoma cell lines and 5 normal melanocyte preparations revealed a 6-10 fold difference in drug sensitivity (EC_{50}=15±10 nM vs 100±10 nM, respectively). Thus, while normal melanocytes responded by cell cycle arrest, melanoma cells activated intrinsic and extrinsic apoptotic pathways. Interestingly, the cytotoxic effect of bortezomib was independent on the status of p53 and the melanoma associated genes survivin, p16INK4a, p19ARF, Ras or BRAF ($p<0.005$). Unexpectedly, and to the contrary to other tumor types, classical proteasome targets such as NF-κB, Bcl-xL, XIAP, FLIP or TRAF-2 (among others) where not affected by bortezomib. High throughput functional proteomic analyses indicated that the basis for the selective effect of bortezomib relies on the capability of melanoma cells (but not normal melanocytes) to mobilize intracellular calcium, activate the stress kinases p38 and JNK, and promote a massive release of pro-apoptotic inducers from the mitochondria. Those results underscore the different regulation of survival and apoptotic signals in melanoma cells and point to "weaknesses"on these tumor cells that can be exploited with therapeutic intentions. In fact, whole-body tumor imaging systems indicated that the selective effect of Bortezomib is extensive to in vivo settings (mouse xenograft models), in particular in the control of melanoma metastasis ($p<0.01$). We consider these results as the framework for the rational design of new melanoma therapies.

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Pharmacokinetics of etanercept in patients with psoriasis after long term intermittent or continuous treatment

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Our objective was to evaluate the concentration time profiles of etanercept in subjects with psoriasis during intermittent treatment or during prolonged therapy. After an initial 24 week double-blind period with etanercept SC doses 25 mg once weekly (QW), 25 mg twice weekly (BIW), 50 mg BIW, or placebo, patients were classified as responders (≥ 50% decrease from baseline PASI) or incomplete responders (failed to achieve PASI 50). Responders were discontinued from etanercept and followed until their disease relapsed, at which time patients either resumed blinded etanercept therapy or, if initially treated with placebo, began 25 mg BIW treatment. Incomplete responders began treatment with open-label etanercept 25 mg BIW. Serum samples were collected during both the double-blind period and the withdrawal and retreatment period. The etanercept concentration time profiles obtained for intermittent treatment of responders or prolonged treatment of incomplete responders were consistent with the steady-state pharmacokinetic profiles obtained during the initial double-blind period of the study. For the responders, the steady-state predose mean (± SD) etanercept concentrations of the 25 mg QW, 25 mg BIW, and 50 mg BIW dose groups were 980 ± 510 ng/mL (N = 18), 2350 ± 760 ng/mL (N = 21), and 3390 ± 1830 ng/mL (N = 29) respectively, after 12 weeks of retreatment following withdrawal and disease relapse. For the incomplete responders, the steady-state predose mean etanercept concentrations at 12, 24 and 36 weeks after the beginning of the open-label period with 25 mg BIW were 1930 ± 1150 ng/mL (N = 47), 1800 ± 1410 ng/mL (N = 34), and 1980 ± 1000 ng/mL (N = 33), respectively. Once or twice-weekly administration of etanercept in psoriatic patients produces stable steady-state concentration time profiles, maintained for prolonged periods of time. There is no change in the pharmacokinetics of etanercept after either intermittent or continuous therapy, supporting the use of etanercept in either treatment paradigm.

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A randomized, double-blind, placebo-controlled study evaluating the S-Caine™ Patch for induction of local anesthesia before minor dermatologic procedures in geriatric patients

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Local anesthetics have an obligate role in dermatologic practice. While injectable lidocaine is the agent of choice for most minor procedures, there is a demand for noninvasive anesthetic delivery systems. The purpose of this study is to evaluate the efficacy and safety of the S-Caine Patch, a 1:1 (wt:wt) eutectic mixture of 70 mg tetracaine and 70 mg lidocaine, in the induction of local anesthesia before minor dermatologic procedures in geriatric patients. In a multicenter, randomized, double-blind, placebo-controlled study, we assign 79 patients over the age of 65 years who require a shave biopsy, superficial excision, or electrodessication procedure to receive a 30-minute application of either the S-Caine Patch or placebo immediately prior to the procedure. The primary measure of efficacy was patient rating of procedural pain using the Visual Analog Scale. Secondary efficacy endpoints included patient, investigator, and independent observer assessments. There was a statistically significant difference ($p=0.041$) in patient ratings of pain by VAS score in the S-Caine group (9.5 mm) versus placebo group (22.5 mm). When patient data from a center that did not use the study's randomization schedule were excluded (n=16), median VAS scores were 7.0 mm for S-Caine and 24.5 mm for placebo ($p=0.020$). None of the secondary endpoints showed a statistically significant difference between S-Caine and placebo. There were no adverse events reported. In conclusion, the S-Caine Patch is a safe and effective method for noninvasive induction of local anesthesia for minor dermatological procedures in patients over the age of 65 years.

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Measles virus-mediated oncolytic treatment of cutaneous T cell lymphomas

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Primary cutaneous lymphomas constitute a spectrum of diseases characterized by a clonal accumulation of lymphocytes in the skin. Cutaneous T-cell lymphoma -derived lymphoma cell lines may present deficient type I IFN signaling, preventing an anti-viral status. Therefore CTCL represent an attractive target for viral oncolysis. We undertook a phase I, open-label, dose-escalating trial to investigate the effect of measles vaccine virus (MV) intralesional treatment in patients with advanced cutaneous T-cell lymphomas (CTCL). Five CTCL patients have received a total of 13 intratumoral MV injections (Edmonston-Zagreb strain, provided by Berna Biotech AG, Bern, Switzerland). Injected doses ranged from 100 TCID50 to 1000 TCID50 MV. Each MV injection was preceded by subcutaneous injection of IFN-a (9 Mio Units on day 1 and day 3). Treatment was well tolerated with no significant treatment-related side effects. Biopsies from injected lesions prior to and after the treatment were analyzed by immunohistochemistry as well as by PCR. The expression of measles receptors CD46 and CD150/SLAM was assessed on tumor cells and found positive in all cases. After therapy measles virus nucleoprotein was detected as well as the formation of syncytia. Quantitative PCR analysis demonstrated an increase in expression of pro-inflammatory serum cytokines (IL-2) and of IFN-?/CD8 ratio in tumor biopsies. Systemic immune response with an elevation of anti-measles antibody titer was detected. Clinical response was observed in 2 out of 5 patients with regression of injected tumor lesions. Our approach induced local and systemic immunological alteration after MV in primary CTCL and local tumor regression, providing information that may be of use in the design of future cancer MV trials.

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Effects of Chinese natural herbs on skin microcirulation

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To assess changes of superficial skin capillaries blood velocity and changes of skin microvascular perfusion due to topical application of different kinds of Chinese natural herb extracts, 20 healthy subjects were respectively treated with 20 different ointments of natural herb chemicals (0.1%) and placebo (vehicle). Capillaries blood velocity and skin perfusion of finger nail folds were measured by capillary microscopy and Laser Doppler flowmetry (LDF) respectively. The measurement was performed successively and results were recorded every 5 minutes (5 min, 10min, 15min, 20min, 25min and 30min) after treatment. Among the 20 Chinese natural herb chemicals, which were reported improving microcirculation by systemic administration, Only H-compound can increase the nail fold microcirculation significantly compared with placebo. The LDF showed a persistent perfusion increase by 2 to 2.5 times from the fifth minutes and lasted for at least 30 minutes. This perfusion pattern was not seen with placebo and other invalid herbs. The velocity of erythrocyte shows a positive relationship with blood perfusion. Under capillary microscopy it increased by 1.8 times compared with base level. Topical application 0.1% H-compound increases nail fold microcirculation. Further studies will be required to explore its mechanism and its effect on modulating skin color.

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Double-blind, placebo-controlled clinical study of cream containing pueraria mirifica for face and breast skin elasticity

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It is well established that body hormones play a central role in skin appearance and are implicated in skin aging. Recently, along with the remarkable increase in interest in natural products, the application of phytoestrogens in anti-aging products has become very important. In this focus, we developed Pueraria mirifica(PM), following specific extraction procedures in this in vivo study on skin elasticity, wrinkles, we investigated the anti-aging properties.30 healthy women volunteers, between the ages of 30 and 60, applied the cream formula with 4% of Pueraria mirifica or placebo, on the face area twice a day for 16 weeks. The evaluation was made both clinically and by silicon replica analysis and Cutometer SEM575 and Corneometer followed by statistical treatment using the student t- test($p<0.05$).Skin elasticity measurement results showed that topical application on the skin increased the total surface of skin elasticity in comparison with placebo group($p<0.05$). However, PM does not have prominent effect on the depth of wrinkles and skin moisturization. These results are correlated with breast enlargement and firmness clinical test in 43 women for 8 weeks. the results were confirmed both clinically and by the impressions of volunteers which indicated a visual improvement of 74%. Skin elasticity increased statistically more significant than before treatment($p=0.038$). But placebo group do not increase skin elasticity($p=0.24$). This in vivo study demonstrates that, PM exhibits a significant skin elasticity increasing effect therefore, is of great interest in anti-aging and firmness skin care products.

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PCL-016 gel (NV-02) in the treatment of acne vulgaris: a pilot study

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PCL-016 is a metabolite of tryptophan and is produced via amino acid breakdown in vivo. It affects zinc binding within zinc finger proteins and has been shown to have antiviral, antibacterial, and immunomodulatory properties. The primary objective of this open-label, pilot study was to determine the safety and efficacy of PCL-016 10% gel (NV-02) in the treatment of mild to moderate acne vulgaris. After obtaining informed consent, male and female patients with mild to moderate acne vulgaris over the age of 18 were enrolled. Participants were instructed to apply study drug twice daily to the face for 12 weeks. Facial lesion counts along with photography were performed at each visit. Adverse events and local skin reactions were assessed at each visit. In addition, routine laboratory testing (complete blood count, serum chemistry) and plasma levels of PCL-016 were evaluated at each visit. A total of 20 subjects were enrolled, with 15 subjects completing the study. NV-02 reduced the mean total lesion count, mean inflammatory lesion count, and mean non-inflammatory lesion count by 54.3%, 51.4%, and 56%, respectively. A statistically significant reduction in these parameters was noted (p = 0.0006, p = 0.001, and p = 0.006, respectively). Application of NV-02 was well tolerated by most patients in this study. One patient discontinued the study due to burning noted after application of study drug. There were no significant laboratory abnormalities noted at any visit for any patient. NV-02 applied topically appears both safe and effective when applied twice daily in the treatment of mild to moderate acne vulgaris. A double blind, randomized, placebo controlled study is warranted to further investigate the role of NV-02 as therapy for patients with mild to moderate acne vulgaris. This is the first reported used of NV-02 in humans, and the first reported use of a zinc finger drug in acne vulgaris.

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Safety of imiquimod 5% cream in subjects with kidney or liver transplants

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The primary objective of this phase IV, open-label study was to estimate the acute rejection rate in otherwise clinically stable liver and kidney allogeneic organ transplant subjects who had external genital warts (EGW) treated with topical imiquimod 5% cream (AldaraTM). Secondary objectives for this study were to determine the safety and tolerability of imiquimod 5% cream and to determine the clearance rates in this subject population following treatment with imiquimod 5% cream. After obtaining informed consent, subjects 18 years or older with allogeneic kidney or liver transplants and EGW were planned to be enrolled. Patients received imiquimod 5% cream to be applied 3 times weekly at night. Subjects were treated for 16 weeks or until genital warts cleared, whichever occurred first. Adverse events and local skin reactions were assessed at each visit. Vital signs, general physical examination, total wart area, routine laboratory testing and immunosuppressant drug levels were also evaluated at each visit. Only 4 patients were enrolled due to difficulty recruiting patients. All patients had allogeneic kidney transplants and were receiving immunosuppressive therapy with either cyclosporine or tacrolimus. No deaths or transplant rejections were reported. Two patients experienced 3 serious adverse events, each of which were reported as intercurrent events and probably not related to study drug. Other adverse events reported were mild to moderate and localized to the application site. No significant laboratory abnormalities were noted. One patient experienced clearance of her EGW and the remaining three each experienced reduction in wart area. Statistical comparison was not performed due to the small sample size. Adverse events judged to be drug-related were mild to moderate, localized to the application site, and similar to those seen in other studies with imiquimod 5% cream. In addition, all subjects experienced clearance or reduction in wart area. This is the first reported use of imiquimod 5% cream in organ transplant patients.

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Pharmacological and clinical activities of 5-alpha Avocuta: application for the management of androgenic disorders

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Androgen-dependent disorders, such as seborrhoea, acne and alopecia are among the most common diseases encountered by dermatologists in daily practice. These pathologies are in part related to an hyper-activity of 5-alpha reductase, the enzyme that metabolises testosterone into dihydrotestosterone (DHT) a major potent androgen in human skin. In this work, we have investigated the efficacy of a lipidic fatty ester : 5-alpha Avocuta (butyl avocadate). Butyl avocadate is obtained from refined avocado oil through a biotechnological process, and is purified by molecular distillation.

We have demonstrated using dermal fibroblasts in culture that 5-alpha Avocuta is a potent inhibitors of 5-alpha reductase type I activity (-49% for the smallest dose tested 0.01%). A multi-centric clinical evaluation, under dermatologist control, was conducted on 27 volunteers. The purpose of this study was to test the efficiency of a shampoo (1% of 5-alpha Avocuta) after 3 weeks of application (1 application/2 days). Analysis of sebum secretion (Sebufix F16) by visual scoring and by image analysis (Skin Visiometer, SV600, CK, Germany) demonstrate that this shampoo is able to significantly reduce sebaceous production. Clinical investigations by the dermatologist (analogic scale) show that the shampoo clearly improves greasy hair aspect, and is able to reduce itching and pruritus, as well as dandruffs. The auto-evaluation by the volunteers confirms these data. 5- alpha Avocuta is a new inhibitor of 5-alpha-reductase type I activity with proven efficiency on human scalp disorder. This natural active ingredient may open the way to the formulation of original and efficient products dedicated to the treatment of androgenic disorders.

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A new method in the management of skin neoplasm using a flexible radioactive patch

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We developed a flexible radioactive polyurethane patch incorporating β–emitting radionuclide (^{166}Ho) to treat malignant skin lesions. We covered tumor surface (n=133) with the radioactive patch and plastered tightly for 33.58± 5.33 minutes. Radiation dose delivered was 35 Gy for Bowen's disease (n=78), Kaposi sarcoma (n=25) and actinic keratosis (n=5), 50 Gy for BCC (n=17) and squamous carcinoma (n=8). Complete response was observed in 91.7% after first therapeutic trial, 96.2% after 2nd and 97.7% after 4th trial with excellent cosmetic outcome for 31.15±13.88 months. In conclusion, radioactive patch therapy is effective for superficial skin cancers in impractical location for surgery.

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Efficacy and safety outcomes of long-term efalizumab therapy in patients with moderate to severe chronic plaque psoriasis: an update

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Efalizumab modulates key T-cell events in psoriasis pathogenesis. An ongoing open-label, Phase III study is assessing weekly efalizumab therapy for up to 3 years for moderate to severe chronic plaque psoriasis. Weeks 1-12, 339 patients received 2 mg/kg/wk subcutaneous (SC) efalizumab, and fluocinolone acetonide ointment or petrolatum during weeks 9-12. Patients achieving >_50% improvement in Psoriasis Area and Severity Index (PASI-50) or static Physician Global Assessment of Mild, Minimal, or Clear at week 12 (n=290) entered maintenance treatment (MT) to receive weekly efalizumab. During MT, analyses (last observation carried forward imputation) included intent-to-treat (ITT; n=339) including all patients even if they did not meet the criteria to enter MT and a maintenance group analysis (n=290). An analysis of patients continuing therapy (as-treated) was also conducted. At week 12, 82%, 41%, and 13% of all patients achieved PASI-50, PASI-75, and PASI-90 respectively. Fluocinolone acetonide did not provide additional benefit. Mean trough serum concentrations were measured during months 2-14 and were consistent. At month 21, ITT (n=339) PASI-50, PASI-75, and PASI-90 were 65%, 48%, and 26%, respectively. Maintenance group (n=290) PASI-50, PASI-75, and PASI-90 responses were 75%, 56% and 30%, respectively. As-treated (n=194) PASI-50, PASI-75, and PASI-90 responses were 86%, 67%, and 34%, respectively. During MT, common adverse events (>_5% of patients during any 12-week period) included accidental injury, non-specific infection (eg, colds), increased cough, rhinitis, and sinusitis. There was no evidence that the incidence of adverse events increased with time. Up to 21 months of efalizumab therapy was well tolerated and provided patients with continuous control of psoriasis. Updated results will be presented.

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HuMax-CD4, fully human monoclonal antibody: phase II trial in cutaneous T cell lymphoma

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The fully human monoclonal antibody, HuMax-CD4 targeting the CD4 molecule was investigated for safety and efficacy in the treatment of CTCL. 49 patients with biopsy-proven CD4+ refractory early or advanced stage CTCL were enrolled in 2 ongoing open-label therapeutic exploratory clinical trials. HuMax-CD4 was infused i.v. at doses of 280 mg (11 early stage, 13 advanced stage patients), 560mg (15 early stage patients) or 980mg (10 advanced stage patients) once weekly for 16 weeks. Primary endpoint was Composite Assessment of Index Lesion Disease Activity (CA) score. 36 patients evaluable for response after ≥4 infusions are the subject of this report: 280 mg (11 early stage and 10 advanced stage), 560mg (9 early stage) or 980mg (6 advanced stage). Following 280 mg, 33% of 21 patients obtained a 50% CA score reduction. Following 560mg, 67% (6 of 9 early stage patients obtained a CA score reduction of > 50%. 2 of 9 obtained a 100% reduction, 4 obtained a 50% reduction, and 3 patients had stable disease. Following 980mg, 50% (3 of 6) advanced stage patients obtained a CA score reduction of > 50% and 3 patients had stable disease. Following 280mg, 6 grade 3 adverse events were reported by 4 patients. 5 were unrelated to HuMax-CD4. Following 560 and 980mg, 1 unrelated grade 3 adverse event was reported. No grade 4 events were reported. CTCL responds to HuMax-CD4 treatment and HuMax-CD4 is safe and well tolerated by patients with CTCL.

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Cancer regression induced by TLR9-targeted immunostimulatory CpG treatment in patients with metastatic melanoma: results of a clinical phase II trial

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Stimulation of Toll-like receptor (TLR) 9 by pathogen-derived compounds leads to direct activation of human antigen-presenting plasmacytoid dendritic cells (pDC) and B cells and indirectly dramatically increases cytotoxic T and natural killer cell responses. The synthetic oligodeoxynucleotide CPG 7909 contains CpG motifs optimized to specifically interact with TLR9 and is a strong activator of both innate and specific immunity. CPG 7909 crossreacts with mouse TLR9 and has shown impressive antitumor activity in preclinical tumor models when used as monotherapy. 20 patients with metastatic melanoma (stage IV, no CNS mets) were enrolled into an open-label, multicenter, single arm study. Pts received 6 mg CPG 7909 weekly SC for 24 weeks or until disease progression. Disease status was assessed according to RECIST. Two pts achieved a confirmed partial response (PR, one pt 13+ months), three pts achieved stable disease (SD). CPG 7909 was well tolerated. Adverse events included transient injection site reactions, fever and arthralgias. Toxicities were limited, transient and did not result in any withdrawals.

Phenotyping of PBMC could be performed in 14 patients and revealed (i) activation of BDCA-2+pDC with increased CD86 and HLA-DR expression consistent with the MOA, and (ii) an increased frequency of CD19+CD38++early plasma cells during therapy. Pts exhibiting PR or SD could be distinguished from non-R pts by differential dynamics in NK cell-cytotoxicity (1.6x↑ vs. 1.7x↓ in lytic units, $p<0.05$) during the first 8 weeks of treatment. Assays to determine frequencies of antigen-specific T cells are ongoing. We conclude that CPG 7909 exerts objective anti-tumor activity in pts with metastatic melanoma, can be administered safely and induces a phenotypic signature in PBMC associated with exposure and, possibly, response to therapy.

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Efficacy and safety of infliximab in the treatment of severe or arthropathic psoriasis: preliminary results

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Psoriasis is a physically and psychologically disabling disease that affects 1-3% of caucasian population. About 25% of patients suffer from a moderate to severe plaque psoriasis and about 7% develop an inflammatory arthritis. Recently, a new therapeutic approach, using antibodies directed against Tumor Necrosis Factor-a (TNF-a), has been proposed. TNF-a is believed to have a central role in the pathogenesis of psoriasis.

Aim of our study was to evaluate the efficacy of infliximab in patients with severe plaque psoriasis or psoriatic arthritis, non-responder to conventional treatments. We enrolled 85 psoriatic patients (40M and 45F), 46% affected by severe plaque psoriasis (PASI >26), 36% by psoriatic arthritis with both severe cutaneous (PASI > 25) and articular involvement, and 18% mainly by psoriatic arthritis at baseline observation, all of them non-responders to conventional therapies (cyclosporin A, Methotrexate, retinoids) . The therapy consisted in i.v. drip treatment with 5 mg/kg of infliximab, at week 0, 2 and 6, as induction period, and every 8 weeks as manteinance therapy. The principal endpoint was the evaluation of effectiveness on Psoriasis (assessed by PASI score), and the evaluation of arthritis at week 22. The 72% (n 61) of patients completed the scheduled therapy (second booster dose of the maintenance period), The first clinical result was the improvement of the articular function already observed after the first infusion (week 2), as referred by the patients who reported reduction of pain and improvement of motility. Concerning the skin involvement, all patients achieved an impressive reduction of PASI score (>75%), mostly before the end of the induction period (week 4). The articular result is still present after 22 weeks, whereas some cutaneous recurrences have been observed during this maintenance period.In conclusion, our preliminary therapeutic experience seems to confirm the effectiveness of this drug in the treatment of severe plaque psoriasis when the official therapies are contraindicated.

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Enhancement of human skin barrier integrity by nicotinic acid derivatives

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A weakened skin barrier is characteristic of numerous dermatology conditions including atopic dermatitis. In addition, evidence suggests that certain treatments for skin photodamage may result in decreased barrier integrity and heightened photosensitivity. Derivatives of nicotinic acid tailored for topical delivery of nicotinic acid to the cellular compartments of skin have been shown to increase NAD content to support genomic integrity and energy metabolism functions and to stimulate the release of leptin to stimulate epidermal differentiation. In the present study, a double-blinded protocol was used to compare placebo and myristyl nicotinate (MN) formulations for effects on skin barrier integrity. Formulations containing 5% MN increased skin NAD content by an average of approximately 40% ($p=0.001$ vs. placebo) and epidermal turnover by 6.3% ($p= 0.003$). Skin barrier assessment was made by determination of rates of trans-epidermal water loss (TEWL), effects on minimal erythemal dose (MED), and analysis of skin biopsies. Over a period of 12 weeks, the placebo formulation decreased the rates of TEWL by approximately 9% while MN formulations decreased TEWL rates by nearly 30%, demonstrating a strong effect of MN on barrier integrity ($p= 0.006$). Even greater effects of MN on reduction of TEWL were observed in atopic subjects. The presence of MN also resulted in a photoprotective effect on MED of approximately 9% ($p= 0.08$). Skin biopsies showed that the presence of MN resulted in an increased thickening of the granular and spinous layers and also dramatically increased cellular layers of the stratum corneum. These results indicate that skin delivery of nicotinic acid may provide clinical benefit to skin conditions that are characterized as having a partially compromised skin barrier, and may be useful either alone or in combination with other treatments for such conditions.

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Cutaneous T cell lymphoma responses to a TLR9 agonist CPG: a phase I/II study

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Available systemic therapies for CTCL produce significant clinical responses and may relieve skin symptoms or complications, but there is a lack of well-tolerated systemic therapies with reliable and durable responses in patients with recurrent or advanced disease. A new class of chemically defined CpG immunomodulators target dendritic cell TLR9 receptors with induction of IL12, IFN-gamma, NK-cell function. In several early trials, CPG 7909 has been well tolerated by weekly s.c. dosing and has clinical anti-tumor activity. Patients with recurrent/refractory (stages IB-IVA) CTCL were enrolled to receive weekly s.c. doses of 0.08, 0.16, 0.24, 0.28, or 0.32 mg/kg of CPG 7909. Clinical response was monitored using the Composite Assessment of Index Lesion Disease Severity Index and the Physician's Global Assessment of Clinical Condition. Sixteen patients (8F, 8M) are treated to date with documented time to response between 4-16 weeks. Responses according to dose level are: 0.08 mg/kg, 1 stable disease (SD), 2 progressive disease (PD); 0.16 mg/kg, 1 complete response (CR), 1 partial response (PR), 1 SD; 0.24 mg/kg, 1 PR, 2 SD; 0.28 mg/kg, 1 SD, 2 pending response; 0.32 mg/kg, 1 SD, 3 pending response. Stable disease (SD) and responses (PR/CR) were maintained for up to 6 months of treatment. Doses were generally well tolerated; dose-related injection site reactions and mild-moderate flu-like symptoms were common. CPG 7909 has significant anti-tumor activity in CTCL. Responses will be correlated with immunomodulator biologic responses and PK. Enrollment is ongoing with additional dose escalations to optimize response at tolerable doses.

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Safety of efalizumab in patients with moderate to severe chronic plaque psoriasis

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Psoriasis is a chronic disease requiring long-term treatment. Traditional options are limited by a lack of consistent efficacy, inconvenient administration schedules, and cumulative toxicity. As knowledge increases about the role of the immune system in psoriasis pathogenesis, the development of targeted therapies focusing on immune cells have expanded. Efalizumab, a recombinant humanized monoclonal IgG1 antibody binds to the CD11a subunit of leukocyte function-associated antigen-1 (LFA-1), inhibiting several key T-cell functions, including initial activation, trafficking, and reactivation. Efalizumab has been studied in over 2,700 patients with psoriasis during 3- and 6-month treatment periods and up to 24 months in a small group of patients. Safety outcomes have been pooled for analysis yielding a large cohort of psoriasis patients. Efalizumab was generally well tolerated, with acute flu-like symptoms (headache, fever, chills, nausea, vomiting, or myalgia, beginning within 48 hours of dosing) observed most frequently during the first 1-2 doses; by the third dose, the incidence was comparable to placebo-treated patients. The adverse events reported most frequently at week 12 among efalizumab-treated patients included headache, non-specific infection (eg, common colds), chills, nausea, and generalized pain. During weeks 1-12, serious adverse events occurred infrequently in efalizumab- and placebo-treated patients (2.2% vs 1.7%). Extended treatment was not associated with any new common treatment-emergent adverse events. Comparison with patients receiving placebo and external cohorts of similar psoriasis patients showed that efalizumab did not appear to be associated with increased risk of infection or malignancy. The favorable efficacy and safety profiles support continuous use of efalizumab in patients with moderate to severe chronic plaque psoriasis.

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Use of alefacept in combination with other psoriasis therapies: a study reflective of clinical practice

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Psoriasis is a T-cell–mediated skin disease that has no cure and typically requires life-long treatment. Conventional systemic psoriasis therapies, such as methotrexate and cyclosporine, are generalized immunosuppressants. As a result of their nonselective effects, these agents are associated with substantial toxicities and dosing limitations. Alefacept selectively targets memory T cells. In multiple randomized, placebo-controlled studies, alefacept has been shown to improve the symptoms of psoriasis without the safety concerns of conventional therapies. To understand the best way to manage existing therapies during a course of alefacept and evaluate the consequences of combination therapy, an international study is being conducted under conditions that reflect clinical practice. Approximately 400 patients with chronic plaque psoriasis will be enrolled in this open-label study in which topical treatments, UVB, systemic retinoids, and prednisone will be permitted as concomitant therapies. Cyclosporine and methotrexate will be tapered during alefacept therapy. Patients must be ≥16 years of age, require systemic therapy, have normal CD4+ lymphocyte counts, and be naïve to alefacept therapy. In each course, alefacept 15 mg will be administered once weekly by IM injection for 12 weeks followed by 12 weeks of observation. Currently, over 200 patients have received ≥1 dose of alefacept; topical steroids and methotrexate have been the most common concomitant psoriasis therapies. Alefacept consistently reduced circulating CD4+ T-cell counts, regardless of the concomitant psoriasis therapy. Nearly two thirds of patients have experienced an improvement in their physician global assessment with alefacept. These preliminary results suggest that alefacept can be safely and effectively combined with other psoriasis therapies and there is no interference with the mechanism of action of alefacept.

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Severity of rosacea: measurement issues

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Our goal was to determine what signs correlate best with global severity of rosacea and to examine inter-rater reliability. Four clinicians each made assessments of 82 patients with active signs of rosacea using 60, 0-to-10, Likert-like scales. Clinician's assessment of severity correlated strongly with erythema, especially on the cheeks. Subjects' assessment of severity correlated more strongly with papules/pustules. Different methods of assessing severity [estimation of area involved, intensity or lesion counts] did not produce significantly differentresults. Inter-rater reliability was low on 11-point (0-10) Leikert-like scales, but improved when scales were collapsed to 5 or 4 points. Clinicians and patients assess overall rosacea severity differently with respect to signs. New instruments for assessing rosacea severity must address inter-rater reliability.

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A validation study comparing spectrophotometric measurement of skin color to digital colorimetry

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Given the limitations of subjective assessments of skin pigmentation, it would be desirable to develop objective methods to measure skin color. Spectrometry has been used for color measurement but is limited by several factors. For instance, values are derived from indirect measures of reflectance of red and green colors only, erythema index becomes skewed at high melanin levels, and spectrometers are not widely available outside research settings. This study was undertaken to test the validity of digital colorimetry (i.e., color measurements derived from standardized digital photography), versus the gold standard of spectrometry. 558 subjects had standardized spectrometric and colormetric readings taken from the inner arm. Melanin and erythema values of spectrometry were compared to red, green, and blue color values. A moderate to high correlation between color and spectrometric indices was found ($0.67 < R2 < 0.74$, $p < 0.01$), although this relationship tended to be stronger in more darkly pigmented Fitzpatrick subgroups. Participants with type V skin showed high correlations between spectrometry and color ($0.83 < R2 < 0.99$, $p < 0.01$) while type II subjects ($0.29 < R2 < 0.37$, $p < 0.01$) showed much less robust associations. Interestingly, among the colors, blue correlated best with the spectrometric measures ($0.74 < R2 < 0.73$, $p < 0.01$). These data validate a novel technology utilizing digital colorimetry to measure skin color using commonly available digital equipment and commercially available software. Furthermore, they suggest that the color blue, associated with deeper levels of melanin, may be an important element of skin color assessments. Objective measures that account for chromophore content and light scattering and allow for simple standardized evaluation of color may enhance subtyping of diverse patient populations for epidemiological research.

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Impact of health care delivery models on melanoma thickness and stage in a university-based referral center: an observational study

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To characterize the relationship between melanoma outcome and health care delivery models, in particular, the gatekeeper (GK) versus direct access (DA) models. Retrospective review of newly-diagnosed cutaneous melanoma patients referred to Stanford University Medical Center pigmented lesion clinic (1996-2000). Data abstracted from medical records included patient age, type of access route (GK vs DA to dermatology), time in months between the patient first noticing the lesion and medical presentation (patient delay), time in months between medical presentation and biopsy (MD delay), tumor thickness, histologic ulceration, melanoma subtype, and stage at diagnosis. Descriptive, non-parametric, and chi-square statistics were applied using SAS statistical software. 234 subjects were analyzed: 72% (166/234) employed the DA route and 28% (66/234) employed the GK route. A significant association existed between MD delay and access route, showing that DA patients were biopsied sooner (< 3 months vs > 3 months) than their GK counterparts ($p<0.0001$). However, no significant difference ($p>0.05$) was observed in stage at diagnosis (predominantly IA), proportion of nodular melanomas (DA 4% vs GK 2%), patient delay, or median tumor thickness between DA and GK routes (1.10 mm vs 0.85 mm, respectively). A trend towards a greater proportion of histologically ulcerated melanomas was observed in the DA compared to the GK group (12% vs 5%, p= 0.06). This pilot study demonstrates no difference in outcome between GK and DA routes as measured by melanoma thickness and stage. However, DA patients receive biopsy sooner and may have tumors that are more likely to be ulcerated compared to their GK counterparts. The potential impact of health care access routes on prompt diagnosis of more aggressive melanoma subtypes requires further study.

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Relationship of treatment delay with surgical defect size from keratinocyte carcinoma (basal cell carcinoma and squamous cell carcinoma)

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Keratinocyte carcinoma (KC) is the most common cancer in the United States. Larger KC lesions are associated with higher recurrence and disfigurement than less advanced disease, yet modifiable predictors of size are not established. The objective of this study was to determine the association of potentially modifiable characteristics, including delay in treatment, with KC morbidity, which we defined as defect size at the time of KC removal by Mohs micrographic surgery (MMS). A stratified random sample of patients treated for KC with MMS during the 12 months beginning September 2000 were selected for interview (n=400). 108 were ineligible for participation. 219 telephone interviews were completed (refusal rate 24%). Interviewed and non-interviewed cases differed only in mean age (66 vs. 72 years, $p<0.0001$). Anatomic site, age, histologic subtype, and gender predicted defect size ($R^2=0.39$). Self-reported delay between initial physician examination and MMS ("system delay") predicted defect size ($p=0.0004$), but delay before initial physician exam did not independently predict size. The effect on size was attributable to system delay greater than 1 year ($p=0.0016$), which was associated with a doubling of defect size (OR 2.0; CI 1.3-3.1). Delays of this duration were associated with initial examination by a primary care provider (OR 3.9 CI 1.7-8.8), initial misdiagnosis (OR 6.8 CI 2.5-18.7), treatment performed by the first provider (OR 23.3 CI 6.5-83.7), and by removal prior to MMS (OR 6.2 CI 2.5-15.5). All but specialty were independent predictors of delay. Delay greater than 1 year between initial medical evaluation and MMS is associated with initial diagnosis, treatment and number of prior removals. This "system delay" is an important predictor of final defect size. Attention to processes of care delivery for KC may have a greater impact on morbidity than efforts at earlier public detection.

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Is there a relationship between long-term antibiotic use and urinary tract infections in acne patients?

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Individuals with acne are often exposed to antibiotics for prolonged periods of time, thus putting them at risk for developing resistant strains of pathogenic bacteria as well as the colonization of their normal flora by pathogenic microbes. The purpose of this retrospective cohort study was to explore the potential correlation between long-term antibiotic use and the incidence of urinary tract infections (UTIs) in acne patients. We used a 20% random sample of female acne patients between 15 and 30 years of age (N=4,656) in the General Practice Research Database (GPRD) from the UK (1991-95). Three cohorts of acne patients were examined: 1) patients on oral antibiotics, 2) patients on topical antibiotics only, and 3) patients not on antibiotics. An individual was noted as a long-term acne antibiotic user if she used the drug for more than 4 weeks. All patients were followed for one year after qualifying for inclusion in a cohort. The outcome was an UTI. Logistic regression was used to make comparisons between cohorts. For acne patients in the GPRD, 81.7% were using long-term antibiotic therapy (oral and or topical) to treat their acne and 18.3% were not using antibiotics. Of those using antibiotics, 67.3% were using oral therapy alone or with topical therapy and 32.7% were using only topical therapy. The odds ratio of developing a UTI comparing the oral users to those not exposed to antibiotic therapy was 0.61 (CI=[0.43,0.87], p=0.006). There was no difference in the risk of developing a UTI after exposure to topical antibiotic therapy (OR=0.92[0.63,1.34]). Adjusting for age did not have a significant effect on these findings. This study demonstrates that long-term oral antibiotic therapy has a protective effect against the development of UTI in female acne patients 15-30 years old. Topical antibiotic therapy did not appear to offer this protection. According to these findings, the current degree of physician apprehension regarding long-term antibiotic therapy may be unwarranted.

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Acceptable risk of therapies for psoriasis

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How physicians make decisions about prescribing medication and how they balance this decision against the perceived risk of its side effects is not well understood. This may be especially important with the advent of new systemic agents to treat severe psoriasis, in that they may be associated with the onset of cancers or other serious medical conditions. We administered a questionnaire to 59 health care providers attending an annual Dermatology conference at an academic hospital using a scale that was devised to quantify the risk of developing lymphoma or skin cancer versus the therapeutic benefit of treating an individual with psoriasis and a new therapeutic agent or with standard therapy (topical steroids). The amount of tolerable risk increased with the likelihood of therapeutic success. For example, for a new treatment with 10% likelihood of therapeutic success, providers tolerated a 3 times increased risk for non-melanoma skin cancer, whereas for a 75 % likelihood of therapeutic success they tolerated a 7 times increased risk of non-melanoma skin cancer versus standard therapy (and its inherent success and toxicity). As expected, they were less tolerant of an increased risk of lymphoma. With respect to a drug that has a 10% likelihood of therapeutic success, providers tolerated a 1.7 times increased risk for lymphoma, whereas for new drug with a 75% chance of success the tolerable risk as compared to standard therapy was only 4.0 times. For both malignancies, the tolerated increased risk of malignancy due to a new therapy for psoriasis was greater then we had expected a priori. This study was important in that we were able to measure physician attitude about acceptable risk versus the benefit of a new therapy for psoriasis. In addition, in the past studies have been designed to find less than a 2 times increased risk but due to the apparent acceptance of risk by healthcare providers in our study, physicians may view psoriasis as a more serious illness than we originally appreciated necessitating a re-evaluation of our trial designs.

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Patients with more extensive psoriasis have greater reductions in quality of life: female patients and young patients are affected to a greater extent

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Psoriasis is a common, chronic, immune mediated disease of the skin that can have substantial effects on quality of life. Quality of life studies have typically been performed in sub-specialty clinic or patient advocacy group populations. The purpose of this study was to describe quality of life in psoriasis patients randomly selected from the United States population. 27,220 patients were randomly selected from the United States population and interviewed over the telephone. 601 patients who identified themselves as having been diagnosed with psoriasis by a physician were invited to complete a more detailed telephone survey about quality of life using a modified version of the psoriasis disability index. 266 (44%) of people who identified themselves as having been diagnosed with psoriasis completed the detailed survey. Self report of body surface area of involvement with psoriasis showed the strongest association with decrements in quality of life (Spearman 0.50, P <0.0001). Younger patients (Spearman -0.16, P <0.001) and female patients (P=0.02) also had statistically significant reductions in quality of life. Increasing psoriasis severity was associated with seeking care from multiple physicians (Spearman 0.31, P<0.001) and having decrements in income (Spearman -0.29, P<0.001). These results support that patients with more extensive skin involvement have greater reductions in quality of life. Female patients and young patients are affected to a greater extent.

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High levels of UVB exposure increase the risk of nonmelanoma skin cancer in PUVA-treated patients

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Sunlight and PUVA are risk factors for the development of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). To quantify the risks due to ultraviolet-B (UVB) therapy, we prospectively analyzed skin cancer incidence from 1975 to 2001 among a cohort of 1380 patients with exposure to PUVA, UVB, and other psoriasis therapies. Stratified Poisson regression was used to quantify the association between UVB and the development of nonmelanoma skin cancer (NMSC), controlling for PUVA, age, gender, skin type, region of residence, methotrexate use, tar use, and sun exposure. A stratified analysis limited to patients with <100 PUVA treatments was also performed. We documented >431,000 UVB treatments among 1130 patients (average per treated subject=381, median=150, range 14 to 6223). High (>=600 treatments) but not intermediate (300-599 treatments) UVB exposure was associated with a significant increase in SCC (adjusted RR=1.48 (95% CI=1.23-1.77)) and BCC (adjusted RR=1.44 (95% CI=1.18-1.78)) risk. In an analysis limited to patients with <100 PUVA treatments, the risks associated with high levels of UVB were greater. For high levels of UVB exposure, the adjusted RR= 3.10 (95% CI=1.72-5.57) and 2.04 (95% CI=1.16-3.58) for SCC and BCC respectively. Intermediate levels of UVB use were a modest risk factor, adjusted RR= 1.86 (95% CI=1.07-3.21) and 1.79 (95% CI=1.13-2.84) for SCC and BCC respectively. UVB use was most strongly associated with tumors on the trunk and legs, which are typically exposed during therapy. For high vs. low UVB, adjusted RR of SCC was 4.88 (95% CI=2.75-8.66) on the trunk or legs vs. 0.71 (95% CI=0.20-2.56) on the head or neck. For BCC, the adjusted RR was 4.38 (95% CI=2.26-8.50) on the trunk or legs vs. 0.53 (95% CI=0.18-1.53) on the head or neck. These results demonstrate that compared to PUVA, UVB has substantially lower NMSC risk. The increased risk of tumors is limited to the highest UVB levels and is predominantly on the anatomic sites typically exposed during therapy.

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Profile of 513 patients with alopecia areata in the United States

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Several lines of evidence support a genetic component to alopecia areata (AA), including differences in patients based on severity of AA, associated diseases, and family history. Here, we examine epidemiological, clinical, and genetic features of patients with AA in the United States. From 1998-2001, a self-selected sample of 513 patients with AA completed interviews consisting of demographic information, clinical information specific to the diagnosis of AA, patient's medical history, and family history of AA. Forty-one percent of respondents reported 100% scalp hair loss with or without body hair loss (alopecia totalis and universalis (AT/AU)). Of those patients with AT/AU, 64% reported an age of onset before 20 years vs. 37% of patients with patchy AA (less than 100% scalp hair loss) (p<0.001). Nail changes were reported more frequently in those with AT/AU (51% vs. 30% of patchy AA) (p<0.001). Fifty-six percent of participants reported having at least one atopic disorder or autoimmune disease, including allergic rhinitis, atopic dermatitis, asthma, thyroid disease, psoriasis, and diabetes mellitus. Patients with AT/AU as well as those reporting nail changes were more likely to have at least one associated disease (p=0.047; p<0.001). Thirty-three percent of participants reported a positive family history of AA. Duration of disease (p=0.033), number of associated diseases (p<0.001), having AT/AU (p<0.05), and having nail changes (p=0.046) were associated with the number of relatives affected by AA. Our findings show marked associations between severity of AA, age of onset, duration of AA, atopy, autoimmune disease, and family history of AA. Further research in atopy, autoimmunity, and genetics in AA will be helpful in clarifying our understanding of AA, leading to improvements in diagnosis and treatment.

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Statistical reviewing policies in dermatology journals: results of a questionnaire survey of editors

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Problems with statistical methods and reporting have been noted in dermatology journal articles. Conclusions in published reports may be misleading if based on inappropriate statistical analysis.our objective was to assess dermatology journal editors' policies and perceptions regarding statistical review of submitted manuscripts,using a questionnaire survey 43 editors, representing 36 dermatology journals from the USA and abroadwere surveyed. 32 editors (74.4%), representing 30 journals (83.3%), returned questionnaires. 24 editors (75%) requested statistical reviews on <5% of published manuscripts containing original quantitative analysis (i.e., excluding reviews and case reports), while 3 editors (9.4%) requested statistical reviews on >75% of such manuscripts. Most editors reported requesting statistical reviews on a case-by-case basis either after initial favorable review by subject-matter (non-statistical) reviewers (12 editors, 37.5%) or at the same time that subject matter review was requested (6 editors, 18.8%). Four editors (12.5 percent) reported requesting statistical review for all manuscripts at the same time they are sent for subject-matter review. 10 editors (31.3%) said their journals had no general policy on statistical reviewing, and statistical review is almost never needed. For 15 editors (46.9%), ideal statistical reviewing policy was identical to their current policy, while 13 (40.6%) favored a more rigorous and 3 (9.4%) a less rigorous policy. Dermatology journals infrequently perform statistical reviews of submitted manuscripts. Dermatology journal editors' statistical review policies range from no general policy to (most frequently) requesting reviews on a case-by-case basis to reviewing all submitted manuscripts. Many editors favor more rigorous statistical reviewing policies for their journals. Increased use of statistical reviewing may increase the reliability of conclusions published in dermatology journals.

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Pilot study to develop a test of skin self-exam accuracy

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While skin self-examination (SSE) for early detection of melanoma has been associated with lower incidence and increased survival from melanoma, people's ability to interpret their own SSE has not been subject to detailed study. We describe a test to evaluate people's sensitivity and specificity in performing SSE through the use of digital photography and computer manipulation of these photographs. Subjects were administered a short questionnaire regarding attitudes toward skin examination, were given instructions in how to perform SSE, and were asked to become familiar with their own skin by practicing SSE at home. Digital photographs were taken of subjects' backs, and these photographs were altered using photo-editing software to mimic early changes of melanoma. These changes included adding a lesion or changing an existing lesion by altering its size or coloration. Each photograph of a subject's back was randomized to have a lesion added, to have an existing lesion changed, or to have no change. At a follow-up visit, subjects were shown the altered photographs of their own back to identify any changes which may have been introduced. Overall, sensitivity and specificity for detecting these artificially introduced "melanoma" changes were 43% and 71%, respectively. In addition, no correlation existed between performance on the study task and either having a history of skin cancer, reporting previous SSE of the back, or having performed SSE during the study period. While this pilot study used a small number of subjects, the preliminary data from these 37 photographs suggest that this test may provide a useful measure of ability to detect changes mimicking melanoma by SSE. Further studies are underway to evaluate the use of this tool in research on early detection of melanoma.

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Risk factors associated with striae gravidarum

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Striae gravidarum is a poorly characterized but common disfiguring condition of pregnancy. Although a relationship between striae gravidarum and weight gain, hormonal factors or genetics have been proposed, no reports exist regarding the strength of these associations. The purpose of this study was to better characterize the epidemiological factors associated with striae gravidarum. An anonymous survey administered at Stanford Ambulatory Clinics sampled 116 women who had given birth. 31 out of 61 women (50.8%) with striae gravidarum had mothers with striae gravidarum; in contrast, only 7 out of 55 women (12.7%) without striae gravidarum had mothers with striae gravidarum, (p<0.001). 34 out of 61 women with striae gravidarum (55.7%) reported additional relatives (sisters, daughters, aunts, cousins) with striae gravidarum; in contrast, 8 out of 55 women without striae gravidarum (14.5%) reported additional relatives with striae gravidarum, (p<0.001). There were no statistically significant differences between women with and without striae gravidarum with respect to percent weight gain in pregnancy (p=0.392) or change in body mass index during pregnancy (p=.302). Recall between women with and without striae gravidarum were similar between the two groups with 39.3% of women with striae gravidarum marking unknown with regard to family history and 38.9% of women without striae gravidarum marking unknown for family history. This study is the first in the medical literature to suggest that family history, and therefore genetic factors appear to be more predictive of the development of striae gravidarum than weight gain or changes in body mass index.

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The quality and impact of case reports and case series

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Case reports and small series are considered weak clinical evidence, but they are often the first indication of potential usefulness of innovative treatments. We analysed case reports and case series (n smaller or equal to 10) from the Lancet published from 1 January 1996 to 30 June 1997 in order to determine the quality and impact of case reports or small series describing innovative treatments. The quality of papers was evaluated by two independent reviewers according to criteria developed from available sources. To assess the impact of the papers we investigated how often they were cited in the literature and how often the suggested treatment was subjected to a clinical trial. We searched MEDLINE, the register of current controlled clinical trials and the Cochrance controlled clinical trials register to find controlled clinical trials. We also searched the science citation index to determine how frequently reports were cited. Sixty-four case reports and thirty-nine small case series were identified. The papers surveyed were quoted on average 17 times (median 6,5; range 0-336). Twenty-four follow-up trials were identified, nine still in the register of current controlled clinical trials. Seven papers (7%)reported treatment failure; 50% reported adverse events or lack thereof; and 39 (38%) reported the patient perception of the treatment. We conclude that case reports and small series published in the Lancet have significant impact on the subsequent literature. They are frequently cited and many of them lead to clinical trials. Frequently the papers report rare conditions for which trials may not be feasible. There was a strong publication bias favouring positive results. Less than half of case reports and series report the patients perception of the treatments outcome. With this survey we hope to stimulate a discussion of case reporting that may lead to the development of guidelines to improve reporting of cases and case series similar to the CONSORT statement for clinical trials.

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Voluntary health advocacy groups: representative? informative? promotional?

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Patient advocacy groups have an increasing prominent role in health care. These organizations serve as advocates and representatives of patients with specific diseases and provide information about these diseases and their treatments. To assess the extent to which members of a patient advocacy group are representative of all those affected by a disease and to assess the differences in knowledge and use of therapies between these two groups. Using random digit dialling, we ascertained psoriasis patients ascertained from the general U.S. population whom we interviewed. Randomly selected members of the Psoriasis Foundation were also interviewed. Main outcome measures: Multivariate logistic regression models designed for surveys were used to estimate differences (odds ratios and 95%confidence intervals) in demographic and clinical characteristics, and the awareness and use of therapies between members and others diagnosed with psoriasis. Of 601 individuals with psoriasis ascertained from the general population survey, 185 provided a second interview and were defined as nonmembers. We interviewed 289 randomly selected members of the Psoriasis Foundation. Although members were significantly older, wealthier and more severely affected by the disease, they reported the disease to be significantly less of a burden and were more satisfied with therapy than others affected. Compared to nonmembers, members were significantly more likely to have heard of and used most of 10 therapies assessed. However, the proportion of respondents who were aware of a therapy who also used it did not differ between both groups. Our findings suggest that members of this specialty society are older, wealthier, and more medicalized and informed about therapies but are not more likely to use therapies of which they are aware. These findings support the premise that patient advocacy groups are generally representative and effective in providing information about diseases and treatments.

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Is Mohs surgery a cost-effective treatment for facial nonmelanoma skin cancer? A decision analysis

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The purpose of this study was to determine whether Mohs surgery (Mohs) is a more cost-effective method of treatment for facial nonmelanoma skin cancer (NMSC) than traditional standard excision (TSE). Since cost-effectiveness analyses (CEAs) incorporate efficacy and outcomes in addition to cost, CEAs can quantify the overall value rather than just the costs of one therapeutic strategy over another. The data from our prospective trial of 98 consecutive patients with primary facial NMSC was used to obtain baseline cost (Connecticut Medicare 2002 reimbursements) and efficacy (margin analysis) information for our CEA. We approached the CEA using a decision analysis model via Treeage Data 4.0 software. Our model also incorporated efficacy using 5-year recurrence rates from the literature, and outcomes (quality-adjusted-life-years (QALY)) using data from a focus group of patients. We performed a sensitivity analysis to determine the influence of key estimates in the model. Our baseline CEA demonstrated Mohs to be less costly and more effective than TSE ($956.60 vs. $1248.10, and 0.6 QALY gain). The sensitivity analysis showed that varying values for QALYs, recurrence rates, and percentage of frozen and permanent section margin analysis did not change the results of our CEA. However, varying the proportion of defect repairs (granulation, primary closure, flaps, grafts) following the two procedure strategies influenced the results of our CEA such that TSE was less costly and marginally more effective than Mohs. Therefore, before the most cost-effective treatment for facial NMSC can be definitively established, further research into actual practice patterns of defect repair selection for both procedures must be examined.

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Clinical research ethics in dermatology

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The word research was introduced in 1639 to describe an investigation directed at the discovery of a fact through careful consideration of a subject. In dermatology, many therapeutic advances emerge from the common practice of off-label use of medications. Because the successful application of innovative therapy is sometimes published as a case study, it becomes important to recognize when the novel use of therapeutics constitutes research, rather than routine clinical care. For example, if an innovation is undertaken not simply to offer an alternative therapy, but with the intent to publish, then it constitutes research, requiring independent review and participants′ informed consent. All clinical evaluations involving the treatment of human subjects, published during 2002 in the Journal of the American Academy of Dermatology, the Archives of Dermatology, and Arthritis and Rheumatism, were reviewed to determine whether institutional review board (IRB) approval was documented in the methods. Case reports involving only one patient were excluded because these reports were thought more likely to constitute innovative care, whereas case series were more likely to have required at least a chart review. Chi square test for proportionality was used to evaluate significance. IRB approval was explicitly documented in 34 of the 83 (41.0%) clinical evaluations involving more than one patient reviewed in the dermatology literature compared to118 of the 198 (59.6%) clinical evaluations of more than one patient reviewed in the rheumatology literature (P=0.04). Of the clinical evaluations involving more than one patient published during 2002, 80.7% in the dermatology literature were open label studies compared to 4.5% in the rheumatology literature. There is a balance between encouraging dissemination of valuable clinical observations and protection of human subjects′ rights. Under the current approach to evaluating the novel application of therapeutics in dermatology, the ethical requirements for human subjects research are probably being insufficiently documented and are possibly being inadequately met.

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The genetic epidemiology of alopecia areata in Chinese

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Alopecia areata (AA) is hypothesized to be an organ-specific autoimmune disease with genetic predisposition and an environmental trigger. There is few clinical data in Asians. The aim of our study is to describe the genetic epidemiologic features of AA patients in China and presume the possible genetic model of AA. Data for 1032 patients with AA was obtained by questionnaire. Complex segregation analysis and heritability were performed using Falconer′s method, Epi Info 6.0 and SAGE-REGTL programs. We found that the mean age of onset was about 29 years old. About three quarters of all patients experienced their first episode of AA within the first four decades of life. A positive family history of AA was obtained in 87 patients (8.4%). A greater severity and longer duration of AA were seen in early onset group than in late onset group. In relatives of AA patients, the Occurring risk of AA increased with increasing blood relatedness. Based on the REGTL results, the best model was a polygenic additive model for AA with approximately 50% heritability. It can be concluded that effect of genetic factors is strong in the episode of AA, but environmental factors may still play an important role. Our findings on the genetics of AA are consistent with a polygenic additive mode of inheritance.

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Skin cancer examination teaching in US medical education

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There are more than 1.3 million skin cancers annually in the US, but most physicians report receiving minimal instruction in its diagnosis. To determine physician preparation for the skin cancer examination (SCE), we evaluated medical students′ practice of, training in, observation of, and self-reported skill level for SCE as well as hours spent in a dermatology clinic. Fourth year medical student coordinators were recruited to survey classmates at 7 US medical schools during the 2002-2003 academic year. All coordinators had input in the final questionnaire. Of the 934 graduating students enrolled at the 7 schools, 659 (71%) completed surveys: 43% of students graduating from medical school had never examined a patient for skin cancer, 27% had never been trained to perform a SCE, and 23% had never observed a SCE. Only 28% of graduating students rated themselves as somewhat or very skilled in the exam. This rate dropped to 19% among those who had not completed a dermatology elective (n= 549). However, graduating students who reported having been trained at least once in the SCE were 8 times more likely to report themselves as somewhat or very skilled as students without such training. 68% of students agreed that too little emphasis in their medical training was placed on learning about the SCE. These results confirm and expand upon previous findings at a single study site. They suggest that even brief curricular additions would boost student skill level and impact practice patterns and competencies of future physicians. More and better SCE might result in earlier detection of melanoma and non-melanoma skin cancers by non-dermatologists, and thus significantly impact public health.

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Obesity and smoking as risk factors in psoriasis

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The mean weight of patients with psoriasis in recent clinical trials has been over 90 kg. The Utah Psoriasis Initiative (UPI) carefully phenotypes patients with psoriasis with the intent of genotyping selected phenotypes. To date we have phenotyped over 350 patients. Data reveals a mean body mass index (weight/height2) of 29.4, which is greater controls in the Utah population (Behavioral Risk Factor Surveillance System Prevalence Data, 2002). The prevalence of obesity in the UPI is higher than the general Utah population (p<0.001). Using a standardized diagram to assess body image, psoriatic patients viewed themselves as normal weight at 18 and at the onset of their psoriasis (mean age of 27.3), but perceived themselves as overweight at enrollment into the UPI (mean age 50.6). This did not support the primary hypothesis, obesity increases the risk of developing psoriasis in those genetically predisposed. Secondary hypotheses were explored. Patients in the UPI who are obese have significantly more body surface area covered with disease than their non-obese counterparts. When correcting for weight and height, it is noted that body surface area with psoriasis is proportional. Obesity does not appear to predispose to arthritis. Obesity did not positively or negatively affect the response or adverse effects of topical corticosteroids, light based treatments, and systemic therapies. The morbidly obese (BMI > 35) appear to have a higher prevalence of inverse psoriasis. When compared to the Utah population, there was a higher prevalence of smoking in the UPI population (p <0.001). Smokers had the same average age of onset and similar amounts of body covered with disease as non-smokers. There was a higher prevalence of obese smokers in the UPI compared to obese smokers in the general population (p < 0.001). This causes us to conjecture that smoking adds additional pressure to emergence of psoriasis in the genetically predisposed. We conclude that psoriasis has a pivotal yet unknown role in developing obesity and that smoking may increase the risk of psoriasis.

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Prevalence and risk factors of dysplastic nevi in the general population

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Demographic changes and the significant increase of malignant melanoma point to a need for a population-based epidemiological investigation of marker lesions, such as dysplastic nevi (DN). Little, however, is known on the frequency and associated risk factors of DN in the general population. For the KORA (Cooperative Health Research in the Augsburg Region) survey 2000 a random sample, stratified by age (25-74 years) and gender, of registered inhabitants of the City of Augsburg, Germany and two adjacent counties was drawn. A total of 2822 (response 67%) subjects were examined dermatologically in a standardised way and dysplastic nevi (DN) were recorded following clinical criteria (ABCD rule, computer-assisted epiluminescence). Relevant risk factors were obtained by a computer-assisted personal interview. A total of 5.2% of the subjects exhibited at least one DN, whereby men were affected more frequently (6.0% vs. 4.5%). The prevalence of DN (9.1, 5.7, 3.1, 2.1%) decreased with age (25-34, 35-44, 45-54, 55-64, 65-74 years). A familial history of a skin tumor or frequent (>50) common nevi was associated with a higher prevalence of DN (11.1% vs. 5.1%, 9.7% vs. 4.7% resp.). Subjects, who used sunblockers or reported sunburns during childhood were also affected more frequently (5.7% vs. 3.8%, 5.9% vs. 4.6% resp.). Furthermore the prevalence of DN depended on the number of weeks which were spent in sunny areas per year (0=3.1%, 1=6.1%, 2=7.1%, 3=6.3%, >3=4.6%). Multiple regression analysis revealed age (OR 0.74, 0.63-0.86), female gender (OR 0.62, 0.42-0.90), family history (skin tumor: OR 2.42, 1.09-5.36; >50 nevi: OR 1.90, 1.22-2.96) and annual time spent in sunny areas (3 vs. 0 wks.: OR 1.93, 1.02-3.63) as significant risk factors. DN are frequent in the adult general population and depend on sociodemographic and genetic as well as parameters of an individual UV exposure.

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TIA-1- positive lymphocytes are associated with metastases in patients with melanoma

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Although factors such as the absence of tumor-infiltrating lymphocytes (TILs) are known to be associated with a higher risk, assessment of the risk for metastases in melanoma patients using current prognostic models is imperfect. We performed a case-control study to determine whether immunohistochemical staining for TIA-1, an intracellular protein associated with cytotoxic granules of lymphocytes, was associated with metastases and therefore a potential contributor to future prognostic models. To test our hypothesis, we used CART (Classification and Regression Tree) analysis to define distinct risk groups based on the number of TIA+ cells and the presence or absence of TILs. The absolute number of TILs reactive to TIA-1 antibody over a 0.125 mm^2 area at the tumor base was measured using light microscopy in 28 cases who developed metastases and 27 controls who did not develop metastases over the first 10 years of follow-up. Subjects were chosen so that the distribution of tumor thickness was similar between the two groups. The number of TIA+ cells within the designated area ranged from 0-290 (median=10). CART analysis identified 3 risk groups: 1) those without TILs, in whom 66.7% (14/21) had metastases; 2) those with TILs and a TIA+ cell count of less than 12, in whom 16.7% (2/12) had metastases; and 3) those with TILs and a TIA+ cell count of 12 or more, in whom 54.5% (12/22) had metastases. The metastatic rates among the three risk groups were statistically significantly different (p=0.02 by Fisher exact test). Metastasis was not associated with TILs alone (p=0.10) nor with TIA+ cell count alone (p=0.43). Using both TIA+ cell counts and TILs to define risk groups for metastasis appears to be better than using either risk factor by itself, and TIA+ cell counts could potentially be a useful addition to prognostic models for metastasis risk in melanoma.

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Youth access to indoor UV tanning: a telephone survey of operators in Colorado, Texas, Illinois, and Wisconsin

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To describe the compliance of indoor tanning facilities with youth access regulations, we assessed the youth access practices of indoor tanning facility operators in three states with varying degrees of youth access regulations (Texas, Illinois, and Wisconsin) and one state without youth access regulations (Colorado). Using a cross-sectional survey study design, 100 randomly selected licensed facilities providing non-medical UV tanning in each state were telephoned. Each state was assessed for the percentage of facilities with an operator reporting permitting indoor UV tanning youth access in violation of state regulations a) for a potential 12-year-old patron and b) for a potential 15-year-old patron. For a potential 12-year-old patron, 38% of facilities in states with youth access regulations had an operator report that they would permit indoor UV tanning youth access in violation of regulations (Texas 77%, Illinois 26%, Wisconsin 11%). For a potential 15-year-old patron, 44% of facilities had an operator report that they would permit indoor UV tanning youth access violating current regulations (89% in Texas would allow access without guardian accompaniment, 20% in Illinois would allow access without guardian consent, and 23% in Wisconsin would allow access). Operators in Colorado reported self-governing regulations for 12-year-old (age limits 18%, guardian consent 62%, and guardian accompaniment 8%) and 15-year-old patrons (age limits 10%, guardian consent 70%, and guardian accompaniment 6%). Compliance with youth access regulation varied greatly among states. Higher compliance levels in states with long standing youth access regulations (Illinois and Wisconsin), and frequent self-imposed youth access limitations in Colorado demonstrate the potential for successful tanning industry youth access regulation, both from outside and within the tanning industry.

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Cancer risk and developmental abnormalities in xeroderma pigmentosum patients and heterozygotes

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Xeroderma pigmentosum (XP), a rare (about 1 in 10^6) autosomal recessive disease, is characterized by severe photosensitivity, abnormal pigmentation and increased risk of skin cancer in association with defective DNA repair. Eight XP genes [(*XPA-XPG*) and XP variant (*pol-eta*)] have been identified. The frequency of clinically normal appearing XP heterozygotes (about 1 in 300) is much greater than XP patients but their cancer risk is not well studied. One study (Swift and Chase, 1979) was performed before the XP genes were identified and reported a 16-fold increase in non-melanoma skin cancer among the blood relatives of XP patients in 4 families compared to spouse controls (p=0.0001). For 50 patients ascertained at the NIH we have assigned an XP gene and for 37 we have identified a causative mutation. The type of skin cancer, frequency of internal tumors and manifestations of neurological or developmental abnormalities differ among these patients. To date, we have collected 19 detailed multi-generation pedigrees. We are performing a molecular epidemiologic study of all consenting relatives in these XP kindreds and will determine which relatives are mutation carriers. We plan to obtain information about skin cancer risk factors, and pregnancy outcomes and confirm reported cancers in study participants. We intend to compare the risk of cancer and developmental abnormalities in the heterozygote carriers with that of non-carrier relatives, spouse controls and population databases. This study may define the cancer risk of carriers of XP DNA repair genes. It may provide a model for study of carriers of other recessive disease genes and increase the understanding of DNA repair mechanisms in cancer and human development.

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Psoriasis is less common but not rare in African Americans

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Psoriasis has been described as rare in African Americans, however, to our knowledge, there have been no population based epidemiologic studies of the impact of race on psoriasis prevalence. The purpose of this study was to determine the epidemiology of psoriasis in African Americans. 27,220 people were randomly selected from the United States population using a stratified sampling method and were interviewed over the telephone. Respondents were classified as having psoriasis if they reported having been diagnosed by a physician as having the disease. 21,921 respondents indicated that they were white, of which 541 indicated that they had psoriasis. 2,443 respondents indicated that they were African American, of which 27 reported having been diagnosed by a physician as having psoriasis. The prevalence of psoriasis, adjusting for the sampling procedure, was 2.5% (95% CI 2.2, 2.7) in whites and 1.3% (0.7, 1.8) in African Americans. The relative prevalence ratio in African Americans compared to whites was 0.52 (P< 0.0001, Fishers exact test). The distribution of psoriasis based on sex was similar between the two groups, as was impact on quality of life and satisfaction with treatment (based on single global questions). This study suggests that African American race is protective of developing psoriasis, however psoriasis is not rare in this population.

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Prevalence and impact of industry funding of dermatology clinical trials

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Recent studies examined the extent and impact of industry sponsorship of clinical trials in medicine and raised concern for conflict of interest; there has been little systematic research, however, on its extent and impact in dermatology. We performed a MEDLINE search of clinical trials published between October 2002 and 2003 in the four most highly cited dermatology journals. Sponsorship, author affiliation, study size, Jadad quality score[1], and outcome were recorded. The search identified 61 articles from the *Journal of Investigative Dermatology, British Journal of Dermatology, Archives of Dermatology*, and *Journal of the American Academy of Dermatology*. Sixty-four percent of the studies were sponsored by the company producing the intervention. One or more authors was an employee of the company in 39% of trials. Twenty of 22 trials in which at least one author was an employee of the company demonstrated a benefit of the experimental intervention over the control. This compares to 65% of trials in which none of the authors were employed by the company (Fisher's exact p=0.032). Jadad study quality scores were similar in trials with author-employees and non-employee authors (3.32 vs 3.47, Mann-Whitney p>0.05). The majority of published dermatology trials rely on industry funding, which is associated with a higher incidence of favorable findings for the intervention. A greater number of dermatology authors reported industry affiliations (39%)compared to general medical investigators (23-28%)[2]. The substantial extent of employee-authorship in dermatology and its documented influence on trial outcomes merit further critical evaluation

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Development of a reliable patient self-rated acne severity questionnaire

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Research in acne outcomes has been limited by the physical requirement that patients visit a trained specialist for severity assessment. Our goal was to develop a reliable Patient Self-Rated Acne Severity Questionnaire, by which patients could assess acne severity in a manner similar to that of a physician, thus obviating the need for the physician visit. From our initial work using standard survey development and improvement probing techniques, we constructed a 16-item survey for patients to evaluate 8 acne-associated lesions on their face and on their trunk for the prior seven days. The survey consisted of pictures and word descriptions of the lesions and was constructed with a 5-point Likert scale for countable lesions (acne bumps, bumps full of pus, blackheads/whiteheads, scabs, and nodules/cysts) and a 4-point Likert scale for lesions better measured qualitatively (oiliness, blemishes, and scars). Subjects' responses were compared to those of a physician rater, who completed the survey simultaneously. We defined a reliable measurement between subject and physician responses to be a mean difference and 95% confidence interval (CI) that fell within +/- 1 point. Twenty-three subjects with mostly mild-moderate acne completed the survey. The mean age was 29 ± 8 yrs. Ethnicities included 39% Caucasian, 30% African American, 26% Asian descent, and 5% Other. Educational levels included 13% high school; 35% college, and 52% post-graduate education. We found acceptable differences for all lesions. For example, mean differences (95% CI) for bumps full of pus were 0.35 (0.10, 0.60) and -0.09 (-0.35, 0.17) for face and chest/back, respectively. These results suggest that there may be no clinically significant difference between patient and physician rating of acne severity with our questionnaire. Further work is necessary in populations with more heterogeneous acne lesions and diverse ethnicity and educational levels.

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Toxic Epidermal Necrolysis, Stevens-Johnson Syndrome, and Erythema Exsudativum Multiforme Majus: what is the role of infection?

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Toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome (SJS) and erythema exsudativum multiforme majus (EEMM) are severe, sometimes life-threatening cutaneous adverse reactions. Besides a number of medications, various infections such as influenza-like illness or mycoplasma infection are considered as etiologic factors. Often these infections are treated with antiinfective agents also thought to be associated with severe cutaneous adverse reactions (SCAR). Before large epidemiologic studies on SCAR were undertaken in different European countries, a consensus definition was established. SJS and TEN develop on a spotty, partly target-like wide-spread exanthema with various amounts of blisters and erosions (< 10% in SJS; > 30% in TEN; SJS/TEN overlap 10-30%). In contrast, EEMM presents with target lesions especially on the lower extremities and sometimes also with single lesions disseminated in other parts of the body. The SCAR-study (1989-1995) revealed that in ca. 65% of SJS- and TEN-cases drugs known to induce SCAR were taken shortly before the onset of the adverse reaction. In contrast, recent or recurrent herpes could be identified as a major risk factor for EEMM (46%). The German Registry on severe skin reactions, an intensive surveillance system operating since 1990, could support these results based on ca. 500 cases of EEMM, and ca. 1,240 cases of SJS, SJS/TEN-overlap and TEN. The recent EuroSCAR-study (1997-2001) showed a slightly increased relative risk (RR) of 2 (confidence interval (CI) 0.5-2.8) for infections, which was low compared to the risk of antibiotics (RR 8; CI 2.1-20). Stratified analysis in the individuals using antiinfective drugs did not show any increased risk for infection (RR 0.8; CI 0.3-2.2). Whereas infections do not show an increased risk for SJS and TEN, this is different for EEMM. Antiinfective drugs given for these infections are the causative agents. However, it cannot be ruled out that infections may influence the risk of SJS and TEN imposed by drugs.

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Nonmelanoma skin cancer mortality in Rhode Island, 1988 through 2000

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Nonmelanoma skin cancer (NMSC) is the most commonly diagnosed malignancy in the United States. Despite the magnitude of the public health burden, investigation of NMSC mortality has been limited. The purpose of this study was to elucidate the magnitude of, and factors associated with NMSC mortality. A population-based follow-back study was conducted of Rhode Island residents whose deaths between 1988 and 2000 were attributed to NMSC. The proportion of misclassified deaths was significantly higher for nongenital NMSC (57%) than for genital NMSC (18%, p<0.0001). The majority of deaths misclassified as nongenital NMSC were caused by squamous cell carcinoma of mucosal surfaces. The age-adjusted NMSC mortality rate was 0.91 (per 100,000 per year) of which half (0.45) was due to genital carcinoma. NMSC mortality increased sharply with age. The mortality rate from nongenital NMSC in men was more than twice that in women, but for genital NMSC this ratio was reversed. Skin cancers originating on the ear were responsible for more than a quarter of all deaths caused by nongenital NMSC. No cases of NMSC mortality occurred in organ transplant recipients. Many individuals had psychiatric comorbidities or evidence of unreasonable delay in seeking medical care for their lesions. Nongenital NMSC mortality was lower in 1988-2000 than in 1979-1987. Misclassifying the cause of death as nongenital NMSC accounts for a large source of error on death certificates in Rhode Island. Overall, mortality from genital cercinoma was about equal to total nongenital NMSC deaths. The dermatology community has not focused sufficient effort on prevention of mortality from genital skin cancer.

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Atopic dermititis family impact questionnaire development

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Childhood atopic dermatitis (AD), or eczema, is very burdensome for the family, and has been associated with lower quality of life in parents and children. However, there are no published instruments validated for the United States that measure the impact of AD on the family. This study was undertaken to develop such a measure. Initial development began with focus groups of parents with children diagnosed with AD to determine areas of life affected by AD. Based upon findings from these groups and the literature, a preliminary AD Family Impact Questionnaire (54 items) was developed and tested on 50 parents of children with AD. The scale was then refined, and the resulting 30-item measure is currently being validated on a larger scale. Two-hundred primary caregivers of chidren ages 12 or younger with AD are the targeted population. Parents are being recruited through advertisements in national newsletters, skin disease websites, and through a University of Miami dermatology clinic. Interested parents directly contact study personnel (by mail, e-mail, or phone) to request a mail survey. Twenty-five percent of the participants who return the survey are randomly being called or e-mailed and asked to complete the instrument again one month later for test-retest reliability. Pearson correlations, Cronbach's alpha, and factor analysis will be used to determin the reliability and validity of the new instrument. To date 109 surveys have been completed. Preliminary factor analyses has revealed three primary factors: time management (9 items), family lifestyle (8 items), and external social issues (3 items). The alpha reliabilities for these subscales and the overall scale are good (.83-.91 for subscales, .94 for overall scale), and initial construct validity is also promising. Once final analyses are completed, the scale will be useful as a process or outcome measure for intervention studies on families of children with AD.

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Etanercept in patients with moderate to severe psoriasis who fail methotrexate: a cost-effectiveness analysis

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The purpose of this study is to evaluate the cost-effectiveness of etanercept (ETA) in patients with moderate to severe psoriasis who have failed methotrexate (MTX). We compared 2 treatment strategies: 1) MTX and 2) MTX followed by ETA (MTX-ETA). A computerized decision analytic model using a Markov process was constructed to assess benefits (in quality-adjusted life-years [QALY]) and costs (2003 US$) for both strategies over 5 years. Clinical inputs for the model were obtained from a literature review and expert opinion. Cost estimates for medications, laboratory tests, physician fees and drug-related complications were obtained from Henry Ford Hospital. In the MTX strategy, patients received 20mg of MTX weekly during a 3-month induction phase, and if successful, they were maintained on 15mg weekly. Treatment failures received repeat induction doses and/or escape therapy (i.e., topicals/phototherapy). In the MTX-ETA strategy, patients first received MTX; however, those who failed MTX were started on 25mg twice-a-week of ETA during a 3-month induction phase and then continued on lower maintenance doses (i.e., 25mg per week). As data are limited regarding long-term remission rates with MTX and ETA, extensive sensitivity analyses were performed. In the base-case analysis, we found that the MTX strategy led to approximately $17,400 in costs and 2.8 QALYs. The cost of the MTX-ETA strategy was 44% greater ($25,000), but resulted in greater benefits (3.1 QALYs). The incremental cost-effectiveness ratio for the MTX-ETA strategy was $25,300 per QALY. Sensitivity analyses suggested this result was sensitive to estimates of ETA's long-term efficacy and cost. We conclude that patients with moderate-to-severe psoriasis who have failed MTX are likely to receive additional benefits with ETA at a reasonable cost. As expected, better estimates of ETA's cost-effectiveness will depend on future data regarding its long-term efficacy and safety in such patients.

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Association of eyeglass use with periocular keratinocyte carcinoma (BCC or SCC) risk

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Eyeglasses have the potential to reduce considerably the flux of ultraviolet light on the periocular skin, and are commonly used, although adequate empirical support for their use to reduce skin cancer risk is lacking. Hence we investigated whether the non-use of eyeglasses was associated with keratinocyte carcinoma risk. We hypothesized that relatively recent use would be protective against squamous cell carcinoma (SCC), and more remote use against basal cell carcinoma (BCC). We used the baseline data of the VATTC Trial, a randomized chemoprevention trial for BCC and SCC among VA patients at 6 centers who had at least two of these cancers in the 5 prior years. We determined at baseline whether any of the qualifying cancers arose on periocular skin. We also interviewed participants to determine their eyeglass use during leisure and recreational activities at different ages. 1131 participants were randomized, of which 1031 (91%) responded to the questions about eyeglass use below age 40 and 956 (85%) at ages above 40. Under age 40, 55% reported no use of eyeglasses, and over age 40, 30% reported no such use. Periocular BCC was diagnosed prior to randomization in 11% and 10% in the under 40 and over 40 respondents, and periocular SCC in 1.5% and 1.4% respectively. Periocular BCC was not associated with eyeglass use in these data (OR 1.4, 95% CI 0.9-2.0 for use under age 40, and OR 1.3, 95% CI 1.3-2.0 for use after age 40). However, our data did suggest an association with periocular SCC: OR 3.3, 95% CI 0.9-11.6 for use before age 40 and OR 3.9, 95% CI 1.3-12.0 for use after age 40. These data provide empirical support for the association of recent non-usse of eyeglasses with SCC and hence for the use of eyewear to prevent periocular SCC of the skin.

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Quality-of-life outcomes of treatments for nonmelanoma skin cancer

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Quality-of-life outcomes are important for patients and physicians choosing therapies for non-fatal cancers. Nonmelanoma skin cancer (NMSC), which is only rarely fatal, is the most common malignancy. The usual therapies are electrodessication and curettage (ED&C), excision, or Mohs histologically-guided surgery (Mohs); most tumors can be removed effectively by many therapies. To compare quality-of-life outcomes of NMSC therapy, we performed a prospective cohort study of consecutive patients with NMSC at a VA and a private practice. Skin-related quality of life was measured before and 2 years after therapy with Skindex-16, a validated measure. Skindex scores vary from 0 to 100 (most effect), and are reported in three domains: Symptoms, Emotional effects, and effects on Functioning. The minimal meaningful change in Skindex scores is 10. To compare differences in changes in Skindex scores, we used analysis of covariance models adjusted for pretreatment clinical and demographic features, including baseline quality of life. In 838 patients, treatments were ED&C in 20%, excision in 40%, and Mohs in 40%. 56% of patients responded at two years after therapy. Patients treated with ED&C improved only minimally in quality of life after treatment. Patients treated with excision and Mohs improved in all three quality-of-life domains (the mean change in Symptom scores for both groups was 10; the mean change for Emotions scores for excision was 18 and for Mohs was 25; changes in Functioning were less). In the multivariable models, quality-of-life outcomes of patients treated with excision and Mohs improved similarly. We conclude that quality-of-life outcomes differ among different therapies for nonmelanoma skin cancer. Improvements were slight for ED&C, but similar for excision and Mohs. This information must be combined with data about other outcomes to inform decision-making for these non-fatal cancers.

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Measuring quality of life of patients with psoriasis using the SF-36

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This study aimed at describing quality of life (QoL) in patients with psoriasis using the SF-36 questionnaire. All adults hospitalised with psoriasis at IDI-IRCCS from Jan. 2001 to Feb. 2002 were given the SF-36, a questionnaire intended to measure QoL in all conditions, including good health. It measures QoL on eight scales: Physical Functioning (PF), Role-Physical (RP), Bodily Pain (BP), and General Health (GH) are linked to physical health; Vitality (VT), Social Functioning (SF), Role-Emotional (RE), and Mental Health (MH) relate to mental health. Lower scores indicate poorer QoL. Clinical severity was measured using PASI. SF-36 data were available for 380 patients. Overall scores showed that our study population, compared to the health profiles of US patients had physical health scores intermediate between minor and serious medical conditions, and mental health worse than both minor and serious medical conditions and quite similar to psychiatric illnesses. In our study, women had a significantly poorer QoL on all SF-36 scales (e.g., RP=44 vs. 65, RE=36 vs. 58, in women vs. men). Patients with age >= 65y had lower scores on all physical health scales and RE (e.g., RE=39 vs. 58, in older vs. younger). Patients with onset after 39y had poorer QoL on all scales except SF and MH. Duration of disease (+/- 10y) influenced only GH (58 vs. 64, for 10+ vs. <10y). As for subtypes of psoriasis, the lowest scores were observed in palmoplantar (RP, BP, and RE), pustular (PF, VT and MH), generalized (SF), and arthropatic psoriasis (GH and VT). Arthropatic psoriasis had very poor QoL in all eight scales. No significant differences in SF-36 scores, except for PF, were seen in quartiles of PASI. SF-36 scores showed that psoriasis is a disease with major QoL impairment, comparable to that of relevant serious medical conditions. Also, SF-36 seems to be a useful tool to describe QoL in different sociodemographic and clinical subsets of patients.

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Sustained improvement in functional status and vitality of psoriatic arthritis patients treated with etanercept

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The purpose of this study was to determine the sustainability of improvements in the functional status and vitality of psoriatic arthritis patients treated with etanercept for up to 72 weeks.

205 patients with psoriatic arthritis were enrolled in a multicenter, double-blind, placebo-controlled trial for 24 weeks. After the blinded phase, patients continued to receive etanercept in an open-label phase of the trial. We reported the preliminary data on the functional status and vitality of the patients who have received etanercept in both the blinded and open-label phase, as measured by the Health Assessment Questionnaire (HAQ) and the SF-36 vitality scale. There were 101 patients who were randomized to etanercept in the blinded phase of the trial. The mean age was 47.6 years and 57% of the patients were male. Ninety percent of the patients were Caucasian. The mean HAQ disability index score improved from 1.1 at baseline to 0.5 at 24 weeks and 0.4 at 72 weeks. The change in the HAQ component scores (dressing and grooming, eating, hygiene, activities, reach, grip, arising and walking) ranged from negative 0.5 to negative 0.6 units at 24 weeks; these were sustained at 72 weeks. At 24 weeks, mean improvement in the SF-36 vitality score from baseline was 14.6; at 72 weeks, the mean improvement from baseline was 15.3. In conclusion, psoriatic arthritis patients treated with etanercept had clinically meaningful improvements in functional status and vitality and these changes were sustained for up to 72 weeks.

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Factors associated with skin self-examination in an intervention study

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Purpose: To identify factors associated with adherence to skin self-examination (SSE) in the context of a study designed to assess the impact of a brief nurse-delivered intervention on patients adherence to performing SSE. Methods: The study population was comprised of 100 patients at high risk for melanoma skin cancer (five or more dysplastic nevi) from the outpatient Pigmented Lesion Clinic at Memorial Sloan-Kettering. Patients were randomized to receive either a teaching intervention with a photobook (personal whole-body photographs compiled in the form of a booklet) or teaching intervention only without a photobook. Self-administered questionnaires were provided at three intervals: baseline, post teaching intervention, and at four months post baseline visit. To assess adherence with SSE, we asked patients, How many times in the past four months did you (or someone else) thoroughly examine your skin? Information was obtained on demographics, phenotypic characteristics, sun practices, skin cancer knowledge and awareness, personal and family history of cancer, psychosocial and behavioral factors, and screening/health behaviors. Logistic regression was used to model the relationship between the potential predictor variables and adherence with SSE at the four month follow-up. For these analyses, use of the photobook was evaluated as a dichotomous predictor variable. Results: Younger age, use of photobook, higher skin cancer knowledge, higher self-efficacy at completing SSE, lower cancer worry and lower perceived risk of developing melanoma were predictors of adherence to SSE. Conclusions: This study included a comprehensive set of potential predictors of SSE, including psychosocial factors. Identifying predictors of SSE will enable health care providers at both the clinical and psychosocial level to target individuals who may not be performing SSE but who are at increased risk for developing melanoma and to further enhance behavioral programs aimed at modifying risk behaviors.

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Cost efficacy comparison of biologics used to treat psoriasis

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This study compared the cost-efficacy of three biologics with completed phase III trials in psoriasis, using the Psoriasis Area Severity Index (PASI) 50% and 75% response rates as efficacy measures. We analyzed the three-month cost-efficacy, from a managed care perspective, of the following treatments: alefacept 15mg weekly, efalizumab 1mg/kg weekly, and etanercept either 25mg twice-weekly or 50mg twice-weekly. Costs of drugs, physician visits, laboratory tests, and treatment of adverse events were included. Drug costs were average wholesale price. Other resource costs were Medicare reimbursement rates and the MEDSTAT DRG Guide. Efficacy measures were percent of patients achieving PASI 50 and PASI 75 response rates, reported from comparable clinical trials. Adverse event rates were assumed equal among treatments and 5% of patients were assumed to drop out at 6 weeks due to adverse events. Total three-month costs were $12,454 for alefacept, $4,372 for efalizumab, $4,326 for etanercept 25mg twice weekly and $8,335 for etanercept 50mg twice weekly. PASI 50 response rates were 0.42 for alefacept, 0.52 for efalizumab, 0.58 for etanercept 25mg twice weekly, and 0.74 for etanercept 50mg twice weekly. Cost per PASI 50 responder was $29,652 for alefacept, $8,408 for efalizumab, $7,458 for etanercept 25mg twice weekly and $11,263 for etanercept 50mg twice weekly. PASI 75 response rates were 0.21 for alefacept, 0.22 for efalizumab, 0.34 for etanercept 25mg twice weekly and 0.49 for etanercept 50mg twice weekly. Cost per PASI 75 responder was $59,304 for alefacept, $19,873 for efalizumab, $12,723 for etanercept 25mg twice weekly and $17,010 for etanercept 50mg twice weekly. In conclusion, etanercept 25mg twice weekly was less costly and more effective compared to alefacept and efalizumab. As a result, it was the most cost-efficacious treatment option. Efalizumab was the second most cost-efficacious based on PASI 50 response. Etanercept 50mg twice weekly was the second most cost-efficacious based on PASI 75 response rate.

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Declines in air travel cost correlate strongly with increasing melanoma incidence

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Background: A relationship between intermittent high-dose UV radiation leading to sunburn and melanoma has been reported, but the times between the exposure, development, and diagnosis of this neoplasm have not been clearly established. As the cost of air travel decreased substantially in the 1970's and 1980's, leisure travel to vacation destinations during winter months expanded. This recreational trend likely increased the incidence of significant UV exposure and sunburns in a broad population who could not previously afford this kind of travel. Purpose: The purpose of this study was to explore the relationship of airfare prices to melanoma incidence. Methods: Mean inflation-adjusted airfare prices for 4 airports linked to leisure destinations (Miami, Los Angeles, San Diego, Phoenix) was obtained from The Domestic Airline Fares Consumer Report. Melanoma data was collected from the SEER 9 Registry Database, 1975-2000, which contains a sample of 77,617 individuals diagnosed with melanoma of the skin. Mean age at diagnosis was 54.4 years. The costs of air travel were compared to melanoma rates. Results: The increase in melanoma rates corresponded strongly with decreasing airfares, R2=0.92, p<0.01. Modeling a short time lag between airfare and melanoma diagnosis did not strengthen the association. Longer times lags could not be modeled due to data limitations. Conclusion: Correlation does not equal causality, but this strong relationship suggests that further research exploring the connection between adult sunburns and development of melanoma is needed.

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Validation of an instrument to collect medically relevant information from psoriasis patients in a standardized manner

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Establishing well-defined psoriatic phenotypes and collecting detailed medical information important for psoriasis pathogenesis is critical for both research on psoriasis genetics and in understanding different individual responses to biologic therapies (i.e., pharmacogenomics). Currently, geneticists and clinical researchers utilize an extremely heterogeneous group of questionnaires to gather medical information from psoriasis patients. This heterogeneity is problematic in that it complicates the use and analysis of information collected by different groups in different studies. The purpose of this investigation was to design and validate a detailed survey instrument that could be uniformly used in psoriasis genetics and clinical research studies. We used the Delphi method, a multiphase process through which expert opinions are developed into a consensus, to generate a novel survey instrument. Fifty subjects with psoriasis were enrolled in the validation study. Each subject was asked the survey questions by each of 3 investigators. Answers from each subject were recorded by each investigator and were compared to determine agreement (expressed as K). Data showed high agreement for questions relating to place of birth (K=0.85-1.0), family origin and psoriasis history (K =0.88-1.0), patient medical history (K=0.76-1.0), distribution of lesions (K=0.73-1.0), precipitating factors (K=0.79-1.0), joint problems (K=0.74-.91), and treatment history, including use of oral retinoids, methotrexate, and etanercept (K=0.73-1.0). Questions with poor to fair agreement between investigators included past history of alcoholism (K=0.16-.30), nature of psoriatic lesions (K=0.33-.66), factors that improve lesions (K=0.02-.26), and joint disease diagnosis by a rheumatologist (K=0.2-.54). In conclusion, we developed a novel validated survey instrument that can be used to gather information from psoriasis patients in a standardized manner.

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Decreased incidence of non-melanoma skin cancer in patients with type II diabetes using insulin

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Keratinocytes in the skin express the receptor for IGF-1 and require IGF-1 for proper maintenance of the epidermis. Because keratinocytes do not produce IGF-1, they are dependent on dermal fibroblasts to produce IGF-1. As dermal fibroblasts age in vitro, they lose the ability to secrete IGF-1. Therefore, we hypothesized that in aged skin, keratinocytes are supplied with decreasing levels of IGF-1. We have previously demonstrated the importance of IGF-1R activation in the response of normal human keratinocytes to UVB exposure in vitro and we have suggested that this reduced activation of the IGF-1R may be correlated with an increased susceptibility to skin cancer. A corollary of this hypothesis would be that individuals with an increased activation of the IGF-1R might have some protection from UVB-induced skin cancer that could be detected by a decrease in skin cancer incidence. Patients with Type II diabetes mellitus frequently have to take exogenous systemic insulin to overcome their insulin resistance. We hypothesized that the increased serum levels of insulin in Type II diabetic patients may activate the IGF-1R in skin and lead to a decreased frequency of skin cancer in these patients. To test our hypothesis, we compared the frequency of skin cancer of patients seen at our outpatient clinical facility from 1980 to 1999 that were taking insulin (n=1,440 patients) with patients from an age-matched control group that were taking cimetidine (used to treat peptic ulcers; n=4,135 patients). Insulin-using patients had a 1.25% incidence of non-melanoma skin cancer compared to a 2.35% incidence in the control group (p=0.016, two-sided, Chi-square test). No protection from non-melanoma skin cancer was observed in type II diabetic patients that were using non-insulin therapy (2.4% incidence). These data provide preliminary evidence that high levels of systemic insulin may provide protection from skin cancer, as suggested by our in vitro data.

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Epidemiology of pemphigoid in Olmsted County, Minnesota: 1950-2000

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We investigated the epidemiology of pemphigoid, its course and management in Olmsted County using the unique resource of the Rochester Epidemiology Project (REP). Reviewing the medical histories of the REP for the time period January 1, 1950 to December 31, 2000, we identified 79 cases. All cases met one or more laboratory methods to establish the diagnosis (histology, direct and indirect immunofluorescence [IF]). The overall age- and sex-adjusted incidence was 2.4/100,000 (95% CI, 1.9-3.0). There were 49 women (62%) and 30 men (38%) that developed pemphigoid. The mean age was 77.4 years (range, 41-98 years). Pruritus and pain were the most common presenting symptoms. Histology + direct and indirect IF were used to diagnose 39 cases (49%), 29 cases (37%) were diagnosed by both histology and direct IF. The remaining 11 (14%) cases were diagnosed by one or a combination of methods. Blisters, erosions and denuded skin were the most common presenting features (97%). Blisters alone were rare (3%). The extremities, chest and back, were common initial sites of the disease. Forty-eight cases (61%) were in remission or free of signs and symptoms, though 25 of these 48 cases (52%) had relapse at some point. Treatment was recorded for 74 patients. Adjuvant therapy, in addition to systemic corticosteroids, was required in 78%. The remaining 22% were managed with local corticosteroid treatment for mild disease. The incidence of pemphigoid is much more common (5-10 fold) than pemphigus and affects an older age population when comparing the epidemiology of these diseases in Olmsted County, Minnesota.

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Epidemiology of pemphigus in Olmsted County, Minnesota from 1950 - 2000

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The epidemiology of pemphigus, including its incidence, course and management in Olmsted County was examined using the unique resource of the Rochester Epidemiology Project (REP). Reviewing the REP for the time period January 1, 1950 to December 31, 2000, we identified 12 cases. All cases met one or more diagnostic criteria for pemphigus (histology, direct and indirect immunofluorescence [IF]. The overall age- and sex-adjusted incidence was 0.35/100,000 (95% CI, 0.15-0.54). Five men and seven women from different ethnic backgrounds were affected. The mean age was 60 years (range, 41-85). Pemphigus subtypes identified included: vulgaris (6), foliaceous (5), and erythematosus (Senear-Usher) (1). Pain, sore throat and pruritus were the most common presenting symptoms. The diagnosis was established by histology only (5), histology and direct and indirect IF (4), and the remainder of the cases diagnosed by IF or histology and either IF method. Erosions and crusted lesions represented the typical morphology. Face, oral mucosa and extremities were the common initial sites of disease. The median delay to diagnosis was 4.3 months (range: 13 days to 4 years). Eight cases eventually completely remitted or were free of signs and symptoms, of which five cases subsequently relapsed. Therapy was recorded in 11 patients. Therapy in addition to systemic corticosteroids was required in seven patients and three patients were managed with local corticosteroid treatment due to mild disease. Pemphigus occurs in multiple ethnic groups in Olmsted County, Minnesota with an overall incidence of <1/100,000. Oral corticosteroid and adjuvant therapies provide remission though relapses were observed.

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Smoking cessation as a therapeutic intervention for acne: initiation of a Cochrane Collaboration Systematic Review

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Studies examining the cutaneous effects of smoking on acne have yielded conflicting results (Mills 1993, Schafer 2001, Jemec 2002). Given these mixed results, we have initiated a Cochrane Collaboration Systematic Review of the effects of tobacco smoking on acne vulgaris. Inclusion criteria for candidate trials are: 1) evaluation of efficacy of tobacco cessation modalities and 2) epidemiological studies that examine the relationship of acne and tobacco. Case reports, case series, consensus panels and expert opinion articles will be excluded. Main outcome measures include: 1) the proportion of participants with clinically significant resolution or improvement of acne after smoking cessation and 2) the association of smoking and acne as determined by epidemiological studies. Databases to be included in this review include: a) The Cochrane Skin Specialised Register, b) The Cochrane Tobacco Addiction Group's Specialised Registers, c) The Cochrane Central Register of Controlled Clinical Trials (CENTRAL), d) MEDLINE, e) EMBASE (from 1980), f) SciSearch (from 1966), g) International Pharmaceutical Abstracts (from 1966), h) PsycInfo (from 1987), i) AMED (Allied and Complementary Medicine,1985), j) LILACS (Latin American and Caribbean Health Science Information database, from 1982), k) the metaRegister of Controlled Trials and l) the NHS Trusts Clinical Trials Register. Performing the search strategy in MEDLINE resulted in additional qualifying articles, however the results remain in conflict regarding the relationship of smoking and acne. We are additionally seeking any unpublished literature and conference proceedings relevant to this topic known to the membership of the SID. A summary of search outcomes to date will be presented. Preliminary analysis suggests that the relationship between smoking and acne will need to be studied prospectively.

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Dermatological research in Chile

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An objective assessment of research in dermatology in Chile was done. All the scientific papers in dermatology originated in Chile published between 1963 and 2003 registered at Medline were reviewed. In addition, all the abstracts originated in Chile presented at the Society for Investigative Dermatology between 2000 and 2003 were included. During those years, 47 papers were published. Less than 20% were published in internationally recognized dermatological journals and most of them were published in domestic journals in Spanish. Most of the relevant research were focused on environmetal issues such as ozone and arsenic poisoning and epidemiology. In conclusion, in a 40 year period, there are few papers published suggesting a need to improve the research in dermatology in Chile.

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The impact of managed care to dermatologists when providing care for the indigent population

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Managed care is growing nationally, causing a shift away from direct access toward the gatekeeper system. Specialists such as dermatologists express great concerns about managed care due to poor reimbursement and limitations in clinical decision-making; these issues are compounded by the generally poor Medicaid fee schedule for Medicaid managed care patients. Studies have shown that direct access to dermatologists is cost-effective and results in greater patient satisfaction. To determine who provides dermatological care to the poor regionally and nationally, we surveyed the major Medicaid HMOs in southeast MI, 249 dermatologists in MI, and 100 academic dermatology programs. The National Ambulatory Medical Care Survey (NAMCS) provided data regarding outpatient utilization of medical services. Nationally, 55% of Medicaid patients participate in a managed care program. Nearly 100% of Medicaid patients are enrolled in HMOs in MI, compared to 27% of the state's total population that is enrolled in a managed care plan. University Dermatology, the clinical practice of the Department of Dermatology at Wayne State University, was found to be the major provider for Medicaid HMO patients in southeast MI, comprising 35% of the practice. In nearly 70% of MI dermatologists, < 1% of their clientele is comprised of Medicaid patients, and even fewer see Medicaid HMO patients. Nationally, 69% of academic dermatology programs consist of <5% Medicaid patients, and most major metropolitans have city, county, or state-funded hospitals. The NAMCS similarly revealed that dermatologists see a disproportionately low share of patients with managed care, and to a greater extent, Medicaid managed care. In summary, Medicaid managed care patients face extreme difficulties in access to dermatological care. Without an appropriate safety net, direct access for specialty care would cripple those few practices that treat the majority of this patient cohort.

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Trends in coverage of skin cancer in the print media

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Our purpose was to observe the trends in coverage for skin cancer and related factors in the New York Times and to analyze the subject content of articles related to skin cancer in fashion magazines, Cosmopolitan and Cosmo Girl. The Lexis-Nexus database, a commercial database that covers a wide array of news articles on current events, legal, business, and medical issues, was used to identify and quantify articles related to skin cancer in the New York Times between 1980 and 2002. The EBSCO Host Master File Premier database, a similar commercial multidisciplinary database, was used to search and identify articles in Cosmopolitan and Cosmo Girl between 1992 and 2002. We further classified these articles on subject content as it related to skin cancer and other factors. The number of articles on skin cancer and related topics such as sunscreen, tanning, and tanning salons ranged from 2 per year to approximately 30 per year during the 22-year observation period for the New York Times. We observed a cyclical trend in coverage with a peak in coverage for articles on basal cell and squamous cell skin cancers in 1985 and melanoma skin cancer in 2000 which coincided with the diagnosis of skin cancers in public political figures. We reviewed a total of 139 articles in Cosmopolitan over a 10-year period, of which 91 were relevant and further analyzed. Sixty-four percent (n=58/91) of the articles discussed primary prevention of skin cancer, with 93% (n=54/58) of these articles promoting the use of sunscreen. Eleven percent (n=10/91) mentioned secondary prevention of skin cancer, all of which were educational oriented about the ABCD characteristics of melanoma. Although fewer in number, the content of articles and distribution in Cosmo Girl was similar. Despite the paucity of articles published in Cosmopolitan and Cosmo Girl, we believe that these magazines made an attempt to educate the adult and teen population about skin cancer and sun protective behaviors, utilizing cosmetic factors as the driving force in getting the message across.

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Availability of the top 100 prescribed topical medications in private insurance, Medicaid, and VA formularies in Michigan

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Medicaid is the largest financial resource for medical services and prescription drug benefits for the poor and disabled people in the U.S. In Michigan, nearly 100% of Medicaid recipients are enrolled in a Health Maintenance Organization (HMO). In February 2002, Michigan implemented a Medicaid formulary, the Michigan Pharmaceutical Product List (MPPL). Medicaid HMOs are not required to adhere to this formulary. Only government-selected physicians and pharmacists provided input in the development of this list; no dermatologists were included. We compiled a list of the top 100 topical medications prescribed by dermatologists and across all specialties in Michigan, as determined by retail sales. The availability of these medications was determined for two private HMOs, the MPPL, several Medicaid HMOs, and the VA. Dermatologists prescribe 33% of all topical medications in Michigan. From our data, private insurance covers 61% of the most prescribed topical medications, compared to 58% by the MPPL, 27% by Medicaid HMOs, and 24% by the VA. In contrast to oral medications in which 82% are available on the MPPL, topical medication formularies are much more restrictive (58% available on the MPPL). Interestingly, access to topical retinoids for acne (tretinoin, adapalene) is highly restrictive for the MPPL and Medicaid HMOs—only one Medicaid HMO has only one of these products available without restrictions. Dermatologists should participate in the development of topical medication formularies to ensure proper treatment options for skin conditions. In summary, Medicaid patients face major obstacles in receiving appropriate medical care; with a severely restrictive formulary, these patients, especially those in managed care, have the added burden of poor access to topical medications for their skin diseases.

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A comparison of the accessibility of the top 100 prescribed oral medications in Michigan

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Medicaid provides health care coverage for disabled and indigent patients, and is subsidized by federal and state funds. In Michigan, virtually all Medicaid recipients are enrolled in a Health Maintenance Organization (HMO). In February 2002, Michigan created its own Medicaid formulary—the Michigan Pharmaceutical Product List (MPPL). Medicaid HMOs are not obligated to follow this formulary. To determine the availability of medications for the Medicaid population, formularies from two private insurances, the MPPL, several Michigan Medicaid HMOs, and the VA were analyzed. A list of the top 100 prescribed oral medications across all specialties was compiled, as determined by retail sales in Michigan. This list was then compared to each formulary to determine the availability of these medications. Our data reveals that private insurance covers 93% of these medications, compared to 80% by the MPPL, 76% by the Medicaid HMOs, and 73% by the VA. Moreover, 89% of medications are available without restriction by private insurance formularies, as compared to 61% by the MPPL, 55% by the Medicaid HMOs, and 63% by the VA formulary. From these data, we conclude that private insurances provide the broadest coverage; whereas government programs have more restricted formularies. Additionally, Medicaid HMOs do not parallel the MPPL, but rather, deviate extensively. The VA drug list is quite restrictive and heavily based on generic medications. Thus, Medicaid patients face significant challenges not only for access to care, but also, access to medication. This year, a federal bill passed that allowed prescription drug coverage for Medicare recipients. Given the difficulty experienced by Medicaid patients and the non-conformity of Medicaid managed care formularies, these data have profound implications for drug availability for senior citizens.

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Effect of an intervention promoting thorough skin self-examination (TSSE) on the use of health services: the Check-It-Out Project at 6 months

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The Check-It-Out project was a randomized trial of intervention to encourage TSSE. Participants were recruited at primary care practices. The intervention included a video, cues, aids, and counseling by an intervention specialist, who asked the participant for their plan of action and placed a follow-up phone call 3 weeks after enrollment. The control group received a dietary fat intervention in the same format and intensity. All participants (1356 were randomized) completed a phone evaluation survey before and at 2, 6, and 12 months after enrollment. Participants were asked if they underwent a procedure on their skin. Physician records were consulted for the exact date and CPT code of the procedure(s), which were then associated with the cost of the procedure. Differences in the costs between groups were assessed by ANOVA with group as the independent variable and total cost per patient with a verified procedure as the dependent variable. During the 6 months prior to randomization, 6.5% of the intervention group v. 5.8% of the controls had a verified procedure on their skin (p=0.6), but during the 6 months after intervention, this was 7.9% v. 3.5% (p=.001). Among patients with verified procedures, the cost per patient of procedures performed on the skin was $116 v. $104 respectively (p=.8) during the 6 months prior to randomization, and $219 v. $77 (p=.01) during the 6 months after randomization. If participants without procedures are included, the average cost was $16.62 for those receiving intervention and $4.83 for the control group. Check-it-Out successfully encouraged intervention participants to perform TSSE more than controls, and also caused greater expenditures on skin procedures in the first six months. Further analysis will assess whether lesions removed were a significant threat to health.

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The associations of body image, weight status, and health limitations on performance of thorough skin self-examination (TSSE)

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Attitudes about weight and body image might influence ones general attitude toward and performance of TSSE. This hypothesis was explored in the Check-It-Out project, a randomized trial of an intervention designed to encourage TSSE. All participants who completed the baseline survey, regardless of whether they were later randomized into the clinical trial (N=2112) were included in this analysis. TSSE was defined as having answered yes to all seven questions regarding examining specific body parts at least once in the previous month; body image was assessed by one question; weight status calculated as BMI (kg/m2) based on self reported height and weight, and categorized according to WHO criteria; health limitations reported as having a condition that makes it difficult to examine ones skin. Body image, weight status and health limitations were assessed using chi square analysis, in which all three were associated with one another (p=.0001 for all). TSSE was not associated with weight status or health limitations, but was associated with body image (p=.0536) in univariate logistic regression. To further explore this association, body image, weight status and the interaction of these variables were entered into a subsequent model, where the interaction term met a liberal threshold at p=.1539. Separate models were then constructed with body image as the sole dependent variable. Body image only was associated with TSSE among the highest weight category (BMI>30), and not associated among any other weight category. Body image appears to be an important factor in TSSE performance among the obese, but not in individuals with weight status. Obesity is associated with many health issues. Infrequent early detection of melanoma may be added to that list among those with poor body image. Given the epidemic of obesity, interventions promoting TSSE should consider body image as a potential barrier to successful TSSE among the obese.

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Keratin expression in epidermal melanocytes of normal human skin

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Human melanomas occasionally express cytokeratins in addition to traditional markers of differentiation, such as S-100 and melanoma specific antigens. Also, similarly to melanoma cells in vivo, several established melanoma cell lines express cytokeratins. Most recently, keratin expression has also been detected in cultured melanocytes derived from normal skin. To determine if normal epidermal melanocytes express cytokeratins in vivo, fifteen samples of normal glabrous adult skin were first incubated with anti keratin 16 antibodies (LL025, 1:20), specifically chosen for this study as this keratin is not expressed by keratinocytes of normal epidermis. The reaction was followed by incubation with the chromogen 3,3'-diaminobenzidine (DAB). Sections were stained using the Dako Envision Double Stain System and photographed. The same sections were then reacted with anti tyrosinase related protein-1 antibodies (Mel-5, 1:15), followed by incubation with the Fast Red chromogen and rephotographed. All specimens showed discrete positive keratin 16 staining of scattered cells in the basal epidermal layer, consistent with melanocytes, and no staining of keratinocytes. Indeed, the stained basal cells were also positive for tyrosinase related protein-1 expression, verifying their identity as melanocytes. The specificity of the staining was confirmed using psoriatic skin from five different donors, as keratinocyte of hyperplastic psoriatic epidermis, known to express keratin 16, positively stained with LL025 antibodies. Conversely, anti CD22 antibodies (B-cell marker), used as a negative control, did not stain either melanocytes or keratinocytes in five of the fifteen normal skin specimens, where the melanocytes stained positively for keratin 16. Our study is the first to show keratin expression in melanocytes of normal human skin. Thus, it appears that melanocytes, similar to keratinocytes, express and utilize keratin filaments to provide mechanical strength, structural integrity and assistance in basement membrane and cell-cell adhesion.

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Three-dimensional analysis of human epidermal structure by *in vivo* reflectance confocal microscopy

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in vivo reflectance confocal microscopy (CM) has made it possible to observe non-invasively the inside of living human skin, mainly epidermal layers. We have developed a method to reconstruct the three-dimensional (3D) structure of the epidermis from a sequence of automatically obtained CM images. A new image analysis method of 3D edge detection and 3D reconstruction based on the Snakes method was used. The Snakes method[1)] is to evolve a curve, which consists of initial broadly positioned control points on the image, through an image-derived energy-minimization process so that it ultimately conforms to the feature of interest, such as the dermo-epidermal junction. To analyze the volumetric confocal images, we have developed the 3D Snakes method, which uses the same image-derived energy functions as the Snakes model, but deforms them into 3D space. This new method makes it possible to detect the surface area of epidermis automatically and to visualize the precise structure of the epidermis from the surface of the stratum corneum to the dermo-epidermal junction. This 3D reconstruction of the epidermal structure allows us to analyze the thickness of the epidermis and the undulation of the dermo-epidermal junction in detail. Our study of the structural variations of the epidermis in normal skin with this method revealed substantial differences between subjects of different ages, and between males and females, not only visually, but also quantitatively. Furthermore, detection of melanin particles, which appear as characteristically bright reflective clusters in the confocal images, was investigated to elucidate the 3D distribution of melanin particles within the epidermis. We conclude that *in vivo* CLSM will be a very useful tool for the precise 3D analysis of epidermal structure.

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Effects of dietary glucosylceramide on the cutaneous functions

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Ceramides in the epidermis play a critical role in the barrier and water holding functions of the stratum corneum (SC). In atopic dermatitis and in xerosis, there are decreases in ceramide content in the SC, etiologically leading to the barrier disruptions and dry skin; topical application of ceramides or enhancement of epidermal ceramide synthesis improve these skin conditions. Recently, the dietary intake of glucosylceramides has been reported to increase the epidermal water holding capacity, however this mechanism remains unclear. Here we examined the effect of oral administration of glucosylceramide (derived from plants) on epidermal water holding capacity, cutaneous barrier function, and sphingolipid content in murine epidermis, including the SC. HR-1 hairless mice were treated with a single dose of glucosylceramide (plant) or triolein (4mg in sample emulsion) by gavage once a day for 2 weeks. Following oral administration, skin conductance, an indicator of epidermal water holding capacity, increased significantly although there were no changes in transepidermal water loss (TEWL), an indicator of cutaneous barrier function. Following administration of glucosylceramide ceramides, glucosylceramide and sphingomyelin contents increased in whole epidermis, especially in living cells (Stratum Basale, Stratum Spinosum, Stratum Granulosum).

We then examined the effect of oral glucosylceramide on barrier recovery. Mice were treated with the diet containing 0.1% of glucosylceramides (maize) for 5 weeks. The recovery rate of barrier perturbation induced by tape stripping was enhanced following glucosylceramide treatment. These findings suggest that dietary glucosylceramides increase the epidermal sphingolipids level by absorption via the intestinal tract and distribution to the epidermis or by enhancement of their de novo synthesis in the epidermis. The end result is an increase in the epidermal water holding capacity and an enhancement of the cutaneous barrier function.

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New insights in cutaneous nociception and neurogenic inflammation: expression of the Vanilloid receptor subtype 1 in the skin

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The cutaneous genesis of itch and pruritus is not yet fully understood and seems to be regulated by a variety of mediators. The vanilloid receptor subtype 1 (VR1/TRPV1) is a non-selective cation channel which is known to modulate nociceptive sensations in the central nervous system. Since the VR1-ligand capsaicin is known to suppress pruritus after cutaneous long-term administration, it was of interest to determine the localization of VR1 in the skin. We investigated VR1-immunoreactivity as well as mRNA and protein expression in a series of normal and capsaicin-treated human skin. VR1 immunoreactivity could be observed in cutaneous sensory dermal and epidermal nerve fibers, mast cells, as well as appendage and epidermal cells. Upon RT-PCR and Western blot, expression of VR1 was confirmed in primary mast cells and keratinocytes from human skin. During capsaicin therapy, VR1-receptor distribution was unchanged while a reduction of neuropeptides (substance P, calcitonin gene-related peptide) was observed in nerve fibers. After cessation of capsaicin therapy, neuropeptides re-accumulated in skin nerves. In conclusion, VR1 is widely distributed in the skin. Expression of VR1 on cutaneous sensory nerve fibers supports the notion that vanilloids and their receptors contribute to the induction and modulation of nociceptive cutaneous sensations such as pain and pruritus.

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Sustained increases in stratum corneum pH cause profound alterations in barrier functions and SC integrity

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The function of the acidic pH of the stratum corneum (SC) was largely unknown until recently, when we showed that transient increases in SC pH are accompanied by transient alterations in both permeability barrier homeostasis and SC integrity/cohesion. Yet, sustained pH elevations characterize clinical situations, such as neonatal and dermatitic skin. Hence, we assessed here whether prolonged neutralization of the SC alone, by repeated applications of the superbase, 1,1,3,3-tetramethylguanidine (TMG) (twice daily, 5-16 days), provokes profound alterations in SC function, as well as their mechanistic basis. In contrast to short-term pH alterations, sustained SC neutralization profoundly altered not only barrier recovery kinetics, but also basal permeability barrier function. This barrier abnormality was attributable not only to decreased in β-glucocerebrosidase (β-GlcCerase) and acidic sphingomyelinase (aSMase) catalytic activity, but also to a sustained pH-induced increase in serine protease (SP) activity, resulting in degradation of both lipid processing enzymes (shown by immunohistochemistry and Western immunoblotting). The role of SP in this process was shown by the normalization of both enzyme activities/content by co-applied SP inhibitors (SPI). In addition, SC integrity/cohesion also deteriorated progressively with sustained SC neutralization, attributable to SP-mediated degradation of corneodesmosomes (CD) and CD-constituent proteins (desmoglein 1), extending to the SG-SC interface, abnormalities that all were again reversed by co-applied SPI. In summary, prolonged SC neutralization provokes profound abnormalities in both permeability barrier homeostasis and SC integrity/cohesion, resulting from sustained proteolytic degradation of lipid processing enzymes and CD proteins. These results suggest that the pH abnormality could dictate many of the functional abnormalities in neonatal skin and chronic dermatoses.

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Molecular cloning and expression of keratinocyte differentiation related protein—a novel differentiation marker isolated from primary cultured keratinocytes using suppression subtractive hybridization

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We describe a novel human cDNA keratinocyte differentiation related protein called KDRP that was isolated using the suppression subtractive hybridization technique in a calcium-induced keratinocyte differentiation model. The full coding region was obtained using the RACE method. Sequence analysis against an NCBI Map viewer revealed that the KDRP gene is located on chromosome 1q21 in the region of the epidermal differentiation complex (EDC). Northern blot analysis showed that the amount of a 2.5 kb mRNA transcript was markedly increased at both 7 and 14 days after treatment with 1.2 mM calcium in cultured normal human epidermal keratinocytes (NHEK). A mouse tissue blot assay detected expression of KDRP at 17.5 embryonic days, and seen in stomach and skin tissues. The KDRP geneis predicted to encode a protein of approximately 70 KDa that consists of 580 amino acids with 18% proline and 11% glutamine. In situ hybridization demonstrated that the KDRP gene is expressed in the upper granular layer of normal epidermis with a characteristic intermittent pattern and increasing expression in the psoriatic lesion with a continuous pattern. Immunohistochemical analysis also showed that the KDRP protein is localized at the granular layer of both normal and psoriatic epdermis, similar to results from in situ hybridization . Consistent with this, Western blot analysis revealed that the amount of the 70 KDa KDRP protein was significantly increased by calcium treatment in a time-dependent manner. KDRP appears to be a novel epidermal marker that has a potential role in keratinocyte differentiation.

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Stratum corneum lipid abnormalities in the patient skin with senile xerosis

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Stratum corneum (SC) lipids play a predominant role in maintaing the water barrier of the skin. The protein-enriched corneocytes are embedded in an intercellular lipid matrix which is composed primarily of ceramides, cholesterol and fatty acids together with smaller amounts of cholesterol sulphate, glucosylceramides and phospholipids. These lipids form multilamellar sheets within the intercellular spaces of the stratum corneum. Depletions in lipid levels as a result of environmental challenges can lead to disturbances in skin function. Changes in stratum corneum lipid levels have been linked with aberrant skin conditions, such as xerosis. The incidence of skin xerosis appears greater with increasing age. To clarify the relationship between the senile xerosis and lipid abnormalities within SC, SC lipids were extracted from the patient skin with senile xerosis and the lipids were analyzed and compared with those of the young and age matched skin. The extracted lipids were purified by DEAE A25 column chromatography (acetate form) separating the neutral and acidic fractions. The neutral fractions contained cholesterol, cholesterol esters and ceramides 1 to 7, which were analyzed by thin-layer chromatography, and the contents were estimated by a densitometric method. In the patient SC, the contents of ceramide 2 and ceramide 7 increased while that of cholesterol esters and ceramide 6 decreased significantly. The acidic fraction contained free ω-hydroxy fatty acids as well as α-hydroxy fatty acids in addition to the several saturated and unsaturated fatty acids. The spectra obtained by the positive ion mass/mass spectrometry revealed that chain length of the free ω-hydroxy fatty acids were very long, C30 and C32:1. The lipid abnormalities in the patients with senile xerosis were different from those of aged skin, suggesting that the different biochemical events may exist in senile xerosis.

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Barium sulfate with a negative ζ potential, accelerates skin permeable barrier recovery and prevents epidermal hyperplasia induced by barrier disruption

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Barium sulfate a stable inorganic material has been used for contrast media or cosmetic products because of its stability. In the present study, we demonstrated that barium sulfate particles in aqueous solution have different potentials depending on their surface structure. Since a negative external electric potential accelerates the skin barrier repair after barrier disruption, we hypothesized that topical application of barium sulfate affects the skin barrier recovery rate depending on its ζ potential. There was a significant correlation between the barrier recovery rate and potential of barium sulfate applied topically. Barium sulfate with a negative ζ potential, significantly accelerated barrier recovery, with a positive potential did not accelerate or even delayed barrier repair. Barium sulfate, with a negative potential had an X ray refraction pattern different from that with a positive potential. The distribution of calcium in the epidermis was also influenced by the polarity of ζ potential. Altogether topical application of barium sulfate with a negative ζ potential, prevented the epidermal hyperplasia induced by barrier disruption under a dry environment. These findings suggest a new pharmacological approach toward altering barrier function or epidermal hyperplasia with inorganic particles in healthy and diseased skin.

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TNF-α, IL-1α and IFN-α enhance IFN-γ-induced fractalkine production in normal human keratinocytes

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Fractalkine/CX3CL1, a chemokine, is synthesized as a membrane bound form and converted to a soluble form by protease(s). Since fractalkine recruits CX3CR1 positive cells such as NK cells and CD8 T cells, it is supposed to be involved in recruiting these cells into the epidermis in the inflammatory reaction. To address this issue, we investigated whether normal human keratinocytes can produce fractalkine in the presence of cytokines. The production of fractalkine was analyzed using real time PCR, Western blot and ELISA. The mRNA expression of fractalkine was very low in unstimulated keratinocytes. However, its expression was induced by IFN-γ (50 IU/ml) in a time dependent manner optimally 67 folds at 24 h. The concentration of fractalkine in the medium was undetectable at 48 h, but increased to 900 pg/ml after addition of IFN-γ (10 IU/ml). In contrast, either TNF-α (10 ng/ml) or IL-1α (10 ng/ml) had no effects. Interestingly, IFN-γ-induced fractalkine mRNA expression was synergistically upregulated by TNF-α and IL-1α compared to that treated with IFN-γ alone, 19 and 3.7 folds at 3h, respectively. IFN-γ-induced soluble fractalkine was markedly increased by IL-1α and TNF-α, 2 and 4 folds, respectively. We also examined the effect of IFN-α on fractalkine expression. IFN-α (0.5 IU/ml) upregulated fractalkine mRNA optimally 5 folds at 1 h, but did not induce the production of fractalkine protein in the medium. IFN-α enhanced IFN-γ-induced fractalkine mRNA expression by 2.5 folds at 1 h. Furthermore, IFN-α increased IFN-γ-induced soluble fractalkine production in a dose dependent manner. Taking together, TNF-α, IL1-α and IFN-α synergistically enhanced IFN-γ-induced fractalkine production in normal human keratinocytes. In conclusion, this is the first report showing that normal human keratinocytes produce soluble fractalkine protein.

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Peroxisome proliferator-activated receptor(ppar)-γ activation stimulates keratinocyte differentiation

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PPARs are nuclear hormone receptors that are activated by endogenous lipid metabolites and thiazolidinediones (TZDs). Previous studies have demonstrated that PPARα and δ activation stimulate keratinocyte differentiation; is anti-inflammatory; and improves barrier homeostasis. Here, a TZD, ciglitizone, increased involucrin /transglutaminase 1 mRNA levels in cultured human keratinocytes,. Moreover, topical ciglitazone and troglitazone increases loricrin, involucrin, and filaggrin expression in hairless mouse. Topical PPARγ activators also improved permeability barrier homeostasis, indicated by accelerated barrier recovery following acute disruption (tape stripping or acetone). Further, TZDs reduced the cutaneous inflammation in both the TPA model of irritant contact dermatitis, and the oxazolone model of allergic contact dermatitis. PPARγ-deficient mice displayed a modest increase in epidermal thickness, associated with increased PCNA staining, while skin functions, including permeability barrier homeostasis, surface pH, and stratum corneum water content were similar in PPARγ-deficient and wild-type epidermis. Moreover, PPARγ activators did not stimulate loricrin and filaggrin expression in PPARγ-deficient mice, indicating that the stimulation of differentiation by PPARγ activators is mediated by PPARγ. However, TZDs inhibited inflammation comparably in both PPARγ-deficient and wild-type mouse skin, indicating that the inhibition of inflammation does not require PPARγ, similar to other studies on macrophages. Together, these observations predict that TZDs, and perhaps other PPARγ activators, could prove useful for the treatment of skin disorders, characterized by abnormal differentiation, barrier function, and inflamation.

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Primary isolation and serial propagation of normal human keratinocytes in a defined, animal product-free cell culture environment

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Cell culture media for in vitro propagation of human keratinocytes are typically supplemented with undefined or defined animal-derived products that can include: sera (e.g., FBS), serum proteins (e.g., BSA), and/or extracts from pituitary glands (BPE), embryos, brain, or other tissues, and other growth-promoting factors such as Insulin. Xenotrophic feeder layer cells have also been utilized to promote the propagation of these cells. Use of these reagents in cell culture media can increase the risk of contaminating cell-based and cell-derived therapeutics with infectious agents such as prions (BSE) and viruses, and can represent a health risk hazard to patients treated with these therapeutic agents. The efficient primary isolation and serial expansion of normal animal cells in a defined animal product-free (d-APF) cell culture system has not been previously reported. We have recently discovered that a d-APF supplement containing growth factors, hormones and other proteins (S-70203) is capable of supporting the primary isolation and serial propagation of adult human epidermal keratinocytes (HEKa). Using the d-APF system, HEKa can be isolated and expanded in primary culture within 7-9 days. The cells can then be serially propagated for up to 40 post-primary cumulative population doublings. The d-APF HEKa cultures display initial growth rates that are typically greater than one population doubling per day, possess normal morphology, express epidermis-specific cytokeratins, and can be induced to express Involucrin as well as form a reconstructed human epidermis ex vivo. Collectively, our results describe a new keratinocyte culture system in which the requirements for animal products such as BPE and other undefined reagents, are eliminated, and replaced by d-APF components. d-APF keratinocyte cell culture systems may eventually allow investigators to more safely utilize both human keratinocytes and human keratinocyte-derived products for wound and gene therapy protocols.

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Vegf is both required for permeability barrier function, and regulated by barrier requirements

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Restoration of a competent permeability barrier after external perturbations requires up-regulation of metabolic responses that lead to normalization of function. We assessed here whether these homeostatic responses include the generation of signals that could facilitate nutrient/precursor delivery from the vascular compartment. Indeed, 2-4hrs after acute barrier perturbation of hairless mouse skin by either repeated tape stripping or acetone, epidermal mRNA levels of VEGF increased 20-40-fold. Artificial barrier restoration by the application of a vapor-impermeable wrap blocked the increase in VEGF specifically linking the increase to barrier requirements. The putative requirements for VEGF were shown further in transgenic mice with epidermis-localized VEGF k.o. (VEGF-A, △K5-Cre/△K5-Cre), which displayed a significant delay in barrier recovery after acute perturbation. Despite the apparent link between VEGF expression and barrier requirements, we could not detect increased blood vessel growth after either acute or repeated barrier insults (as changes in CD31 and factor 8 levels). Moreover, both CD31 and factor 8 expression appeared unchanged in VEGF k.o. skin. Although these studies clearly demonstrate a link between VEGF and permeability barrier homeostasis, it is likely that other growth factors (i.e., FGF) sustain vessel growth in the absence of VEGF. Finally, these studies suggest that VEGF influences barrier homeostasis by mechanisms distinct from its well-known paracrine effects on vessel growth.

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Analysis of N-terminal domain of connexin26: a novel N14Y mutation does not affect secondary structure of N-terminal domain, but increases hydrophobic features of intracytoplasmic head of connexin26

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Connexins (Cxs) are transmembranous proteins and their hexamer "connexon" constructs channel structure at the plasma membrane. Connexons form transmembranous channels with adjacent cells, which are known as gap junctions. Ions and second messengers can permeate through the gap junction and it is thought that gap junction may have certain roles in cell-cell communication and maintenance of tissue homeostasis. The N-terminal 21 amino acids of Cx26 is located at the cytoplasmic side of the channel pore and is thought to be essential for the regulation of channel selectivity. Missense mutations of the Cx26 gene have been reported in cases of keratitis-ichthyosis-deafness (KID) syndrome and two of the four reported mutations to date were at the N-terminus of the Cx26, which suggest the critical role of this domain for the channel functions. We have found a novel mutation N14Y in the N-terminal domain of the Cx26 in a case of KID syndrome. In order to elucidate pathomechanism of KID syndrome, the effect of the mutation on molecular structure and chemical characteristics of the N-terminal domain was investigated using synthetic N-terminus peptides (1-20 residues) of Cx26, both of the wild type and the mutated one. The secondary structures of these two model peptides are basically the same from the analysis using two dimensional 1H nuclear magnetic resonance (NMR) and circular dichroism measurements, though the water solubility of the peptides decreased by the mutation. These results raise the possibility that the amino acid substitution from a hydrophilic residue to a hydrophobic one induces changes in local conformation and electronic charge, which might affect ion permeability of gap junctions and result in a phenotype of KID syndrome.

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Organization of stem cells and their progeny in human epidermis

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The epidermis is a self-renewing tissue that consists of stem cells, transit amplifying cells and differentiating cells. Histological and genetic labeling studies in mouse epidermis are consistent with the presence of highly ordered, columnar structures named epidermal proliferative units. Each unit consists of a stem cell and its descendent amplifying and terminally differentiated cells. In human epidermis, the larger number of strata, and the undulating basement membrane with rete ridge formation make comparison with mouse difficult. These differences have lead to conflicting reports. Some reports indicate clustering of human epidermal stem cells (b-1 integrin bright cells) at the top of the ridges, whereas others show a location at the base of the ridge. To address this issue, we have genetically labeled epidermal stem cells in adult, human skin xenografts using in vivo lentivirus-mediated gene transfer. Stem cell transduction was confirmed by long-term non-invasive monitoring of the skin surface for transgene product (Green or Red Fluorescent Protein). Lineage analysis of serially sectioned skin at 6 months post-transduction indicated the presence of labeled cells organized into narrow columns extending outward from basal layer. This was consistent with the organization of stem cells and their progeny into EPUs. The number of basal cells in an EPU however, was less than that observed for mouse epidermis (10-20 basal cells) ranging from 1 to 4 cells. These EPUs originated from the top, middle and bottom of the rete ridges. No correlation between b-1 integrin bright cells and location of stem cells was found. These data confirm columnar organization of stem cells and their progeny in undulating human epidermis and suggest minimal migration of stem cells or their progeny along the basal layer.

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Lactate and potassium content in relation to the physical properties of the stratum corneum in healthy subjects

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Natural moisturizing factor (NMF) of the stratum corneum (SC) has been established to play important roles in regulating the physical properties of the SC. However, few studies have investigated the specific influences of NMF components other than the amino acids. In this study we focus on the relationship between the ion content and physical properties of the SC in 40 healthy subjects. The hydration, stiffness, and pH of the SC on the forearm were respectively measured by a skin surface hygrometer (Skicon-200), tactile sensor (Venustron), and flat glass electrode connected to a pH meter. The ion levels in the SC were measured after water-extraction of tape-stripped SC. All ion quantities were normalized to the amount of tape-stripped SC. The changes in the physical properties of the SC induced by the NMF extraction were equivalent to the changes that took place from summer to winter, thereby demonstrating the important role of NMF in regulating the physical properties of the SC in healthy subjects. The seasonal changes in the physical properties of the SC from summer to winter were accompanied by significant decreases in the levels of lactate, potassium, sodium, and chloride in the SC. Lactate and potassium were the only components that significantly correlated with the hydration, stiffness, and pH. Interestingly, the lactate and potassium levels of the SC were also significantly correlated with each other. Moreover, potassium lactate was more effective than sodium lactate in restoring the SC hydration depleted by the NMF extraction. The ratios of both lactate and potassium to sodium were confirmed to be lower in the sweat than in the SC, showing that the lactate and potassium in the SC were derived from an epithelium other than the sweat gland. These results suggest that the movement of lactate and potassium from epithelium to the SC may play important roles in regulating the physical properties of the SC.

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Biochemical basis for accelerated permeability barrier recovery induced by PPAR and LXR activators

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Previous studies from this laboratory have demonstrated that topic applications of peroxisome proliferator activated receptor (PPAR) and liver X receptor(LXR) activators enhance epidermal differentiation and accelerate epidermal permeability barrier recovery as determined by measurement of transepidermal water loss (TEWL). We determined here whether increased lipid synthesis is a mechanism that accounts for the acceleration of barrier recovery by these agents. 6 to 8 week old hairless mice were topically treated with 10 mM of test compound twice-daily for three days. Control groups were treated with vehicle (acetone) alone. 14C acetate incorporation into lipids was measured to assess lipid synthesis rates. Osmium tetroxide post-fixation was used to visualize epidermal lamellar body structure and number (density) by electron microscopy. Topical applications of either PPAR alpha (WY14643), delta (GW1514), gamma (ciglitazone) or LXR (22(R)-cholesterol) activators, for 3 days significantly increased epidermal cholesterol and fatty acid synthesis as compared to vehicle-treated controls. In addition, both the density of lamellar bodies, the organelle that delivers lipids to the stratum corneum for barrier formation, and lamellar body secretion following barrier disruption increase significantly in the activator-treated groups. These studies indicate that topical administration of PPAR and LXR activators induce an increase in epidermal lipid synthesis and increased formation of lamellar bodies, which account, at least in part, for the acceleration in epidermal barrier recovery that follows acute barrier disruption. These results further suggest that PPAR and LXR activators might be useful in the treatment of skin disorders with abnormal barrier function, or in the development of skin care products that aim to improve epidermal function.

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Analysis of profilaggrin N-terminus protein-protein interactions using the yeast two-hybrid system

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In this study, we used the yeast two-hybrid (Y2H) system to examine if the human profilaggrin N-terminus can form homodimers in vivo. Profilaggrin, a protein expressed in granular cells of epidermis, consists of multiple filaggrin units and N- and C-terminal domains that differ from filaggrin. The human profilaggrin N-terminus consists of two distinct segments, an acidic A domain that binds calcium and a basic B domain that includes a nuclear localization signal. The A domain is homologous to the S100 family of calcium binding proteins. S100 proteins form homodimers, heterodimers with other S100 proteins, and also bind additional target proteins in a calcium-dependent manner. Here, we demonstrate that the human profilaggrin N-terminus containing both the A and B domains can homodimerize in vivo. However, fusion proteins containing the profilaggrin A domain (either 91 or 99 amino acids) failed to form homodimers in yeast. The 91-residue fusion protein includes the high-affinity calcium-binding loop and the adjoining helices, while the 99-residue fusion protein contains the extended fourth alpha-helix domain. Western blotting showed that both the AB and A domain fusion proteins were expressed in yeast. A mutant profilaggrin AB fusion protein with severely reduced calcium-binding capacity formed homodimers in the Y2H assay. This result is consistent with studies of other S100 proteins, which demonstrated that S100-S100 interactions do not require calcium-binding to the EF-hands. These results demonstrate the profilaggrin N-terminal domain, unlike the canonical low molecular weight S100 proteins, requires part or all of the downstream B domain to form homodimers. Western blot data also suggests that progressive extension of the A domain beyond the S100 domain to include the B domain acts to stabilize the A domain fusion proteins in yeast. Current studies are directed towards determining the downstream targets of the profilaggrin N-terminus, and its possible role in nuclear events during epidermal differentiation.

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Functional properties of the stratum corneum in patients with diabetes mellitus

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Though earlier studies have confirmed the effects of diabetes mellitus in inducing numerous pathophysiological changes of the skin, none have focused on the state of the stratum corneum (SC) in diabetic subjects. The following values were measured in 49 diabetic patients to examine the functional changes of the SC: fasting plasma glucose (FPG) and hemoglobin A_{1c} (HbA_{1c}); the skin surface lipid level on the forehead; and high-frequency conductance (HFC) and transepidermal water loss (TEWL) of the cheek, volar forearm, and extensor surface of the lower leg. The high-FPG group (>110 mg/dL) and low-FPG group (<110 mg/dL) exhibited almost identical TEWL values, though the former exhibited significantly lower skin surface lipids and significantly lower HFC levels on the extensor leg and volar forearm. The HFC did not significantly differ between the high (>5.8%)- and low (<5.8%)-HbA_{1c} groups. Like patients with senile xerosis, diabetic patients exhibited reductions in both SC hydration and sebaceous gland activity without any impairment of the SC barrier function. The SC hydration was more influenced by the hyperglycemic condition at the time of measurement than by the hyperglycemic condition over the 1 to 2 months immediately prior to the measurement. We also measured the HFC of 9 healthy subjects during the oral glucose tolerance test to investigate how the SC hydration responded to the change in the blood glucose. The HFC was significantly decreased when the plasma glucose peaked, suggesting that the SC hydration was influenced by at least a temporal rise in the blood glucose level.

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CGI-58 protein is involved in formation of the trans-Golgi network- lamellar granules and caveolae in human differentiating keratinocytes

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We have reported that truncation of CGI-58 protein, a member of α/βhydrolase family and a putative esterase/lipase/thioesterase, results in abnormal LG formation in Dorfman-Chanarin syndrome (Akiyama et al, JID 2003). LGs have been shown to be a part of TGN and secretion of LG contents is associated with the formation of caveolae, specialized areas of sphingolipid- and cholesterol-rich membrane invagination. To clarify the roles of CGI-58 protein, we studied the effects of the complete abolition of CGI-58 protein enzyme activity on the TGN-LG and caveola formation. Homology search and conserved domain analysis suggested that a nonsense mutation R184X would abolish the α/βhydrolase CGI-58 activity. In the epidermis harboring such homozygous mutations ($CGI-58^-$ epidermis), the ultrastructural formation of TGN-LG and caveola and the distribution of proteins associated with TGN-LG and caveolae were investigated. We studied the distribution of TGN-markers (TGN-46 and protein kinase A type II regulatory subunitα (RIIα)), TGN-LG contents (cathepsin C and D, βdefensin 2 and 3) and caveola components (caveolin 1, 2 and 3) using confocal laser scanning microscopy. TGN-46 was distributed only in the perinuclear area in $CGI-58^-$ epidermis, while it was observed diffusely throughout the cytoplasm in normal epidermis. RIIα and cathepsin D were distributed normally in $CGI-58^-$ epidermis. Cathepsin C expression was seen in the granular layer of the normal epidermis, but not in $CGI-58^-$ epidermis. The expression of both βdefensin 2 and 3 in the upper epidermis was stronger in $CGI-58^-$ skin than in normal skin. Caveolin 1, 2 and 3 expression was reduced in $CGI-58^-$ epidermis. Ultrastructurally, malformed TGN-LG and caveolae were seen in the granular layers. These findings suggest that lipid-carrying TGN-LG and caveolae are specifically affected in $CGI-58^-$ epidermis. CGI-58 protein is thought to be involved in formation of the lipid-transporting TGN-LG and in the caveola-mediated secretion of LG contents.

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Increased expression of squamous cell carcinoma antigen protects keratinocytes from UV-induced cell death: critical role of SCCA as a natural inhibitor of the stress kinase JNK/SAPK

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Squamous cell carcinoma antigen (SCCA) belongs to the serpin superfamily and two different gene products, namely SCCA1 and SCCA2, are known in Homo sapience. Although they have all the characteristics of inhibitory serpins, their physiological roles in keratinocytes are largely unknown. Here we show that SCCA1 and SCCA2 are natural inhibitors of the activated JNK and play a critical role against UV-induced keratinocyte apoptosis. Immunohistochemical and in situ hybridization analyses demonstrated marked elevation of SCCAs in spinous and granular cells of sun-exposed skin, whereas they were expressed weakly in upper epidermis of the sun-protected skin. UV irradiation study in vivo on buttock skin confirmed strong up-regulation of SCCAs in spinous and granular cells. cDNAs for SCCA1 or SCCA2 were stably transfected into 3T3-J2 cells, which do not express SCCAs. FACS analysis revealed that UVB-induced cell death was significantly suppressed in SCCA-transfected cells. Furthermore we established an SCCA knockdown cell line using RNA interference technology. HaCat cells, which express high levels of SCCAs, were stably transfected with the pSilencer vector containing the siRNA target sequence to SCCA. In this cell line (siSCCA) more than 90% of SCCA was suppressed as judged by quantitative PCR analysis. UVB irradiation study (75 mJ/cm2) showed that only 12% of siSCCA survived, while 43% was found alive in control cells. We then investigated interacting molecules for SCCAs using the Signal Transduction Antibody Array (Hypromatrix, Inc). Both SCCAs demonstrated specific binding to active JNK1 but not to inactive JNK1. These results were confirmed with immunoprecipitation studies. In addition, recombinant SCCA1 suppressed kinase activity of JNK1 with a dose dependent manner. These lines of evidence strongly suggest a novel UV protection mechanism in human epidermis, which SCCA inhibits the UV-induced apoptotic signal transduction.

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Decreased hydration and reduced flexibility of strarum corneum are associated with severity of phenotype in severe ichthyosis

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Exact pathomechanisms of hyperkeratosis in severe ichthyosis have not been clarified completely yet. Defective barrier function of stratum corneum is thought to be associated with formation of ichthyosis phenotype. In order to evaluate the stratum corneum barrier function in severe ichthyosis, we studied transepidermal water loss (TEWL), stratum corneum hydration and stratum corneum thickness in the dorsal and volar forearm, the forehead and the back of patients with severe ichthyosis aged 2 to 46 years (4 males, 3 females) (TEWL was not measured in the back). Seven patients (4 non-bullous congenital ichthyosiform erythroderma, 1 bullous congenital ichthyosiform erythroderma, 1 lamellar ichthyosis, 1 ichthyosis bullosa of Siemens) and age- and sex- matched controls were included in the present study. TEWL was measured using an evaporimeter (AS-TW1, ASAHI BIOMED, Japan). Stratum corneum hydration was measured as low frequency conductance by a corneometer (ASA- M2, ASAHI BIOMED). In addition, stratum corneum thickness was also measured by the corneometer and stratum corneum flexibility was assessed as (stratum corneum hydration) / $(\text{stratum corneum thickness})^2$. Severity of ichthyosis phenotype was evaluated using a visual analogue scale (VAS). Stratum corneum hydration and flexibility were significantly reduced in severe ichthyosis patients as compared to normal control ($p<0.005$). TEWL was significantly increased in severe ichthyosis skin ($p<0.02$), except for the forehead. Positive correlation was observed between the ichthyosis severity and stratum corneum thickness and negative correlation was confirmed between ichthyosis severity and stratum corneum hydration and flexibility. These results indicated that permeability defects in severe ichthyosis lead to increased TEWL and decreased stratum corneum hydration and it was suggested that low stratum corneum hydration and decreased flexibility of stratum corneum are closely associated with severity of ichthyosis phenotype in severe ichthyosis.

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Serine Proteases alter barrier homeostasis by a protease activated receptor 2-dependent mechanism

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Keratinocytes express protease activated receptor 2 (PAR2) in both the basal layer and stratum granulosum, where it further co-localizes with the stratum corneum (SC) tryptic and chymotryptic enzymes. Here, we addressed the effects of serine protease (SP) and PAR2 activation/inhibition on permeability barrier homeostasis in two mouse models of SP activation: 1) transient SC neutralization, and 2) acute barrier perturbation. To provoke SC neutralization, 1,1,3,3-tetramethylguanidine (TMG, 1/100), a superbase, was applied in propylene glycol:ethanol (7:3) and the downstream effects on barrier recovery was evaluated in comparison to both neutralized TMG and TMG+co-applied SP inhibitors (SPI). In parallel, either SPI or PAR2 agonist peptides (SLIGRL, 10mM) were applied to tape-stripped flanks of hairless mice, and barrier recovery kinetics were compared to vehicle alone (propylene glycol:ethanol, 3:7), and to stripped skin sites treated with the PAR2 reverse peptide (LRGILS, 10mM) alone. Both SC neutralization and acute barrier perturbation increased SP activity, assessed by in situ zymography. Application of either the superbase or the PAR2 agonist peptide delayed permeability barrier recovery. In contrast, either co-applied SPI, shown to inhibit SP activity, or applications of the PAR2 reverse peptide, significantly accelerated barrier recovery in tape-stripped skin. The latter could be further attributed to enhanced generation and secretion of lamellar bodies, observed by electron microscopy. These results provide a mechanism whereby pH-dependent increases in SP activity perturb barrier function. Finally, blockade of PAR2 represents a potentially novel form of therapy for dermatoses associated with barrier dysfunction, increased SC pH, and/or SP activation.

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Rambazole^{TM}, a potent inhibitor of all-*trans* retinoic acid metabolism, applied topically to mouse tail skin exerts retinoid mimetic activity

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Rambazole has been identified *in vitro* as a potent and selective inhibitor of retinoic acid (RA) cytochrome P450 4-hydroxylase. Repeated oral administration of Rambazole to mice exerted retinoidal effects in tail and ear skin. The aim of the present study was to investigate whether topical application of Rambazole would also generate retinoidal effects. Mice were treated topically once daily for 14 days on their tail skin with vehicle, RA or Rambazole. 24 hours after 1st, 4, 7, 11 & 14th application, tail and ear skin samples were collected for histologic assessment of (1) local drug activity by quantification of the degree of para- to orthokeratotic conversion of tail epidermis and of (2) systemic drug activity by measuring of pinnal epidermal hyperplasia. Repeated application to tail skin of either RA (0.5-4 μg) or Rambazole (0.5-16 μg) resulted in a dose-related para- to orthokeratotic transformation of tail epidermis. The potencies with which RA and Rambazole affected keratosis were comparable: RA and Rambazole yielded a statistically significant response from 1 μg and 2 μg on, respectively. However, Rambazole caused no thickening of ear epidermis indicating that its activity is restricted to the application site and that a systemic action of the drug or endogenous RA can be ruled out. Time-course data reveal a faster onset of action with Rambazole (16 μg) than with RA (4 μg); the transformation was almost complete at day 8 in Rambazole-treated mice *versus* at day 15 in RA-treated mice. The orthokeratotic transformation of mouse tail epidermis by RA (16 μg) and Rambazole (16 μg) was markedly attenuated by concomitant application of the RAR antagonist AGN193109 (16 μg). In conclusion, our data provide evidence that topical Rambazole exerts local retinoidal effects in mice. The observation that its retinoid mimetic activity could be reversed by RAR antagonism supports the idea that Rambazole′s actions result from enhanced endogenous RA levels and triggering of RA receptors.

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A new peptide that displays an uncoupling-like effect on the mitochondrial respiration process of adipocytes and reduces adipocyte lipid synthesis

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Uncoupling is an essential mechanism of respiration and energy dissipation. Data has shown that animal and plant homologues of uncoupling proteins (UCPs) are universal. Interestingly, recent studies have noted that these UCPs play a part in fatty acid and glucose utilization, and that they are involved in thermogenesis and obesity disorder. As we were interested in agents and mechanisms that can modulate fat cell metabolism in order to reduce adipocyte storage of triglycerides in lipid droplets, we developed an uncoupling-like peptide with an amino acid sequence similar to a conserved sequence present in many UCPs, and investigated its effect, in vitro, on lipid synthesis in adipose cells. For these studies, we used established pre-adipocytes 3T3-L1 and studied the effect of this new UCP-like peptide on their differentiation and formation of lipids. Oil red staining experiments demonstrated that application of 0.5% of the UCP-like peptide on the cells at different times during their differentiation significantly reduced their lipid droplet content. This effect was significant when the active ingredient was applied early in the first hours of their culture, before the beginning of differentiation. In order to confirm that the new peptide mimics the mechanism of action of UCPs, ATP and cAMP assessment, as well as glycerol release experiments were performed, in comparison with an agent that has a lipolysing effect. These experiments confirmed that the new peptide presumably uncouples, or facilitates the uncoupling of the cellular mitochondrial respiration process from its terminal ATP synthesis step, therefore suppressing lipid droplet accumulation in the cells, and enhancing energy release, by the ATP-independent pathway. These studies reveal the interest of using this new UCP-like peptide for reducing adipose cell lipid content, an action which is of great application in the slimming products of modern cosmetics.

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Sulfur mustard vesicant-induced alterations of laminin 5 and gelatinase in mouse skin

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Epidermolysis bullosa (EB), a genetic skin disease characterized by skin blistering, may be caused by mutations in any of a variety of genes that encode proteins required for maintaining the structural integrity of skin. Three of these genes, laminin-α 3, laminin-β 3, and laminin-γ 2 encode separate polypeptides that together create a heterotrimer, forming the basement membrane protein, laminin 5. Disruption of the laminin 5 protein causes skin blisters. The chronic structural skin damage observed in EB may be further exacerbated by the actions of matrix metalloproteinases (MMPs). The chemical warfare agent, bis(2-chloroethyl)sulfide (sulfur mustard, SM), penetrates the skin rapidly and within several hours causes skin blistering similar to EB. After exposure to liquid SM, mouse ear skin was examined for upregulation of expression of the laminin 5 polypeptide chains and MMPs 2 and 9 (gelatinase A and B, respectively). Punch biopsies of mouse ear skin were taken at 6, 12, 24, and 72 hours after SM exposure. They were examined by histology, real-time PCR and gelatinase activity assays. SM caused a time-dependent increase in skin weight and damage relative to vehicle controls. Increased mRNA levels for MMP-9, laminin-α 3, laminin-β 3 and laminin-γ 2, were noted after SM exposure whereas MMP-2 mRNA levels decreased. There was a time-related increase in overall gelatinase activity observable 6 hours after SM exposure and persisted throughout the study. Pretreatment with the MMP inhibitor, Ilomastat, appeared to have no effect on the observations. Taken together, these observations may form the basis for an in vivo assay to test the efficacy of pharmacological countermeasures useful in preventing or alleviated SM induced skin damage.

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Effect of antioxidants on the wound healing of keratinocyte monolayers

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HaCat keratinocyte confluent monolayers are a useful model to study the effect of antioxidants on wound healing (WH). They were grown to confluence in DMEM-10% serum medium and a 1.5 mm wide wound was made along the midline of the monolayer. Immediately, thereafter, hydroalcoholic extracts prepared from green tea leaves (GTL) or ripe Autumn Olive berries (AOB), were added, and compared to the effects of addition of a flavonoid antioxidant, quercetin dihydrate (QD)and vitamin C(ascorbic acid, AA). Untreated and treated wounded monolayers were photographed 24 and 48 hours later using phase contrast optics and again after fixation and crystal violet staining. Untreated monolayers healed completely along the wound gap, Wound Healing Zone(WHZ) between 24 and 48 hours. The rate of WH was accelerated by treatment with of 12.5mM AA. By contrast, addition of plant-derived antioxidants inhibited proliferation and WH in a dose dependent manner. Cell migration into the WHZ was less affected than proliferation at equivalent doses. QD completely inhibited WH at 20uM; partial closure was seen at 10uM. GTL (1% extract) profoundly inhibited growth and WH, and induced cornified envelope formation in unwounded cells of the monolayer above 1% of GTL extract. A 2.5% AOB extract completely inhibited growth and WH, and partially inhibited closure at 1%. A 5-min exposure of live cultures to 1% SDS revealed that cells in the WHZ are more sensitive to detergent disruption than surrounding cells. AA treatment decreased the sensitivity of cells in the WHZ, while plant antioxidants increased their sensitivity. The results suggest that cells in the WHZ are in a less advanced stage of keratinizing differentiation. Also, vitamin C has the opposite effects on WH than the plant antioxidants tested. These studies are relevant to the use of plant antioxidants as potential cosmeceutical ingredients.

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Pro-differentiating effects of liver X receptor activators in keratinocytes

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Oxysterols activate liver X receptor (LXR) stimulating keratinocyte differentiation, which involves the formation of the cornified envelope on the inner plasma membrane by transglutaminase crosslinking of several constituent proteins. We report here that oxysterols increase the expression of one of these crosslinked proteins, involucrin, and that this effect can be abolished by mutations of the distal activator protein (AP)-1 response element in the involucrin promoter. Furthermore, oxysterols increase AP-1 binding in an electrophoretic gel mobility shift assay and induce the expression of an AP-1 reporter-luciferase construct. Within the AP-1 DNA binding complex we identified Fra-1 by super shift analysis of nuclear extracts from oxysterol-treated, cultured keratinocytes. Western blot analysis demonstrated that oxysterol treatment increased the levels of Fra-1 and Jun-D, while Northern analysis revealed that oxysterols increased mRNA levels for Fra-1, c-Fos and Jun-D. Similar alterations in AP-1 proteins occurred when 25-OH-cholesterol or non-sterol LXR agonists (GW3965, TO-901317) were used. Together these data indicate that LXR activators stimulate involucrin expression by increasing the levels of specific proteins of the AP-1 complex. The expression of key proteins required for keratinocyte differentiation, such as transglutaminase 1, involucrin, and loricrin, are regulated by AP-1 and therefore the LXR induced increase in AP-1 factors could activate many of the genes required for epidermal differentiation.

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Generation and characterization of constitutively active recombinant human caspase-14

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Caspase-14 is a member of the caspase family of cysteine proteases, which is expressed and activated in the granular layer of epidermis and some other keratinizing epithelia. Its substrates, function, and mode of activation during epidermal terminal differentiation are currently unknown. Caspase-14 possesses a particularly short prodomain of 13 amino acids similar to the caspase 1/3 subfamily. In order to obtain constitutive active recombinant caspase-14 for in vitro studies, we generated reverse-engineered forms of caspase-14, mimicking an approach used to obtain active precursors of other short prodomain caspases (J. Biol. Chem. 273:10107, 1998). Both the wild type enzyme (small subunit-prodomain-large subunit, denoted RevC14) and a second similar but catalytically inactive form with a cysteine to serine mutation (denoted MRevC14), were prepared. The histidine-tagged proteins were expressed in E. coli and purified by nickel chelate resin affinity chromatography. The RevC14 recombinant enzyme showed catalytic activity against a number of fluorogenic caspase substrates, with the highest activity against DEVD-AFC and YVAD-AFC. The MRevC14 recombinant enzyme showed greatly abrogated activity against all substrates tested, demonstrating the importance of the active site cysteine for catalytic activity. The autocatalytic activity towards DEVD-AFC was inhibited by the pan-caspase inhibitor Z-VAD-fmk. Optimal buffer conditions depended on the substrate being tested. These studies demonstrate that caspase-14 is an aspartate protease indicative of the caspase family. Studies in progress will be directed towards determining the substrate specificity of caspase-14 and determining its substrates in epidermal keratinocytes.

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Comparative analysis of the activity of three generations of retinoic acid metabolism blocking agents in cultures of differentiating normal human epidermal keratinocytes

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Retinoic Acid Metabolism Blocking Agents (RAMBAs) enhance (skin) tissue concentrations of endogenous all-*trans* retinoic acid (RA) to/or sustain pharmacologically active tissue levels of RA by blocking cytochrome P450-enzymes. One of these compounds, liarozole, was shown to be effective in the treatment of psoriasis and ichthyosis. Hence, they represent a novel alternative in the treatment of retinoid sensitive (skin) diseases. Rambazole™, a new azole derivative with increased potency and selectivity in a variety of biochemical and animal assays, was identified. RA modulates Ca^{2+}-induced differentiation of normal human epidermal keratinocytes (NHEK), by downregulating the expression of transglutaminase(TG)-1. The present study compares, *in vitro*, the retinoid potentiating effect of Rambazole with that of its predecessors ketoconazole and liarozole in a culture of differentiating NHEK. NHEK, isolated from foreskin, were plated in serum free, low Ca^{2+}, keratinocyte growth medium. At 60% confluency, the monolayers were switched to high Ca^{2+} and treated with RA alone (10^{-8}-10^{-12} M) or in combination with a RAMBA (10^{-6}-10^{-8}M). At day 5, after monitoring cell viability with MTT, cells were processed for measurement of TG-1 with an *in situ* ELISA. RA dose-dependently downregulated TG-1 expression (IC_{50}= 2-5 nM). Dose-response curves shifted to the left when RA was combined with each of the 3 RAMBAs. At 1 µM, minor differences in potency among the 3 compounds were observed. All three lowered the IC_{50} of RA at least 2000-fold to ≤ 1 pM. The decrease in TG-1 expression by ketoconazole, however, was partially due to toxicity. At 0.1 µM, Rambazole was 5 to 10 times more potent than ketoconazole and liarozole, respectively, and at 0.01 µM even ~ 100 times. In conclusion, Rambazole is superior to ketoconazole and liarozole in reinforcing the inhibitory effect of RA on TG-1 expression in human keratinocytes.

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Lack of the mouse channel-activating protease 1 causes epidermal malformations

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Serine proteases are proteolytic enzymes involved in the regulation of various physiological processes such as epidermal remodeling. We recently identified the mouse orthologue of the human serine protease prostasin as the amiloride-sensitive epithelial sodium channel (ENaC) activating protease 1 (CAP1), thereby presenting a new mechanism for autocrine activation of ion channels. CAP1 is expressed in different epithelial tissues such as lung, kidney, colon and skin and colocalizes with ENaC. In order to study the consequences of CAP1 deficiency in epidermal function, we generated mice harbouring a conditional knockout allele of CAP1 and crossed these mice with mice expressing the Cre recombinase under the control of the keratin 14 promoter. In newborn double transgenic mice, the CAP1 wild type allele is successfully deleted in epidermis, but these mice die within the first three days after birth. Histological analysis shows that loss of CAP1 in epidermis causes severe epidermal malformations, suggesting an important role of CAP1 expression in the formation and maintenance of the epidermal permeability barrier.

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Automated assessment of cell growth, confluence & compound-induced inhibition using unlabeled skin-derived cells and brightfield microscopy

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High Information Content Screening (HICS) aims at quantifying the full complexity of biological phenomena inside cells, such as e.g. protein activity in multiple signalling pathways that execute cell division, differentiation, motility and cell death. With the advent of microscopy readers, full automation has been achieved of the image acquisition and image segmentation process, as well as of image data reduction to quantitative data, predominantly for fluorescent readouts. In spite of this evolution, very little satisfactory tools have been generated to automate assessment of basic features of unlabeled cells in culture, still the most commonly used microscopy mode in hands-on cell culture assessment. A key hurdle is the variable background that results from the air-liquid meniscus inside wells. This uncontrolled lens over the cells makes it impossible to apply background subtraction models or threshold-based image segmentation to images. Cell counting, cell number, confluency of monolayers and colony size of unlabeled living cultures, therefore remains elusive or is substituted for by surrogate endpoints, such as mitochondrial assays. We have applied automated full-well, brightfield image acquisition to unlabeled, living cultures of skin-derived primary epidermal keratinocytes using the MIAS-2 microscopy reader. Using Scale-Space theory and the eaZYX-IMAGING software, we have developed image analysis tools for background-independent object identification, which facilitates image segmentation from tiled images covering the entire well surface. Based on this novel image segmentation technology, brightfield image analysis applications have been built for cell counting and measuring confluency of monolayers, which are currently applied to study the modulatory effect of drug-like compounds on growth and cell death of keratinocytes. The developed applications will facilitate complete automation of the cell culture and compound screening process.

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Cholesterol sulfotransferase (SULT2B1b) is a late marker of human keratinocyte differentiation

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The importance of cholesterol sulfate as an epidermal factor stems from the discovery of high levels of this sulfolipid in the epidermis of patients afflicted with recessive X-linked ichthyosis. Several reports have indicated that cholesterol sulfate accumulates during keratinization suggesting that it plays a role in keratinocyte differentiation and development of the barrier. The enzyme that produces cholesterol sulfate is as a member of the cytosolic sulfotransferase (SULT) superfamily. We investigated expression of this enzyme in primary cultures of normal human epidermal keratinocytes (NHEK) undergoing induction of terminal differentiation by calcium. Importantly, RT-PCR and western analyses showed that, of the three isozymes known to sulfonate steroids/sterols, only SULT2B1b was expressed by NHEK. This was also the case for normal human skin. Quantitative real-time PCR analysis demonstrated significant induction of SULT2B1b mRNA by calcium. Furthermore, cholesterol sulfotransferase protein and activity paralleled the increase in SULT2B1b mRNA during calcium induction of NHEK terminal differentiation. Immunocytochemical analysis revealed that SULT2B1b was localized to the granular layer of the epidermis of normal human skin suggesting that it is a late marker of keratinocyte differentiation. A similar analysis in disorders involving the integument revealed striking differences in SULT2B1b expression.

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Transcriptional profiling of keratinocyte differentiation in culture: the roles of cell confluency, calcium and detachment from the basement membrane

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In epidermis, proliferating basal keratinocytes eventually detach from the basement membrane, terminate cell growth and initiate differentiation into cornified cells that protect our body from the environment. The factors that initiate and regulate these processes are not known, although cell-cell contacts, calcium and detachment from the basement membrane have been suggested to play a role. To define comprehensively the transcriptional changes essential for keratinocyte differentiation, we used large DNA microarrays and profiled the changes in keratinocyte gene expression depending on culture density, calcium, and detachment from the substratum. Confluent keratinocytes express genes associated with cornified envelopes, lipid barrier, tight junctions and suprabasal tonofilaments, while suppressing basal keratins, integrins, ECM proteins, and growth-related, suggesting a spontaneous maturation of keratinocytes by increased cell density. Suspension cultures, in which keratinocytes are prevented from attaching to the basement membrane, showed even stronger effects on these genes. Additionally, our data identify many genes that have not been previously recognized as basal- or suprabasal-specific. In contrast, addition of 1.2mM calcium, while dramatically altering keratinocyte morphology and allowing formation of dense colonies, only marginally affected the gene expression. These results suggest that detachment from the substrate and culture density are much stronger differentiation stimuli than calcium.

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Liver X receptor activators accelerate post-natal acidification of the stratum corneum and improve permeability barrier homeostasis and stratum corneum integrity

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At birth, mammalian skin displays a near-neutral stratum corneum (SC) pH, which declines gradually to an acidic pH, comparable to adults. The absence of an acidic pH in neonates has adverse consequences leading to abnormal permeability barrier homeostasis and SC integrity/cohesion. Therefore, we utilized the neonatal rat model whose SC pH at birth is approximately 6.8 and declines to adult levels to assess the effects of LXR activators. Topical treatment with several LXR-activators (22(R)-hydroxycholesterol and the non-sterol activators TO-901317 and GW 3965) accelerated the acidification of the SC in neonates. The abnormal permeability barrier homeostasis that is characteristically observed in neonates was normalized by treatment with LXR activators. The improved barrier homeostasis was paralleled by maturation of the extracellular lamellar membranes in the SC. The biochemical basis for the accelerated maturation could be attributed to an increase in beta-glucocerebrosidase activity. The abnormality in SC integrity/cohesion was improved by treatment with LXR activators. The improvement in SC integrity/cohesion was associated with increased numbers of corneodesmosomes and in desmoglein 1 expression. Finally, treatment with LXR activators increased SC secretory phospholipase A2 activity, which could be one mechanism that accounts for acceleration of SC acidification. These results demonstrate the beneficial effect of LXR activators in the post-natal development of an acidic SC pH, and the subsequent improvement in permeability barrier homeostasis and SC integrity/cohesion. Coupled with the previously described anti-inflammatory and pro-differentiating effects of LXR activators, these results further suggest that LXR activators maybe useful agents in the treatment/prevention of neonatal skin disorders.

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Ceramide hydrolysis is an important protective mechanism against ceramide-induced apoptosis in UVB irradiated keratinocytes

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Whereas Ceramide (Cer) are key constituents of the stratum corneum permeability barrier, they also can function as pro-apoptotic lipid molecules within epidermal keratinocytes. Our prior studies demonstrated that increased de novo Cer synthesis accounts, in part, for UVB-induced apoptosis in cultured human keratinocytes (CHK) (Uchida et al., JID, 120: 622, 2003). Since epidermis is continuously at risk from exposure to UV, we hypothesized that keratinocytes must have mechanisms that regulate intracellular Cer levels to protect against Cer-induced apoptosis in response to UVB. We recently demonstrated that acidic ceramidase (aCDase) is expressed in both undifferentiated and differentiated keratinocytes (concurrently presented at this conference), and therefore, we investigated here whether aCDase, which hydrolyzes Cer, is an important regulator of intracellular Cer levels following UVB. Both low dose UVB (low-UVB, <35mJ/cm2) and higher dose UVB (high-UVB, >45mJcm2) inhibited DNA synthesis of CHK, but apoptosis occurred only in CHK exposed to high-UVB. Cer synthesis, assessed by [3H]-palmitate incorporation, increased similarly (i.e., 2.7-fold) in both low-UVB and high-UVB-treated CHK by 6 h following irradiation. While Cer synthesis returned to normal in low-UVB-CHK, Cer production remained elevated in high-UVB-CHK even at 24 h (3.3-fold). In addition, although apoptosis (i.e., LDH release and TUNEL-positive staining) was not evident in CHK treated with either low-UVB or N-oleoylethanolamine (NOE), an aCDase inhibitor, alone, CHK simultaneously treated with low-UVB plus NOE were sensitized to apoptosis. Finally, lipid quantification revealed significantly increased Cer content in NOE plus low-UVB-treated cells (1.8-fold; 6h after exposure), while that of low-UVB or NOE alone-treated cells were unchanged. Thus, at least one CDase isoform, aCDase, appears to have an important role in protecting keratinocytes from Cer-induced apoptosis in response to UVB.

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Wounding-induced up-regulation of hyaluronan in organotypic cultures of keratinocytes

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Hyaluronan (HA), a high molecular weight glycosaminoglycan, is generally regarded as a component of connective tissue extracellular matrix. However, epidermal keratinocytes synthesize HA in the intercellular space between basal and spinous keratinocytes. Recent studies suggest that accumulation of HA is correlated with cell proliferation and migration during skin development and renewal. To examine the role of epithelial HA after wounding, we investigated HA expression in pin-pricked organotypic keratinocyte cultures. Rat epidermal keratinocytes (REKs) were grown at the air-liquid interface in the absence of fibroblasts and, after 5 days a fully differentiated epithelium was formed. These cultures were pricked with a needle and harvested at 15 min, 6 h, 24 h, and 48 h. Paraffin-embedded sections were stained with HA-binding protein and analyzed by immunofluorescence and computer-assisted image analysis. A significant increase in HA levels was first detected at 15 min, became maximal at 24 h, and decreased again by 48 h. Induction of HA was seen as far away as 1 cm from the point of injury, suggesting the production of a soluble paracrine factor. To test this, we examined the effects of conditioned medium after injury. Media from pricked REKs were harvested at 15 min, 6 h, 24 h, and 48 h, then transferred into wells of unpricked cultures. These cultures were analyzed 24 h later. Strong and rapid induction of HA expression was observed, and was already nearly maximal in cultures exposed to the 15 min harvested medium. These data indicate that, as observed in skin *in vivo* [1], upregulation of HA by keratinocytes may be an important factor in wound repair. Some diffusible factors, which stimulate keratinocytes to synthesize HA, may be secreted from keratinocytes in rapid response to focal injury.

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Assessment of cellular turnover by fluorescent staining of stratum corneum with pyranin

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Cellular turnover assays have traditionally been conducted using a 24-hour Dansyl Chloride (5% in petrolatum) patch and then following the decay of the of the fluorescent signal produced by ultraviolet light, for several weeks. We have used a short-term (2 hours) patch of Pyranin 2% aqueous and followed the blue-light (417 nm) induced fluorescent signal. To record the fluorescence emission, a digital camera system was used with blue-cutting filter on the lens and the flash lamps emitting blue-violet light (417 nm) through a band-pass interference filter. To standardize the luminance measurements on the series of images captured on different days, a rectangular tile painted with pyranin was placed adjacent to the patch site during all image captures. A computer program was developed to determine the luminosity values of three sites in each image; the Pyranin-labeled skin, adjacent control site (background) and the rectangular fluorescent standard. The luminosity of the Pyranin site, after subtracting the luminosity of the control site, was compared to the luminosity of the fluorescent standard. This was done to eliminate the errors due to variations in flashlamp intensity and digital camera imaging, from one image capture to the other. Series of images were obtained over a period of few weeks. When the shape and size of the rectangular tile was similar in all the images we can measure the extinction of the fluorescence emission very accurately. We have also observed that adding lactic acid to the Pyranin solution increases the penetration depth which results in longer extinction times.

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Stem cell frequency in aged epidermis is increased

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The aim of this study was to determine the stem cell frequency of aged vs. young epidermis. Using an in vivo competitive repopulation assay and a limiting dilution design we studied the numbers of long-term repopulating cells in aged (20-24 mo) vs. young epidermis (8-12w). Murine keratinocytes (follicular and interfollicular) were isolated from GFP transgenic mice. We then implanted various dilutions of GFP+ test keratinocytes with 1 million GFP- keratinocytes in chambers on the backs of SCID mice. Numbers of test keratinocytes ranged from 100,000 to 6,250 for aged epidermis, and from 100,000 to 12,500 for young epidermis. An epidermis formed by 2 weeks. Chambers were observed at 3 weeks, and every 2 weeks thereafter, with an epifluorescence microscope (Zeiss Stemi SV) to detect the presence or absence of a GFP+ stem cell and its progeny. Data was analyzed with the help of limiting dilution analysis software (L-Calc, Stemsoft, Vancouver). GFP+ repopulating units were observed to disappear at times up until 7 weeks, but not after 7 weeks, suggesting that at this point we were observing true stem cells rather than transit amplifying cells or early progenitors. Limiting dilution analysis was therefore performed at 7 weeks, at which point chambers were assessed as positive or negative for GFP+ competitive repopulating units. Stem cell frequency in aged epidermis was significantly greater than that in young, 1 in 13,200 (95% confidence interval 1 in 6,300 to 1 in 27,800), vs. 1 in 28,200 (95% confidence interval 1 in 11,300 to 1 in 75,500) p=0.04. In previous studies of hematopoietic stem cells (the only other tissue with a quantitative in vivo stem cell assay) stem cell frequency in aged bone marrow increased 2-5 fold. It has been hypothesized that stem cell frequency in the aged increases in an attempt to compensate for decreased proliferative capacity of each stem cell. We will next conduct studies using our assay to determine the proliferative capacity of individual aged vs. young epidermal stem cells.

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Plakin proteins are coordinately cleaved during apoptosis but preferentially through the action of different caspases

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In epithelial cells, cell-cell and cell-matrix junctions, desmosomes and hemidesmosomes, provide anchorage sites for the keratin intermediate filaments. The plakin proteins desmoplakin, plectin and periplakin represent intracellular constituents of these adhesion junctions. In staurosporine treated apoptotic HaCaT cells desmoplakin, plectin and periplakin became cleaved coordinately with the elimination of keratins 10 and 14, while involucrin, actin and keratin 18 displayed considerable stability. The caspase inhibitor zVAD-fmk prevented both the cell detachment and protein cleavage, indicating the function of caspases in these events. Closer examination in vitro revealed that while caspases 2 and 4 most efficiently cleaved desmoplakin, and plectin served as a target for caspases 3 and 7, periplakin as well as keratins were cleaved by caspase 6. The involvement of multiple caspases in the destruction of epithelial cell integrity ensures the efficient elimination of cytoskeleton, but also provides specificity for selectively targeting individual adhesion molecules.

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Breaking the connection: caspase 6 cleaves periplakin releasing the intermediate filament binding tail region from the actin binding N-terminal domain

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Periplakin is a member of the plakin family of cytolinkers that connect cytoskeletal networks to each other as well as to junctional complexes. Here we demonstrate direct binding between the N-terminal head domain of periplakin and filamentous actin. Furthermore, the C-terminal part of periplakin binds to and colocalizes with the intermediate filaments. We demonstrate that the specificity of intermediate filament binding depends on the length of the rod domain attached to periplakin C-terminal tail domain. Double labeling of the Madin-Darby canine kidney cells expressing the N- and C-terminally tagged periplakin revealed unexpected lack of colocalization of the two signals. Although the C-terminal tag colocalized with intermediate filaments both in normal and apoptotic cells, the N-terminal signal was either absent or was detected as cytoplasmic aggregates in apoptotic cell remnants. Western analysis revealed that periplakin becomes cleaved close to its C-terminal tail during apoptosis, leading to loss of cell adhesion. Digestions with active caspases 1-10 in vitro revealed that caspase 6 only cleaved periplakin, using an unconventional recognition site TVAD-1603. Collectively, the results emphasize a role for periplakin as a cytolinker and suggest that the connection mediated by periplakin needs to be broken during programmed cell death.

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Overexpression of Hoxb13 in an epidermal organotypic system disrupts hyaluronan distribution and results in a psoriasiform phenotype

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Hox genes encode a highly conserved family of transcription factors that function as key regulators of differentiation and cell fate specification. We recently showed that loss of Hoxb13 function in murine skin results in increased levels of epidermal hyaluronan (HA; a large extracellular glycosaminoglycan known to inhibit differentiation) and decreased expression of several epidermal differentiation markers (1). These observations led to the hypothesis that one function of Hoxb13 *in vivo* is to promote epidermal differentiation by keeping levels of HA low. The purpose of the present study was to evaluate the effects of overexpression of Hoxb13 on differentiation *in vitro*. To accomplish this, we utilized a rat epidermal keratinoctye (REK) cell line that, when grown in lift culture, fully stratifies in 5 days and produces all of the morphological and differentiation markers observed *in vivo*. Stably transfected cell lines constitutively expressing Hoxb13 produced several aberrant differentiation phenotypes reminiscent of psoriatic lesions. Included among these are parakeratosis, lack of a granular layer, acanthosis, and suprabasal mitotic figures. In addition, we find that HA levels, normally high in the basal layer of stratified REK cells, are significantly reduced in stratified REK cells overexpressing Hoxb13. The expression profiles of several epidermal differentiation markers are also abnormal in response to Hoxb13 overexpression. Together, these data support a role for Hoxb13 in the regulation of HA expression and of differentiation in the epidermis.

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Keratin expression in cultured skin substitutes suggests a hyperproliferative state that is normalized after grafting to athymic mice

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Cultured skin substitutes (CSS) consisting of fibroblasts and keratinocytes in a biopolymer matrix are an adjunctive treatment for full thickness burn wounds. A recent cDNA microarray analysis revealed that several genes characteristic of hyperproliferative epidermis have greater relative expression in CSS *in vitro* compared to native human skin (NHS). To examine this phenomenon at the protein level, immunohistochemistry was used to localize multiple keratin proteins in NHS, and in CSS *in vitro* and after grafting to athymic mice. Human skin was obtained from breast tissue and was imbedded frozen for sectioning, or was used to establish selective cultures of fibroblasts and keratinocytes. These cells were used to prepare CSS by sequential inoculation of collagen-based substrates with fibroblasts and keratinocytes. After 2 wks culture, CSS were grafted to athymic mice, and biopsies were collected for immunohistochemistry. Keratins 6, 16, and 17 were highly expressed in the epidermis of CSS *in vitro*. Keratin 16 was localized to the suprabasal epidermis, whereas keratins 6 and 17 appeared more widespread. The levels of these proteins were low or undetectable in the NHS samples, and were reduced in CSS after grafting. Conversely, keratin 15 was localized to basal keratinocytes in NHS, but was not detected in CSS *in vitro*. Expression of keratin 15 in CSS was up-regulated after grafting to mice. Keratins 6, 16, and 17 are known to be induced following skin injury and in hyperproliferative skin diseases. Expression of these keratins in CSS *in vitro* suggests a hyperproliferative or activated state, similar to wounded native skin. Keratin 15, known to be expressed in basal keratinocytes and down-regulated after wounding, was absent from CSS *in vitro*. After grafting to athymic mice, the down-regulation of keratins 6, 16, and 17 and up-regulation of keratin 15 suggests a normalization of CSS to a phenotype more closely resembling uninjured human skin.

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Gene expression by CD34-positive hair follicle bulge keratinocytes and expression patterns of bulge-specific genes following abrasion-induced epidermal regeneration

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Stratified epidermis is maintained by the continuous loss and replacement of proliferating basal keratinocytes from a pool of pluripotent stem and progenitor cells, including those in the hair follicle bulge region. The cell surface marker CD34 is expressed on hair follicle bulge cells and can be used to isolate these cells using fluorescence activated cell sorting for molecular studies, including gene expression analysis. Keratinocytes were harvested from 7 week-old mice, stained with antibodies to CD34 and alpha-6 integrin, and CD34 and CD34- keratinocytes were sorted into individual populations. Total RNA was isolated, and microarray analysis was conducted using three separate platforms, including high density microarray chips and a cDNA nylon array. Included among genes identified as differentially expressed in CD34 bulge keratinocytes were known bulge markers such as CD34, S100A4, Tcf3, and Barx2. The bulge-specific profile was characterized by functional categories including calcium ion binding (S100A4, S100A6, cadherin 13), adhesion (CD34, Ctgf), and Wnt signaling (Fzd2, Dkk3, Wnt3a, Wnt10), among others. To gain further insights into how bulge-specific genes behave following an external stimulus, we analyzed their expression patterns following epidermal abrasion-induced regeneration. In this context, bulge markers such as CD34 and S100A4 were lost during the massive hyperproliferation, while tenascin C and S100A6, both expressed in CD34 cells, experienced an overall increase in expression in abraded skin. Thrombospondin 2, down-regulated in CD34 cells, was increased following abrasion. From these data, we have gained insight into genes specific to bulge keratinocytes as well as their behavior following abrasion-induced epidermal regeneration.

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Assessment of toxicity of sidestream cigarette smoke on HaCaT-keratinocytes with the neutral red release assay

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In recent years sidestream cigarette smoke (SS) has been more and more identified as a non-oxidative and oxidative toxic factor altering lipids and membrane functions of the superficial layers of the skin. Therefore the aim of the present study was to investigate acute toxic effects of SS on HaCaT-keratinocytes *in vitro* using the neutral red release assay (NRRA) that particularly reflects cell membrane alteration and leakage. For the experiments HaCaT-keratinocytes were incubated 3h with neutral red in a phenol red free DMEM-medium and then exposed 15 min under PBS to SS that was collected from three University of Kentucky research cigarettes (2R4F) immediately before exposure. Finally the cells were destained and the optical density (OD) of the resulting solution was measured at 540 nm. After determining in a first experiment the optimal amount of PBS overlying the cells during exposure, cells were exposed to 0,9 and 1,5 µmol sodium lauryl sulfate (SDS) before smoke exposure, after smoke exposure and to SDS alone. In a further experiment the effects of ascorbic acid 50 µg/ml prior to smoke exposure were investigated. The results demonstrate that the OD was minimal with a supernatant of 1 ml PBS under exposure and was maximal at 0 and 3 ml. According to these results a supernatant of 1 ml was considered to be the best for the further experiments. Treatment of the cells with SDS before smoke exposure markedly decreased the OD to 0,19 and 0,15 in comparison to 0,57 and 0,45 after treatment with SDS after smoke exposure and 0,89 and 0,81 after SDS alone. The OD after smoke exposure following ascorbic acid was 0,72 and 0,55 without ascorbic acid. In conclusion using the NRRA profound acute toxic effects of SS associated with cell membrane damage could be demonstrated. These effects can be markedly increased by pretreatment with the strong detergent SDS. The fact that ascorbic acid increases OD indicates at least in some extent oxidative damage of the cells through SS. Further studies are necessary to investigate the molecular mechanisms underlying these findings.

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Mechanisms by which psychologic stress alters skin barrier homeostasis and stratum corneum integrity

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Although many skin disorders, including psoriasis, atopic dermatitis, and healing wounds, are adversely affected by psychologic stress, the pathophysiologic link is unclear. In contrast to the extensive studies on the effects of psychologic stress on the immune and neuroendocrine systems, few studies have examined the effects of psychologic stress on epidermal function. Recent studies in both humans and rodents have shown that psychologic stress alters permeability barrier homeostasis. Here, we determined the mechanisms by which psychologic stress alters permeability barrier function and SC integrity (= resistance to mechanical disruption). Insomniac psychologic stress (IPS) sustained for 42 h in mice altered both barrier homeostasis (i.e., delayed barrier recovery after acute perturbations) and SC integrity (i.e., resistance to tape stripping). IPS mice showed decreased epidermal cell proliferation, impaired epidermal differentiation, and decreased numbers of corneodesmosomes (CD). The IPS-induced decline in integrity, was further linked to degradation of CD proteins (e.g., DSG1). The secretion and production of lamellar bodies also declined, which in turn, could be attributed to a global decrease in epidermal de novo cholesterol and fatty acid synthesis. To determine further whether lipid deficiency accounted for the functional abnormalities in IPS mice, a topical mixture of physiologic lipids (equimolar cholesterol, ceramides, and free fatty acids) was applied to IPS mice, which normalized both barrier homeostasis and SC integrity.

This study demonstrates that psychologic stress alters permeability barrier homeostasis and SC integrity by decreasing epidermal lipid synthesis resulting in decreased lamellar body formation, secretion, and decreased CD. Topical treatment with epidermal physiologic lipids may help stress-induced, barrier- related skin disorders (e.g., psoriasis and atopic dermatitis).

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A functional *ex vivo* quantitative human epidermal stem cell assay

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The aim of these studies was to design a functional ex-vivo quantitative human epidermal stem cell assay. Previously we designed a functional in-vivo quantitative murine epidermal stem cell assay using ability for long-term proliferation as a test of stem cell function. We have now developed an ex-vivo human assay based on the same principles. This assay utilizes HLA A2+ (test) and HLA A2- (competitor) human foreskin keratinocytes. A fixed number of HLA A2- competitor keratinocytes are implanted along with variable numbers of HLA A2+ test keratinocytes in a silicone chamber on the dorsum of a SCID mouse. A complete epidermis is formed by 2 weeks. However, chamber epidermis is analyzed at 9 weeks to ensure that all transit amplifying and differentiated cells have been lost, and that remaining HLA A2+ repopulation units represent a true long-term repopulating cell and its progeny. At 9 weeks chamber epidermis is dissociated and analyzed by fluorescence activated cell sorting as positive or negative for HLA A2+ keratinocytes. Limiting dilution analyses of different populations of keratinocytes can be analyzed by specific limiting dilution software based on the Poisson distribution (L-Calc, Stemsoft, Vancouver). In these studies a human epidermis could be produced in a chamber on the fascia of a SCID mouse and maintained for at least 4 months. We were able to determine HLA A2 positivity or negativity of fresh foreskin epidermis with MA 2.1 antibody (gift of Stewart Cooper, San Francisco). After growing the HLA A2+ keratinocytes in the chamber for 9 weeks FACS revealed chambers to be greater than 97% positive for HLA A2. Finally, we could detect small numbers of HLA A2+ cells (1%) by FACS, such that we can be confident of detecting a HLA A2+ repopulation unit in this assay. Thus we now have an assay that can assess human epidermal stem cells by their defining function, namely long-term repopulation. Competition at limiting dilution allows the quantitation of stem cells in a keratinocyte population. Competition at non-limiting dilution allows comparison of the proliferative capacity of two different populations of stem cells.

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Analysis of calcium-inducible genes in keratinocytes using suppression subtractive hybridization and cDNA microarray

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Terminal differentiation of skin keratinocytes is a vertically directed multi-step process that is tightly controlled by the sequential expression of a variety of genes. To gain further insight into the molecular events involved in this process, we used suppression subtraction hybridization (SSH) and cDNA microarray analysis. Messenger RNAs were isolated from the primary skin keratinocytes cultured in vitro after treatment with calcium and/or 12-o-tetradecanoylphorbol-13-acetate (TPA), and then SSH was performed. A total of 840 cDNA clones were obtained from subtracted libraries, and these cDNA clones were used to make the microarray slides. Time-course cDNA microarray analysis (1, 3, 7, and 14 days after calcium treatment) revealed the global gene expression profile during keratinocytes differentiation. Of the 840 genes tested, 290 showed a greater than twofold change in expression level at least once over four time points. The genes were clustered into six groups according to their expression pattern using self-organizing maps analysis, and showed the global feature of function-related regulation. The genes related to keratinocyte differentiation were markedly up-regulated by calcium treatment. In addition, the unique pattern of increase was seen in the expression of genes related to ribosomal protein. On the other hand, transcripts involved in metabolism, DNA repair, transcription, and translation were generally down-regulated. These results demonstrate the complexity of the gene expression profile that contributes to the spatiotemporal regulation of keratinocytes differentiation. The combination of PCR-based cDNA subtraction and cDNA microarray analysis in the present study allowed efficient identification and validation of genes thought to play some roles in keratinocytes differentiation.

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Regulation of epidermal differentiation and permeability barrier homeostasis: effects of protein kinase C inhibitors on the skin barrier

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Calcium has an established role in the normal homeostasis of skin and serves as a modulator in keratinocyte proliferation and differentiation. Profiles of calcium concentration across normal skin are consistent with migration patterns of keratinocyte during terminal differentiation, and previous studies suggested that the epidermal permeability barrier homeostasis is definitely influenced by the epidermal calcium gradient. While the role of epidermal calcium gradient in permeability barrier homeostasis is well studied, few studies about the role of differentiation have been reported.In a previous study, we reported that topical application of Protein Kinase C (PKC) inhibitors to the barrier disrupted skin induced increase of intracellular calcium ion and significantly delayed the barrier repair. PKC have been implicated in various biological processes, including terminal differentiation of keratinocytes and topical application of PKC inhibitors inhibited the terminal differentiation processes. In this study, we investigated the effects of PKC inhibitors on epidermal barrier homeostasis while the increase of intracellular calcium concentration is prevented by calcium channel blocker and calcium ion chelating agents. The barrier function of hairless mice skin was disrupted by tape stripping and PKC inhibitor was topically applied. Calcium channel blockers, calcium ion chelating agents, and vehicles as a control were topically applied in same site and the recovery rate was measured. The formation of epidermal calcium gradient in vivo was observed under electron microscopy and change of intracellular calcium ion in vitro using cultured mouse keratinocytes. The protein and mRNA expression of differentiation markers after PKC inhibitor treatment were also measured.

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The effect of the epidermal barrier functions and structural changes using high dose systemic steroid in hairless mice

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Prolonged exposure of skin to corticosteroid can result in cutaneous abnormalities. Skin barrier impairment is reported as a steroid induced side effect. Here, we determined whether high dose systemic steroid injection would display adverse effects on epidermal functions, permeability barrier homeostasis. Systemic steroid was administered by injecting each hairless mouse, intraperitoneally with 0.3 mg triamcinolone, two times per week for five weeks. 0.9% normal saline was administered by injecting with same method for the controlled hairless mice. Every week, TEWL was checked and skin biopsies were taken. Skin specimens were prepared for H&E stain, ion capture cytochemistry, immunohistochemistry and electron microscopy. The results were following: High dose systemic steroid produced visible cutaneous changes and increasing TEWL, from about 1 week, in the group of 0.3 mg triamcinolone injected hairless mice than the control; Light microscopic observations of steroid-injected hairless mice showed gradually tinning of the epidermis and loss of stratum corneum from about 2 weeks, compared with the control; Ruthenium tetroxide staining of high dose systemic steroid treated specimens revealed that the lipid bilayer was impaired and fragmented from about 3 weeks. Intercellular spaces were widening and the lipid bilayer disappeared or was damaged compared with the control; Electron microscopic studies revealed not only a marked decrease in the number of lamellar bodies but also an abnormal transformation of lamellar bodies in the steroid injected hairless mice compared with the control, from about 3 weeks; For five weeks, gradually, the calcium gradient was disappeared in 0.3 mg triamcinolone injected hairless mice compared with the control, from about 3 weeks; Degree of expression of involucrin, loricrin and filaggrin was decreased in steroid injected hairless mice compared to the control.

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Fos and jun proteins are differentially expressed in normal and psoriatic human epidermis

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Here we studied the expression of AP-1 transcription factors in keratinocytes in monolayer culture, in differentiating skin organ culture, in normal epidermis and in lesional skin from psoriasis patients. All Jun and Fos proteins except FosB, which was negative in all models tested, were highly expressed in proliferating keratinocytes. By contrast in organotypic epidermal culture as well as in normal epidermis the expression was differentiation stage associated. JunB and JunD were expressed in all layers of normal epidermis whereas, in contrast to what has been reported previously, c-Jun expression started suprabasally then disappeared and became very strong again in distinct cells of the outermost granular layer. Since Fos proteins were abundantly present in nuclei of basal and suprabasal keratinocytes our data suggest that in these locations predominantly Jun/Fos heterodimers are formed. By contrast the strong expression of all Jun family members in subcorneal keratinocytes with a weak expression (c-Fos, Fra-1) or absence (Fra-2) of Fos proteins suggest that AP-1 dimers in the last stages of terminal keratinocyte differentiation are mainly composed by Jun proteins. A similar expression pattern was also detected in organotypic epidermal culture except that c-Jun was also present in basal keratinocytes. In contrast to normal skin, c-Jun expression was strongly upregulated in both basal and suprabasal keratinocytes in psoriatic epidermis and JunB expression was dramatically diminished whereas c-Fos expression was unchanged. Our data suggest that Jun proteins in the relative absence of Fos partners are involved in regulating terminal differentiation of keratinocytes whereas the induction of c-Jun and the loss of JunB expression in basal and suprabasal keratinocytes might contribute to the pathogenesis of psoriasis.

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Stratum corneum penetration by protein transduction domain peptide sequences depends on secondary structure

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Protein transduction domains (PTDs), such as that in the HIV tat protein (residues 47-57; YGRKKR-RQRRR), have been reported to penetrate plasma membranes of a wide range of cell types. However, while arginine content has been demonstrated to be important for enabling penetration, secondary structure requirements have not been fully examined. Moreover, there are no published data on PTD penetration of full-thickness skin or cultured keratinocytes. The current study utilizes confocal microscopy to measure the penetration of the tat PTD and variants, into stratum corneum and cultured keratinocytes. In addition, biophysical infrared (IR) spectroscopy techniques were employed to determine peptide secondary structure in water and in lipid membranes. These studies demonstrate that the tat PTD penetrates cultured keratinocytes as well as stratum corneum of intact skin within minutes of exposure. Furthermore, although unstructured in water, the tat PTD interacts intimately with negatively charged phospholipids, adopting a helical secondary structure. To investigate the role of secondary structure the lysines in the tat PTD were substituted with glycines as well as acetylated lysines. Confocal microscopy experiments indicated that neither variant peptide penetrates keratinocytes or stratum corneum. Interestingly, although these lysine substitutions did not significantly affect peptide solubility or secondary structure in water, neither variant interacted with lipid bilayers or demonstrated any evidence of helical structure in the presence of membranes, likely explaining their inability to enter cells. These initial findings demonstrate that the PTD sequence of tat is capable of penetrating stratum corneum and cultured keratinocytes and that helical secondary structure is required for membrane and skin penetration.

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Lipid synthesis and uptake are differentially regulated in human sebocytes (SEB-1) by retinoids, adipogenic hormones, fatty acids and culture conditions

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An understanding of the factors that regulate lipid synthesis and uptake in human sebaceous glands is essential in order to develop therapeutic strategies aimed at reducing sebum production and improving acne. Human sebocytes are like adipocytes in that they respond to adipogenic hormones and ligands of the peroxisome proliferator-activated receptors. The goal of this study was to compare the effects of retinoids, adipogenic hormones, fatty acids and culture conditions on the uptake of fatty acids by immortalized human sebocytes (SEB-1) and on the de novo synthesis of lipids from 14C-acetate in these cells. The uptake of 14C-oleic acid by SEB-1 cells was significantly lower in medium containing insulin compared to medium without insulin. Accordingly, treatment of SEB-1 cells with adipogenic hormones (isobutylmethylxanthine, dexamethasone and insulin) suppressed fatty acid uptake compared to medium without insulin. Interestingly, treatment with 13-cis retinoic acid (10-6M, 10-7M) significantly increased fatty acid uptake by SEB-1 cells, which is in agreement with the recent observation of that 13-cis retinoic acid increases lipid droplets in human ovarian theca cells. Linoleic acid and palmitic acid did not increase the uptake of 14C-oleic acid by SEB-1 cells. The incorporation of acetate into sebaceous lipids was increased with adipogenic hormones and insulin alone whereas isobutylmethylxanthine, dexamethasone and 13-cis retinoic acid decreased acetate incorporation into lipids. No significant differences in acetate incorporation into lipids were noted between cells treated with linoleic acid, palmitic acid and medium alone. These data further support the reliance of sebaceous lipogenesis on the substrate provided. In addition, these data demonstrate that insulin decreases fatty acid and increases de novo sebaceous lipid synthesis in human sebocytes. Futhermore, 13-cis retinoic acid has effects opposite to insulin on these processes.

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Keratinocyte differentiation activates mitochondrial-dependent cell death pathway

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Conversion of live keratinocytes into dead corneocytes involves the destruction of cellular organelles, degradation of DNA and formation of the cell envelope. Our previous findings suggested that keratinocyte differentiation activates mitochondrial-dependent cell death pathway. In this study, we induced apoptotic-like mitochondrial changes with protoporphyrin IX, staurosporine and rotenone in undifferentiated keratinocytes and asked whether these changes can affect keratinocyte differentiation. We showed that all three drugs inhibited cell cycle, decreased mitochondrial membrane potential, increased reactive oxygen species (ROS) production and induced cytochrome c and apoptosis inducing factor (AIF) release from mitochondria. These changes preceded or coincided with expression of keratin 10 (K10) and morphological alterations that normally accompany keratinocyte differentiation. A significant apoptosis was observed only at the high levels of the inducers, suggesting that apoptotic-like mitochondrial changes can trigger keratinocyte differentiation without inducing apoptosis. A kinetic study of mitochondrial alterations and their pharmacological or genetic modulation demonstrated that a decrease in ROS production decreased K10 expression, whereas the exogenous introduction of cytochrome c and AIF into keratinocytes by either microinjection or transfection induced it. In conclusion, these data showed that keratinocyte differentiation can be induced by triggering mitochondrial changes associated with apoptosis thus, supporting the hypothesis that keratinocyte differentiation utilizes mitochondria-dependent machinery of classical apoptosis.

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Induction of sphingolipid production in cultured human keratinocytes by LXR activators through increased expression of serine palmitoyltransferase

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Activators of the nuclear hormone receptor superfamily, including the liver X receptor (LXR), are important in the regulation of epidermal differentiation and development (Komuves et al. JID 118:25, 2002). Treatment of cultured human keratinocytes (CHK) as well as murine epidermis with LXR activators (e.g., oxysterols), increased expression of the differentiation-related structural proteins, involucrin, loricrin, and profilaggrin. Because lipid formation for the epidermal barrier is also differentiation-dependent, we investigated whether LXR activators upregulate the synthesis of sphingolipids in differentiated CHK, as well as the mechanism for such increases. The synthetic LXR activator TO-901317 (5 uM) induced a significant increase in total ceramide synthesis, measured as incorporation of [3H]-serine over 24 h following treatment. Synthesis of specific ceramides, i.e. ceramides-2-7, as well as glucosylceramides, increased 1.4- to 1.8-fold; p <0.001, and corresponding elevations in the content of these ceramide fractions were evident 48 h after LXR-activator treatment. Quantitative rt-PCR revealed a near-doubling of mRNA levels encoding for SPT2, the catalytic subunit for SPT, the key regulatory enzyme in ceramide synthesis, while SPT1 mRNA levels remained unchanged. Likewise, dual-reporter assays with an SPT2-promoter-luciferase construct, showed a comparable increase in relative luciferase activity; i.e., 1.9-fold for LXR activator-treated vs. vehicle-treated controls; p<0.005. Together, these studies suggest that LXR activators increase ceramide production and that the LXR-induced increase results, at least in part, from transcriptional up-regulation of SPT, a key enzyme in epidermal ceramide synthesis. Combined with the prior work demonstrating enhanced differentiation induced by LXR agonists, these studies suggest that LXR activators regulate both the lipid synthetic and protein arms of epidermal differentiation.

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Desmoplakin phosphorylation and regulated intermediate filament association is required for its efficient trafficking and incorporation into desmosomes

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Regulation of intercellular junctions is essential for epithelial morphogenesis and wound healing, but the dynamic behavior and roles of individual desmosome molecules during junction remodeling are poorly understood. We propose that desmosome remodeling requires regulated interactions with the intermediate filament cytoskeleton (IF), which is linked to the plaque via desmoplakin (DP). To test this hypothesis, GFP-tagged molecules including wild type DP (DPSer2849), a series of truncation mutants (DPNTs) lacking the C-terminal IF binding site, two PKA phosphorylation site mutants (DPGly2849 and DPAla2849) impaired in their ability to reversibly bind IF, and desmoglein2 (Dsg2) were introduced into cells. Cytoplasmic particles containing DPSer2849, but not DPNTs, were associated with IF, whereas DPGly/Ala2849 decorated IF more continuously. To determine if DP particles are desmosomal precursors, endocytosed desmosomes, or both, live cell imaging was performed on cells after inducing junction formation. The majority of cytoplasmic DP did not colocalize with endosomal markers, or the membrane marker DiI, in constrast to desmoglein2. DPSer2849 dots moved on straight tracks to newly forming cell contacts where they organized into desmosomes, exhibiting velocities over an order of magnitude slower than the movements of conventional molecular motors. DPGly2849 dramatically suppressed particle motion, resulting in delayed desmosomal incorporation. Particles that moved did so haltingly, alternately binding and releasing from IF. DPNT particles exhibited random cytoplasmic movements before trafficking to newly forming cell-cell borders. These data support the model that non-membrane bound, cytoplasmic DP complexes are desmosome precursors and that efficient incorporation of DP into desmosomes is promoted by Ser2849 phosphorylation and requires regulated interactions with the IF cytoskeleton.

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Impaired epidermal differentiation including reduced ceramide binding involucrin in atopic dermatitis

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A defective permeability barrier leading to the penetration of environmental allergens into the skin and initiating immunological reactions and inflammation is crucially involved in the pathogenesis of atopic dermatitis (AD). Studies have shown that decreased stratum corneum ceramide content may cause the defect in permeability barrier function consistently found in AD. In addition to lipids, we have previously shown that epidermal differentiation-related proteins, keratins and cornified envelope proteins are of crucial importance for skin barrier function. In the present work, we determined involucrin, loricrin, filaggrin, and keratin expression in lesional and non-lesional skin of AD patients by immunohistochemistry and by western blotting. We found premature, but reduced protein expression of involucrin and filaggrin in non-lesional and in lesional skin. Involucrin serves as a substrate for the covalent attachment of ceramides to the cornified envelope. Filaggrin is involved in water-binding; dry skin is consistently found in AD. Loricrin revealed broadening of the stained band and an increased protein expression in non-lesional and lesional epidermis as shown by western blot analysis. Extension of the staining intensity from the basal to suprabasal layers for K 5 and induction of proliferation associated K 16 were also found in lesional and non-lesional skin. Reduction of staining for suprabasal K 10 as well as induction of proliferation associated K 6 and inflammation associated K 17 were found in lesional skin of AD only. We suggest that the pathogenesis of AD involves both reduction in lipid content and impaired differentiation, including reduced protein expression of involucrin, which serves as a substrate for covalently binding ceramides, and reduced filaggrin, which is important for water binding.

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Differentiation-dependent expression of ceramidase isoforms in human keratinocytes and epidermis

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It is well established that ceramides are key lipid components in the formation and maintenance of the epidermal permeability barrier, which localizes to the stratum corneum. However, the presence within the stratum corneum of both ceramidase (CDase) activity, which hydrolyzes ceramides to their corresponding sphingols (e.g., sphingosine) and free fatty acids, as well as the CDase product, free sphingol, suggests a role for ceramide hydrolysis in epidermal function(s). Since CDase activity with a range of pH optima have been reported in homogenates from stratum corneum, epidermis, and cultured human keratinocytes (CHK), we investigated whether the expression of four CDase isoforms is altered during keratinocyte differentiation; i.e., in undifferentiated (serum-free KGM plus 0.07 mM Ca2+); differentiated (KGM 1.2 mM Ca2+); and lamellar body-competent (vitamin C plus 1.2 mM Ca2+ plus serum-supplementation) cells, in comparison with whole human epidermis. Normal and quantitative rt-PCR revealed low levels of both acid- and alkaline-CDase mRNA in low and high Ca2+-containing medium, values that increased significantly in lamellar body-competent CHK (i.e., 2- and 79-fold over KGM controls for acid- and alkaline CDase, respectively), with whole epidermis containing even higher expression of both isoforms. Conversely, mRNA levels for phytoalkaline-CDase were equivalent for undifferentiated and differentiated CHK, and significantly lower (i.e., <0.25-fold of control) in lamellar body-competent CHK and whole epidermis. Neutral-CDase mRNA levels remained comparable at each differentiation state. These results demonstrate significant alterations in the expression of key CDase isoforms during epidermal differentiation, suggesting specific, localized roles for each isozyme in epidermal structure and/or function.

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Expression of murine beta-defensins 1-4 depends on epidermal differentiation and stimulation

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Epidermis is exposed to a wide variety of environmental germs, nevertheless, infections are rare. Defensins comprise a family of cationic antimicrobial peptides with different specificity against gram positive and negative bacteria. In PAM212 cells, an immortal keratinocyte cell line, in primary keratinocytes and in hairless mouse skin in vivo, we investigated m-RNA expression of beta-defensin 1, 2, 3, and 4 using micro array and RT-PCR techniques. In addition, defensin proteins were quantified by FACS analysis and the distribution pattern was determined by immunohistochemistry. RT-PCR revealed constitutive expression of murine beta-defensin 1 in PAM212 keratinocytes, in embryonic, neonatal, and adult skin. Immunohistochemistry showed that beta-defensin 1 was equally distributed in the epidermis. No challenge is necessary to induce beta-defensin 1 expression in unstimulated cultured cells or fetal epidermis. Only in primary keratinocytes does induction with PMA lead to a significant increase in protein expression. Murine beta-defensins 2 and 3 were not expressed constitutively, but inducible by phorbol ester PMA stimulation. Immunohistochemistry revealed beta-defensin 2 and 3 in the outer epidermis, with an expression peak in the stratum granulosum. beta-defensin 3 showed up-regulation on gene and protein level after PMA stimulation in primary keratinocytes and in PAM212. beta-defensin 4 was constitutively, but weakly expressed and localized in the stratum granulosum. PAM212, which represent cells from the spinous layer, showed mRNA up-regulation after PMA stimulation. The results indicate a late expression of beta-defensin 4 in epidermal differentiation. In summary, antimicrobial peptides provide an innate defense system and develop during epidermal differentiation and stimulation.

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Protective effect of tocotrienol on hydrogen peroxide–induced intracellular ROS generation and cell death on normal human keratinocytes

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Epidermal cells are exposed to a large range of oxidative stress i.e. solar radiations, tobacco smoke, pollutants, and ozone, contributing to chronic skin inflammation and premature aging. One of the most powerful natural antioxidant is alpha–tocopherol, a lipophilic cell membrane scavenger for OH° and 1O2. In the present study we investigated the protective effect of the tocopherol derivative, tocotrienol (2, 5, 7, 8–tetramethyl – 2 – (4″ 8″ 12″ – trimethyltrideca–3″ 7″ 11″ – trienyl) –6–chromanol) on intracellular oxidation and cell death induced by H2O2 (1 mM) on normal human keratinocytes. Cell death (apoptosis and necrosis) was measured by following mitochondrial transmembrane potential (collapsed in apoptotic and necrotic cells) with the JC–1 probe, and plasma membrane permeability (maintained in apoptotic and unaltered cells) using the TOTO–3 fluorophore. Caspase–1 and –3 activity were quantified using specific permeant FITC and rhodamine substrates respectively. For intracellular oxidation, cells were loaded with dichlorodihydrofluorescein diacetate, emitting a green fluorescence when oxidized. We demonstrated that the protective effect of tocotrienol during accute oxidative stress takes place through: (1) a reduction of intracellular ROS generation, (2) a protection against cell death with more JC1–high and TOTO3–negative cells, (3) a reduction of the necrotic vs apototic cell ratio mainly due to a decrease of necrotic cells, (4) a reduced caspase-1 activity leading to a lower caspase 1/caspase 3 ratio. In the same range of concentrations, α–tocopherol failed to protect so efficiently the cells. We conclude that this tocopherol derivative could be a potent molecule to protect the keratinocytes from oxidative stress–induced intracellular oxidation and death, and improve removal of irreversibly damaged epidermal cells through a programmed cell death process.

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Class II PI3-kinase mediates cell cycle inhibition and terminal differentiation in epidermis

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The phosphoinositide 3-kinase (PI3K) multi-gene family has been implicated in the regulation of proliferation, apoptosis and differentiation in various tissues. A role for PI3Ks in epidermal homeostasis, however, is undefined. In examining PI3K distribution in epidermis, we found that Class I PI3K proteins are expressed in all layers while Class II PI3Ks are strikingly localized within suprabasal, differentiating cells. To explore a potential role for PI3Ks in epidermal growth and differentiation, we next activated and blocked PI3K function using both genetic and pharmacologic approaches. In contrast to Class I PI3Ks, active Class II PI3K induced differentiation and growth arrest in keratinocytes in vitro. In genetically engineered human epidermis regenerated on immune-deficient scid mice in vivo, active Class II PI3K triggered growth inhibition, hypoplasia and premature differentiation. Mutagenesis demonstrated that these effects are dependent on intactness of both the PI3K lipid kinase and C2 domains. Knocking down Class II PI3K levels in vivo, in contrast, stimulated epidermal hyper-proliferation and hyperplasia while simultaneously down-regulating differentiation marker expression, indicating that intact Class II PI3K function is required for normal epidermal differentiation. Of interest, calcium addition activated Class II PI3K plasma membrane localization and function while active protein itself elevated intracellular calcium concentration, indicating that Class II PI3K participates in a calcium-mediated autoregulatory positive feedback mechanism. These data are the first to identify an important role for Class II PI3Ks in controlling epidermal growth and differentiation and indicate that these effects may proceed in part via autoregulatory amplification of calcium signaling.

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Scanning transmission ion microscopy, particule induced X-ray emission and skin structure

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Studying the morphology and the chemical composition of human skin, a tissue with a total typical thickness under a few hundred micrometers, requires techniques working at the microscopic level. We have developed ion microbeam techniques of imaging and analysis with the capability to determine mineral ions and exogenous elements in the different strata of native skin. Particle Induced X-ray Emission (PIXE) and Scanning Ion Transmission Microscopy (STIM) allow respectively to map most elements present in epidermal sections and to image the skin structure on the basis of its density contrast. Owing to its multielemental capability, micro-PIXE analysis allows the simultaneous mapping of a dozen elements in tissues sections. It is thus particularly well adapted for the measurement of balanced ions (Na, Mg, S, Cl, K, Ca) which appeared highly compartmentalized in the different skin strata. STIM delivers image of tissues with sub-micrometer resolution, both rapidly and non destructively, authorizing localization of cellular structure. In addition, it allows the measurement of the sample mass to normalize X-ray data in terms of concentration. Those techniques were carried out in the frame of physiological studies, to highlight for instance the calcium gradient in the outermost layers of human skin in relationship with the functional barrier effect. The fact that this method may be applied on un-embedded sections, only cryofixed and freeze-dried is undoubtedly an advantage when addressing percutaneous penetration studies. The aim of this work is to show the perfect complementarities of STIM and PIXE when applied to the elucidation of the elemental content and structure of different models of human and animal skin. The method proved to be very useful in providing elemental and structural information that allowed a clear identification of very thin skin layers such as stratum granulosum.

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Mek1, but not Mek2, alters epidermal growth and differentiation

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The highly homologous kinases, Mek1 and Mek2, act downstream of Ras and Raf to activate ERK MAP kinases. In epidermis, Ras and Raf promote proliferation and oppose differentiation, however, they act on multiple Mek-independent effectors and the extent to which Meks can mediate these effects is unknown. Moreover, despite their similarity, the function of Mek1 and Mek2 is not entirely redundant and the degree to which either Mek kinase can mediate Ras/Raf effects and alter epidermal homeostasis is not defined. To address this, we first examined the necessity of Mek2 for Ras/Raf effects. Conditionally inducible epidermal Ras mice (K14-ER:Ras) and Raf mice (K14-Raf:ER) were crossed with MEK2 knock-out mice. Ras induction in K14-ER:Ras/MEK2-/- mice increased proliferation and inhibited differentiation in a manner indistinguishable in kinetics and magnitude from K14-ER:Ras/MEK2+/+ controls. Similarly, no difference was seen between K14-Raf:ER/MEK2-/- mice and K14-Raf:ER/MEK2+/+ mice, indicating that MEK2 is dispensable for Ras/Raf-driven epidermal changes. We next examined the effects of epidermal Mek gain-of-function. To do this, we generated conditionally active Mek fusions to the estrogen receptor ligand-binding domain. ER-fused Mek1 and Mek2 were then expressed in transgenic murine (3 independent lines each) as well as in genetically engineered human epidermis regenerated on scid mice. Both Mek1 and Mek2 triggered epidermal ERK phosphorylation in vivo in human and murine skin in response to topical 4-hydroxytamoxifen. Only Mek1, however, recapitulated Ras/Raf effects in increasing proliferation and integrin expression while suppressing differentiation. Furthermore, a kinase-dead Mek1 mutant demonstrated that ERK phosphorylation was dispensable for these features. These data indicate that Mek1 is sufficient to promote the undifferentiated, proliferative epithelial phenotype in a manner independent of ERK activation and that Mek2 is dispensable for Ras/Raf-driven changes in epidermal growth and differentiation.

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Developmental environments alter the fate of aged epidermal stem cells

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Homeostasis of continuously renewing tissues, such as the epidermis of the skin, is maintained by somatic stem cells. These are undifferentiated, self-renewing cells, which also produce daughter transit amplifying (TA) cells that make up the majority of the proliferative population. TA cells undergo a finite number of cell divisions before leaving the proliferative compartment and moving toward terminal differentiation, whereas it has been assumed that the stem cells persist throughout the lifetime of the organism. Through a series of labeling experiments with tritiated thymidine, we previously showed that stem cells from adult mouse skin did not divide as often as the other basal cells, but they did divide at a steady rate in vivo. We also showed that they continued to proliferate in vivo throughout life, and that they have a high proliferative potential in vitro. Thus, we wondered if epidermal stem cells did not follow the Hayflick theory of aging and possibly lived forever. Using our recently redefined sorting method, we isolated epidermal stem and TA cells from the skin of neonatal and old mice. The stem cells, but not the TA cells, had the capacity to regenerate an epidermis with continuously long term expression of a recombinant gene. We also injected both the neonatal and the aged stem cells into developing mouse embryos and into developing zebrafish embryos. We found that both ages of stem cells had the remarkable ability to participate in the formation of tissues from all three germ layers when introduced into developmental environments, whereas the TA cells could not. These data suggest that there may be a fundamental intrinsic difference between epidermal stem and TA cells, and that the environment, rather than the age of the stem cell, influences the fate determination of a stem cell.

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Influence of keratinocyte culture conditions on development of a senescence phenotype

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It has been reported that with age, keratinocytes *in vivo* become less proliferative and have a greater tendency to become growth arrested (senescent). Recently, we cultured neonatal keratinocytes *in vitro* in an attempt to elucidate this process. The expression of β-galactosidase, a widely confirmed marker of senescence, and population doublings were examined as indicators of the replicative capacity of keratinocytes during their cultured life span. Interestingly, it seems that the culture conditions affect the replicative life span more than the number of actual population doublings on the journey to senescence *in vitro*. Specifically, the expression of β-galactosidase and growth arrest was induced consistently as a function of the number of trypsinizations, irrespective of population doublings. This trypsin-induced growth arrest could also be altered with a different type of media, bringing into question media effects on senescence *in vitro*. It also appears that differentiated keratinocytes *in vitro* develop their β-galactosidase staining properties, while only basal keratinocytes *in vivo* stain positive for this enzyme. Collectively, this data highlights using caution when using cell culture models to measure replicative capacity of keratinocytes.

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S100A8 and S100A9 in human keratinocytes: a novel secretory mechanism

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S100 proteins are thought to be important in epidermal differentiation, as they are expressed in epidermis, clustered in the epidermal differentiation complex at 1q21, differentially expressed during differentiation, and activated by calcium. Moreover, select members of this family are overexpressed in epidermal disease. S100A8 and S100A9 form a hetero-complex, and are particularly important, since they are markedly overexpressed in psoriasis. Previously, we mapped S100A8 and S100A9 expression in normal and psoriatic epidermis. In psoriasis, S100A8 and S100A9 expression is markedly elevated and aberrant expression is detected in the epidermal basal and spinous layers. Secreted S100 proteins are thought to function as chemokines for CD4+ lymphocytes; however, there is no direct evidence indicating how S100s are released from keratinocytes. In the present studies, we examine the distribution of S100A8 and S100A9 in cultured normal human epidermal keratinocytes. Our data demonstrate that S100A8 and S100A9 assume a cytoplasmic distribution in resting cells and are not present in the Golgi or endoplasmic reticulum. Differentiation of keratinocytes induced by okadaic acid treatment causes S100A8 and S100A9 to co-localize with β-actin in the cortical actin ring, followed by the formation of S100A8 and S100A9-positive cytoplasmic buds that protrude from the apical cell surface. These blebs are rimmed by actin which appears to sequester the S100A8 and S100A9-containing cytoplasm. These extrusions appear to release from the surface in a non-classical secretory mechanism. This study suggests that a novel mechanism whereby S100A8 and S100A9 may be released from keratinocytes is the formation of actin-stabilized cytoplasmic buds.

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Identification and refinement of a locus for type I punctate palmoplantar keratoderma on chromosome 15q22-24

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Mutations in different components of the intermediate filaments, desmosomes and gap junctions have been identified underlying several disorders of keratinization. Among them is an extraordinarily heterogeneous group known as palmoplantar keratodermas (PPK), for which only a few molecular defects have been described. Here we report the identification of the first locus for type I punctate PPK. A genomewide scan was performed on an extended autosomal dominant pedigree and linkage to chromosome 15q22-24 was identified. With the addition of two new PPK families, we confirmed the mapping of the locus for punctate PPK to a 9.98-cM interval, flanked by markers D15S534 and D15S818 (maximum two-point LOD score of 4.93 for marker D15S988). In order to refine the location of the PPK locus, we genotyped all available markers within the region and narrowed the locus to an interval of 8.89 cM. According to the current physical map, the critical linkage interval identified here corresponds to a region of 3.7 Mb, syntenic to mouse chromosome 9. We have recently identified three new PPK families from Slovenia, with a total of 10 available family members. The common geographical origin of these three pedigrees will be helpful in further testing the genetic homogeneity of PPK, and also in searching for a possible common haplotype shared among them. In all, these findings will facilitate the identification of a new gene involved in skin integrity that could also contribute to the understanding of the multiple organ involvement in the syndromic forms of PPK.

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A proteomic characterization of the plasma membrane of human epidermis by high-throughput mass spectrometry

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Membrane proteins are responsible for many critical cellular functions, and identifying these cell surface proteins on different keratinocyte populations would improve our understanding on the biology of the epidermis. However, the characterization of membrane proteins, which are mostly hydrophobic and incompatible with 2-dimensional gel electrophoresis, has been difficult to accomplish because high-throughput analytical methods to analyze membrane proteins do not exist. In this study, membrane proteins in human epidermis were analyzed using a two-dimensional liquid chromatography (LC) tandem-mass spectrometry (MS/MS) approach that can identify proteins by determining both the mass and amino acid sequences of tryptic peptides. From foreskin epidermal sheets, a highly enriched plasma membrane fraction was prepared using sucrose gradient centrifugation, followed by a single tube membrane protein extraction/solubilization into 60% methanol, and tryptic digestion. The resultant peptide mixture was fractionated into 80 fractions and analyzed by strong cation exchange/reversed phase LC-MS/MS. This high-throughput shotgun approach identified a total of 2,875 unique peptides corresponding to 1,306 proteins, including 1,083 known proteins and 223 novel proteins. Of the known proteins, 600 (55.4%) were annotated membrane proteins, while 104 of the 223 novel proteins (46.6%) were predicted to contain at least one transmembrane domain, yielding a significantly enriched total membrane proteome of 704 proteins (out of 1,306 proteins; 53.9%). Most known integral membrane proteins, including the cell adhesion complex, proteins involved in immune responses, neural function and molecular transport, and membrane caveolae proteins were identified. These results demonstrate identification of a significant number of the estimated membrane proteins (approximately 1,400 to 2,100) and provide a solid analytical platform to conduct quantitative comparison of protein expression in different keratinocyte populations.

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Role of the Ca-binding protein Scarf, during epidermal differentiation

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During the process of epidermal differentiation, intracellular and extracellular Calcium concentrations induce an array of signaling pathways. Members of the Ca -binding proteins play a central role in the transduction of Ca signals. We identified two novel mouse genes, Scarf (skin Calmodulin-related factor) and Scarf2, which have homology to Calmodulin (CaM)-like Ca -binding protein genes. Southern blot and computational genome sequence analysis show that three mouse Calmodulin like proteins -mClp, Scarf and Scarf2- are clustered on the end of chromosome 13 long arm. Scarf2 is expressed at a much lower level than Scarf in differentiated keratinocytes, even though a high degree of homology between the genes has been found in the upstream regions that contain potential regulatory sequences. To understand the control elements that confer the distinct expression patterns to Scarf and Scarf2, we are performing Scarf promoter analysis using a luciferase assay. Since Ca -binding proteins exert their roles through Ca -dependent interactions with their target proteins, we are in the process of identifying Scarf-interacting factors utilizing yeast II hybrid screens and affinity chromatography followed by Mass Spectrometry (MS) analysis. The identification of the interacting factors will aid in understanding the function of Scarf in the Ca -dependent keratinocyte differentiation.

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Anti-psoriatic drug Anthralin induces EGF receptor phosphorylation in keratinocytes: requirement for H2O2 generation

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Anthralin is a well-established topical therapeutic agent for psoriasis, although little is known about its effects and biochemical mechanisms of signal transduction. In contrast to a previous report, we found that anthralin induced time- and concentration-dependent phosphorylation of epidermal growth factor receptor in primary human keratinocytes. Combined evidence shows that this process is mediated by reactive oxygen species. First, we found that anthralin induces time-dependent generation of H2O2. Second, there is correlation between the time-dependent increase in anthralin-induced epidermal growth factor receptor phosphorylation and H2O2 generation. Third, structurally different antioxidants, n-propyl gallate and N-acetylcysteine inhibited epidermal growth factor receptor phosphorylation induced by anthralin. Fourth, overexpression of catalase also inhibited this response. The epidermal growth factor receptor-specific tyrosine kinase inhibitor PD153035 abrogated anthralin-induced receptor phosphorylation and activation of extracellular-regulated kinase 1/2. These findings link the following sequence of events: (1) H2O2 generation, (2) epidermal growth factor receptor phosphorylation, and (3) extracellular-regulated kinase activation. Our data identify anthralin induced reactive oxygen species and, more specifically H2O2 as important upstream mediators required for ligand-independent epidermal growth factor receptor phosphorylation and downstream signaling.

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Role of erbB3 in proliferation and in stress-response of human keratinocytes

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Key regulators of both the proliferative and differentiation signal transduction pathways in keratinocytes are members of the erbB family of transmembrane tyrosine kinase receptors. Three of the four identified erbB family members are expressed in the keratinocytes of the skin, erbB1, erbB2, and erbB3, and many of their numerous ligands are either produced by keratinocytes or by cells that are adjacent to keratinocytes. The erbB1 protein is the prototype of the family and has been extensively studied in a variety of cell types. Less is know about the function of erbB2 in skin, but it has been suggested that expression and activation of the erbB2 receptor is associated with the induction of differentiation. Although the erbB3 receptor has been shown to be a critical signaling protein in other cell types, very little is known about the function of the erbB3 receptor in the skin. Previously reports from our lab have suggested that the erbB receptor family is integrally involved in the stress-response of keratinocytes induced by exposure to ultraviolet B radiation. To investigate the function of the erbB receptor family more fully, we have constructed HaCaT cell lines that overexpress either erbB1, erbB2, or erbB3. Characterization of these cells lines has demonstrated that overexpression of erbB3 increases keratinocyte proliferation and increases the sensitivity of the cells to UVB-induced apoptosis. Because the erbB3 receptor is a substrate of both the erbB1 and erbB2 receptors but has little to no tyrosine kinase activity itself, these observations imply that the phosphorylation of the erbB3 receptor plays a critical role during proliferation and the response of keratinocytes to UVB exposure.

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Calcium-induced changes in connexin expression and function in the corneal epithelium

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The genetic connexin disorder Keratitis-Ichthyosis-Deafness syndrome (KIDS) is caused by dominant missense mutations in the Cx26 gene GJB2, yet the pathomechanisms of corneal disease have not been elucidated. Therefore, we investigated the expression of Cx26 and 16 other connexin genes in normal human cornea and cultured primary corneal epithelial cells (PCEC) at different stages of differentiation. In contrast to human epidermis expressing up 10 different connexins, immunohistochemisty (IHC) of central cornea and limbus revealed 3 major connexins, Cx26, Cx30 and Cx43. The latter two proteins showed overlapping yet distinct immunostaining in the basal and suprabasal layer, sparing superficial cells. Cx26 staining was less pronounced and mostly limited to basal cells. There was no evidence for corneal expression of other connexins. For in vitro studies, PCEC were obtained from corneal rims and cultured in either keratinocyte serum-free medium or KSFM with 0.3% fetal bovine serum and 1.8 mM Ca^{2+} to promote epithelial differentiation. Under both conditions, 9 connexin genes were expressed by RT-PCR analysis, of which Cx26, Cx30 and Cx43 were upregulated after Ca^{2+} switch. IHC in undifferentiated PCEC showed a characteristic punctate plasma membrane staining for Cx26 and Cx43. Nevertheless, cells were poorly coupled by gap junctions, yielding an average dye transfer to 0.86 ± 1.0 cells. A switch in Ca^{2+} concentration resulted in formation of epithelial sheets, strong upregulation of Cx26 and Cx43, and induction of Cx30 staining as evident by IHC. Gap junction-mediated dye transfer was significantly increased with average dye spread to 5.72 ± 3.1 cells ($p<0.00001$). Based on these observations, we speculate that KIDS mutations in Cx26 directly or indirectly interfere with the function of co-expressed Cx30, Cx43, or both. A resulting functional impairment might lead to perpetually induced expression of Cx26 as compensatory mechanism, thus producing the progressive corneal pathology in KIDS.

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Differential reactivity of basal and suprabasal keratinocytes to H2O2-induced apoptosis in normal human epidermis: an *ex vivo* immunohistological study

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Apoptosis is a physiologic programmed cell death (PCD) that occurs throughout the epidermis. It contributes to epidermal protection and maintenance by removing damaged or unwanted cells avoiding inflammation. Recent evidences suggest that cell susceptibly to apoptosis is dependant to their differentiation state. To explore this hypothesis we carried out an ex vivo immunohistological study of H2O2-induced apoptosis in normal human skin by confocal microscopy. Apoptotic cells were characterized by two different immunohistological staining of skin cryosections after oxidative stress exposure of the biopsies. Specific antibodies were used to detect, active caspase-3 and single strands break DNA. To characterize differentiation state of apoptotic cells in epidermis, double staining were performed to correlate DNA fragmentation, or active caspase-3 versus beta1-integrin expression, a marker of basal cells. During the time course of H2O2 induction, apoptotic cells detected by both methods appeared in a dose dependant manner 24 hours after oxidative stress exposure (0,5 to 5 mM H2O2) and greatly enhanced at 48 hours. In the germinative layer and the next upper layer of the epidermis, a significant caspase-3 activation occurred without DNA fragmentation (even after 48 hours of H2O2 exposure). Interestingly, single strands break DNA was exclusively restricted to the most suprabasal layers (essentially granular layers), without activation of caspase-3. We conclude that human keratinocytes possess differential reactivity to H2O2-induced apoptosis depending on their differentiation state and that this sensitivity could be partially explained by activation of different pathways. On the other hand, in basal cells, caspase-3 activation does not seem to induce cell death.

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TIG3 - a novel regulator of keratinocyte differentiation

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We have identified TIG3 as a novel regulatory protein that reduces keratinocyte viability and proliferation. We recently showed that the TIG3-mediated reduction in viability is associated with activation of type I transglutaminase (TG1) and increased cornified envelope formation (Sturniolo et. al., JBC 278, 48066-48073). A key question is whether TG1 activation is required for the biological response and whether TIG3 interacts directly with TG1. In the present study we show that TIG3 forms a complex with TG1 and that this interaction is associated with TG1 activation. Moreover, the reduction in cell viability is specific for TG1 activation, as inhibition of TG1 activity with monodansylcadaverine prevents the TIG3-associated cellular responses, and a TIG3 mutant that does not interact with TG1 does not increase TG1 activity or produce a biological response. An important finding is that these TIG3-dependent changes occur in the absence of elevated extracellular calcium levels, suggesting that TIG3 alters the TG1 requirement for calcium. This is the first evidence that an agent can activate TG1 in the absence of increased extracellular calcium. TIG3 also serves as a substrate for TG1, as demonstrated by the TIG3-dependent covalent attachment of the TG1 substrate, biotin-x-cadaverine, to TIG3, and by the formation of high molecular weight TIG3-containing complexes. Microscopic localization and isopeptide crosslinking studies suggest that TIG3 and TG1 co-localize in membranes. In contrast, markers of apoptosis, including caspases and PARP, are not activated by TIG3, and caspase inhibitors do not stop the TIG3-dependent biological response. The TIG3-dependent formation of cornified envelope-like structures suggests that TIG3 is an activator of terminal keratinocyte differentiation. Our studies suggest that TIG3 may serve to facilitate the terminal steps in keratinocyte differentiation by activating type I transglutaminase.

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The unique linear migration of human keratinocytes on collagen is driven by an autocrine mechanism

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We have previously shown that the unique feature of human keratinocyte (HK) migration is the linearity of its migration tracks in response to human serum or bovine pituitary extract (BPE) even in the absence of a gradient. In contrast, other types of skin cells, such as dermal fibroblasts, melanocytes, melanomas and microvascular endothelial cells, do not show similar migratory responses to the same stimuli (Chen, Helmold, Kim, Wynn and Woodley, Clinical and Laboratory Investigation, 188:6-12, 1993). We postulated that the factor(s) that causes the linear migration of HKs is not from serum or BPE, but is secreted product from the migratory HKs themselves. We investigated this hypothesis using serum-free conditioned media from HKs (HKCM), prepared from HKs cultured in serum-free media. Our results showed that the HKCM stimulated linear migration of HKs and, surprisingly, all other cell types tested. These included dermal fibroblasts, melanocytes and melanoma cells. More intriguingly, HKCM stimulated migration of these cells even stronger than their published stimuli. The CM, prepared from cycloheximide-pre-treated HKs, completely lacked the linear migration-promoting activity. However, GM6001, MMP Inhibitor III and TIMP-1 had no inhibitory effects on HKCM-stimulated linear migration, indicating that the linear migration-promoting factor is not related to MMPs. Human Cytokine Array analyses (RayBiotech, Inc) suggested that the linear migration-promoting factor in HKCM is not among the previously reported pro-motility factors for HKs, dermal fibroblasts or melanocytes. This study indicates that the unique linear migration by HKs is driven by an autocrine mechanism. This linear migration-promoting factor from HKs may play an important role in skin wound re-epithelialization.

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Ceramide glucosyltransferase expression is regulated by a MAPK pathway involving non-classical PKC isoforms, PI3K, MEK1/2, and ERK

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Ceramide glucosyltransferase (CGT) is required for synthesis of the glucosylceramide (GlcCer) precursors of ceramides, the dominant lipids of the epidermal permeability barrier. We previously reported that GlcCer content and CGT activity are induced during keratinocyte culture differentiation, and that expression of CGT mRNA, protein and activity in low calcium (KGM)-grown keratinocytes is induced by phorbol ester (PMA)-mediated stimulation of protein kinase C (PKC). We have now studied the PKC isoform specificity and tested for involvement of a mitogen-activated protein kinase (MAPK) pathway. Basal or PMA-induced CGT expression in KGM-grown human foreskin keratinocyte cultures was not affected by calcium ionophores or by raising the calcium concentration of the medium, and the PKC alpha, beta, gamma selective inhibitor, Go 6976, did not inhibit PMA induction of CGT mRNA, suggesting that the calcium-dependent classical PKC isoforms are not involved. CGT mRNA induction was suppressed by Go 6983, which inhibits novel, nPKCs and the atypical, aPKC zeta isoform, and by a pseudosubstrate inhibitor of PKC zeta, but not one specific for nPKC eta. High concentrations of rottlerin were required to inhibit CGT mRNA induction, suggesting that nPKC delta may have a lesser role than other isoforms. PMA-induced CGT mRNA was blocked by the MEK1/2 inhibitor, PD98059 and by the phosphatidylinositol 3-kinase (PI3K) inhibitor, wortmannin, but not by the Raf-1 inhibitor, GW5074. Induction was blocked by peptide inhibitors of ERK activation, but weakly inhibited, or activated, by compounds that block p38 (SB203580, SB202190) and JNK (SP600125, JNK inhibitory peptides) activation. The p38/JNK activator, anisomycin, blocked PMA-induced CGT mRNA, in agreement with recent evidence for antagonism of keratinocyte ERK function by p38. Our results indicate that induction of CGT mRNA by PMA is mediated by a MAPK pathway that does not include Raf-1, and involves novel, and possibly atypical, PKC isoforms, PI3K, MEK1/2, and ERK.

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Transcriptional profiling of aging in photoprotected skin

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Relatively little data quantitative data exists on the molecular biology of human aging. Photoprotected skin can provide a window into aging. Prior studies on human aging have used cell-lines, which can miss in-vivo cell-cell interactions, and which tend to be selected for ability to amplify in culture. A case-control design was used to compare gene expression among elderly (case) and young (control) subjects to identify previously unidentified genes displaying differential expression between young and old photoprotected skin. Subjects were healthy caucasian males between 20 and 25 years of age (10 younger subjects), or healthy caucasian males over 80 years of age (10 older subjects). Subjects were excluded for significant skin disease requiring dermatologic treatment, history of systemic chemotherapy, or other history of chronic disease. To retain near in vivo profiles, specimens of buttock skin were immersed in liquid nitrogen within 60 seconds of removal. Specimens remained at -170 until RNA was isolated. Labeled RNA was annealed to Affymetrix U133 microarrays which can assay 22,000 gene transcripts. 10 subjects (5 younger and 5 older) were initially analyzed to generate a list of genes showing adequate expression, fold-change, and p-values. Sample classes were iteratively randomized to generate nominal p-values. This list was then validated with 10 additional subjects (5 younger and 5 older). 12 differentially expressed genes included: (1) genes known to be associated with skin-aging (e.g. trichohyalin), (2) previously known aging-associated genes not known to be involved in aging of skin (e.g. thrombospondin 2), and (3) genes previously not associated with aging (e.g. calgranulin A). Absent photodamage, there are still robust transcriptional differences between young and old skin.

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Characterization of new buccal and gingival epithelial tissue models

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Three-dimensional models of the human oral epithelia, exhibiting a buccal or gingival phenotype have been developed using normal human oral epithelial cells cultured in serum free medium. Two versions of buccal tissue contain 8-12 and 25-35 cell layers (designated B12 and B35, respectively) with cells becoming increasingly squamous toward the apical surface have been developed. No evidence of cornification is present in histological slides and immuno-staining shows the expression of cytokeratin K13 in the suprabasal layers. Cytokeratin K4, the expression partner of K13, has also been detected by immuno-blotting. These features are characteristic of buccal epithelium. The gingival tissue (G13) has 9-13 layers of viable, nucleated cells and is partially cornified at the apical surface. When exposed to the surfactant Triton X-100, an exposure time of 52+/-20 minutes (n=31) reduces the viability of B12 to 50% as determined by an MTT assay. For G13 and B35, a similar exposure of >8 hours is required to damage the tissue to the same extent. Lipid analysis of B12 revealed that, of the ceramides important in the barrier of epidermis, only ceramide 2 (C2) was present, a result which matches that of human buccal tissue. B35 contained ceramides C2 and C3 in a ratio of 3.1:1 and G13 showed the presence of the three least polar ceramides, C1, C2, C3, in a ratio of 1: 8.2 : 4.5, respectively. The absolute amounts of ceramide in B12, B35, and G13 also varied significantly. Cultures contained 1.0, 4.5, and 8.2 micrograms/cm^2, respectively. However, G13 was not as cornified as human hard palate, which contains all ceramides normally present in epidermis, C1-C7. The good correspondence between these tissues and those of native oral epithelia leads us to believe the tissues will be useful for a broad variety of basic and applied oral cavity studies.

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Keratinocytes act as a source of reactive oxygen species by transferring hydrogen peroxide to melanocytes

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Hydrogen peroxide (H2O2) levels in keratinocytes and melanocytes were compared on a per cell basis and found to be significantly higher in keratinocytes. Since H2O2 is a neutral molecule capable of permeating through cellular membranes, we then investigated the possibility that H2O2 transfer might occur between these two types of cells. Because the ratio of keratinocytes to melanocytes in skin is 36:1, keratinocytes may act as a source of reactive oxygen species (ROS) even by passive diffusion and, thus, affect melanocytic functions. In order to measure H2O2 transfer, a fluorescence-based co-culture system was developed in which melanocytes were first pre-labeled with 2',7'-dichlorodihydro-fluorescein diacetate (DCFdA). Once inside the melanocyte, non-specific esterases remove the acetyl moieties leaving 2',7'-dichlorodihydrofluorescein, a reduced form of DCFdA, trapped inside the cell. Upon oxidation, fluorescence increases and can be used as a marker of oxidation specific to the melanocyte. When melanocytes were co-cultured with keratinocytes, fluorescence increased as a function of keratinocyte cell titer. Thus, for melanocytes incubated with 1-, 1.5- and 2-fold the number of keratinocytes, fluorescence increased by 22.6 % (+/- 2.8 %), 25.6 % (+/- 4.8%), and 39.9 % (+/- 4.1 %), respectively. Separating the cells with a transwell membrane did not prevent transfer which indicates that H2O2 migration is not dependent upon direct cell-to-cell contact. To further demonstrate that increased fluorescence was attributable to the transfer of H2O2, catalase was added to the media of co-cultures and observed to significantly reduce the amount of H2O2 in melanocytes. In conclusion, keratinocytes appear to be a previously unexamined source of ROS which may affect neighboring skin cells such as melanocytes and, as a result, may influence the process of melanogenesis or contribute to the progression of vitiliginous lesions.

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Analyses of sebosuppressive activities of phosphatidylcholine and its hydrogenated derivative: a possible molecular mechanism involved by PPARγ

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Overproduction of sebum is one of the primary causes of acne development. Therefore attempts to reduce sebum production are logical approaches for acne treatment. Previous studies showed that phosphatidylcholine (PC) was effective in the treatment of acne vulgaris. The high content of linoleic acids in PC was believed to help alleviate acne. Peroxisome proliferator-activated receptor g (PPARg) is a member of ligand-dependent transcription factors. Linoleic acid is a known ligand and activator of PPARg. PPARg has recently been found to specifically affect sebocyte growth and differentiation and highly expressed in adipose tissue. It was previously suggested that PPARg antagonist would seem possible to interfere sebum production without side effects. We have examined several phospholipids for their sebosuppressive activities and found that hydrogenated PC (HPC) showed strongest sebosuppressive activity followed by PC using a fuzzy rat model. HPTLC analyses of the rat skin lipid revealed that total lipid and wax esters were reduced by 45% and 35% respectively from the HPC treated group. The number of sebaceous gland counted in a same area under light microscopy was decreased by 45-55%, which indicated the decrease of volume of sebaceous gland after treatment. To observe the effects of HPC in human, 0.5% of HPC cream and placebo cream was applied to the forehead of 15 volunteers (10 males and 5 females, average age 27) for 6 weeks and the sebum was extracted using a bentonite clay method. Over 20% reduction of the sebum was observed compared to that of the control skin. The expression of PPARg expression was markedly suppressed in HPC treated skin, while a significant stimulation of PPARg was observed in the PC treated skin.

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Epidermal protein expression in the migrating tongues of normal human incisional wounds, excisional wounds and diabetic ulcer margins

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The purpose of this study was to compare protein expression on the migrating tongue of incisional and excisional wounds, as well as diabetic ulcer margin. Narrow incisional wounds close by 3 days, 3 mm excisional punch wounds close after 7 days and diabetic ulcers may take months to achieve wound closure. We were interested in studying keratinocyte (KC) activation, differentiation and proliferation markers as a function of time to epidermal closure and distance of epithelial migration. Partial-thickness incisional human wounds (narrow normal) were created using a Simplate-II bleeding time device. Excisional human wounds (wide normal) were created using a 3 mm punch biopsy. Tissue samples were taken from the margin of diabetic ulcers of patients scheduled to undergo leg amputation. All samples were frozen in O.C.T. for immunohistochemistry. Differences in protein staining pattern were observed among all three types of wounds. K16 a keratin associated with KC activation, showed dramatic immunostaining in all wounds studied and appeared to excessively stain the entire epidermis of the ulcer margin. The adhesion molecule, integrin $\alpha3\beta1$ also demonstrated excessive immunostaining in diabetic ulcers compared with normal wounds. Keratins K10 and K2e, markers for epidermal differentiation, were present on the migrating epithelial tongue of normal narrow wounds but lost in favor of K16 in wide normal wounds and diabetic ulcers. The proliferation marker (Ki67) showed that the epithelium at the diabetic ulcer margins was actively proliferating even though the KCs were not migrating. Despite the expression of proteins associated with activation, migration and proliferation, the KCs of diabetic ulcers often fail to migrate.

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Salicylic acid induced loss of the epidermal calcium gradient without skin barrier impairment and increased the expression of epidermal cytokines

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In a previous study, we reported that topical treatment of alpha-hydroxy acids (AHAs), such as glycolic acid, did not induce any significant increase in transepidermal water loss. However, topical glycolic acid induced the loss of epidermal calcium gradient and initiated barrier repair process, such as lamellar body secretion, without epidermal barrier impairment. An in vitro study using cultured keratinocytes suggested that glycolic acid could lower the calcium ion concentration, at least in part, through the chelating effects of the glycolic acid on the cationic ions. Salicylic acid, with its structural similarity with glycolic acid, can be hypothesized as a chelating agent for calcium ions in epidermis. In a previous study, we also reported that topical application of salicylic acid did not induce any significant increase in TEWL. In this study, we examined the secretion of lamellar body and epidermal calcium gradient in vivo. We also examined the effects of salicylic acid on the intracellular calcium ion concentration in response to extracellular calcium ion concentration changes in the cultured mouse keratinocyte. In addition, protein and mRNA expression of epidermal cytokines after salicylic acid treatment were measured. These results suggest that salicylic acid, along with glycolic acid, can induce the loss of epidermal calcium gradient and initiated barrier repair processes, such as lamellar body secretion, without epidermal barrier impairment, at least in part, through the chelating effect of the salicylic acid on the calcium ions. As a conclusion, topical salicylic acid as well as glycolic acid could be used to improve the epidermal permeability barrier.

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The aryl hydrocarbon receptor nuclear translocator plays an essential role in epidermal barrier formation in mouse skin

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Arnt is a member of the basic helix-loop-helix PER-ARNT-SIM (PAS) family of proteins which serve as dimeric transcription factors. Among PAS proteins, Arnt is a central dimerization partner playing an essential role in mediation of diverse biological functions including xenobiotic metabolism, hypoxic response, and circadian rhythm. Ablation of Arnt activity in mice results in early embryonic death. Despite the fact that several molecular targets of Arnt-mediated regulatory pathway are key elements of skin pathological conditions in humans and most of Arnt dimerization partners are known to be involved in control of skin homeostasis, still very little is known about possible Arnt functions in skin. As an initial step in elucidation the functions of Arnt in skin, we generated Arnt deficient mice using conditional gene targeting strategy. By crossbreeding of Arnt floxed and Cre transgenic mice carrying Cre transgene under skin-specific Keratin 14 promoter we achieved disruption of Arnt gene in keratinocyte-specific manner. Keratinocyte-specific ablation of the Arnt resulted in spatially-specific impairment of epidermal barrier formation during late embryogenesis which matched spatial patterns of Arnt expression in mouse skin. In addition, while being alive and active during first hours of their live, Arnt k/o newborns were not able to feed. As a result of extensive dehydration and rapid loss of weight, newborns with skin-specific ablation of the Arnt gene die in 12-35 hours after birth. The histological analysis of skin in Arnt-deficient and control newborns revealed no significant difference in hair follicle morphology. At the same time, structure of epidermis in newborns with Arnt deficiency in the skin showed prominent alterations. Thus, generation of mice with skin-specific ablation of Arnt gene elucidates an essential role of Arnt-dependent regulatory pathway in control of skin barrier functions and provides a useful model to study other aspects of Arnt activity in skin.

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The transcription factors c-rel and relA control epidermal development and homeostasis in embryonic and adult skin via distinct mechanisms

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Determining the roles of Rel/NF-kB transcription factors in mouse skin development using loss of function mutants has been limited by redundancy amongst these proteins and by embryonic lethality associated with the absence of RelA. Utilizing mice lacking RelA and c-Rel, which survive throughout embryogenesis on a TNF-a deficient background (rela-/-c-rel-/-tnfa-/-), we show that c-Rel and RelA are required for normal epidermal development. Although mutant fetuses fail to form tylotrich hair and have a thinner epidermis, mutant keratinocyte progenitors undergo terminal differentiation to form an outer cornified layer. Mutant basal keratinocytes are abnormally small, exhibit a delay in G1 progression and fail to form keratinocyte colonies in culture. In contrast to the reduced proliferation of mutant keratinocytes during embryogenesis, skin grafting experiments revealed the mutant epidermis develops a TNF-a dependent hyper-proliferative condition. Collectively, our findings indicate that RelA and c-Rel control the development of the epidermis and associated appendages during embryogenesis and regulate epidermal homeostasis in a post-natal environment through the suppression of innate immune mediated inflammation.

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Characterization of a novel mouse calmodulin-like protein

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Epidermal differentiation is tightly controlled by a calcium gradient, which increases towards the surface of the skin. During differentiation, keratinocytes up-regulate specific proteins including calcium-binding proteins, such as the calmodulin-like protein (CLP). Human CLP was shown to share 85% identity with the ubiquitously expressed calmodulin. Unlike calmodulin, human CLP appears to be expressed specifically in epithelial cells with intense staining in the skin, increasing during keratinocyte differentiation. These studies by M.S. Rogers et al (2001) suggest that CLP may be involved in the program of keratinocyte differentiation. To further explore the role of calcium signaling during this process, we have cloned a potential mouse homologue of human CLP. Mouse CLP shares 89% identity with human CLP. This intronless gene encodes a protein with 149 amino acids, containing four predicted calcium-binding EF-hand motifs, a molecular weight of 16.7kDa, and a pI of 3.9. RNase Protection Assays (RPA) and reverse transcription polymerase chain reactions (RT-PCR) confirmed that expression of mouse CLP was confined to the epidermis and other stratified epithelia, e.g. tongue, esophagus and forestomach. Consistent with observations that human CLP was expressed at an early stage of differentiation, the onset of mouse CLP expression occurred at embryonic day 15.5. In situ hybridization studies demonstrated restricted expression of mouse CLP in the suprabasal layers of the epidermis. This preliminary data suggests mouse CLP may be involved in the process of terminal differentiation. Future interaction studies and mouse models may further distinguish this protein from calmodulin and help to further elucidate the role of calcium signaling in skin development and differentiation.

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Calcium-fluorescence lifetime imaging in *ex vivo* skin II

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In our recent publications, we described Fluorescence Lifetime Imaging Microscopy (FLIM) to assess pH in intact epidermis. Here, we report about our recent progress in measuring and visualizing Ca2+ in ex-vivo biopsies of unfixed epidermis. In our initial Ca-FLIM studies we used Calcium Green-5N which is sensitive in the intermediate range of concentrations expected in epidermis and were able to show overall increasing Ca2+ concentrations from basal (SB) to granular (SG) layers, confirming our prior PIXE and Calcium-precipitation results. We refined and broadened this method to cover the full range of epidermal Ca2+ concentrations, from nanomolar to millimolar values. Using sequential measurements with Calcium Green-1 for low concentrations and Rhod-5N for high concentrations, we find highest Ca-concentrations to be limited to the intracellular domain in an ER-like distribution, specifically in the apical (granulosum and spinosum) layers, while in deeper (basal to dermal) layers this distinction is partially lost. In addition, FLIM demonstrates a shift in the Ca2+ distribution pattern upon barrier disruption: following tape-stripping, Rhod-5N reveals a partial loss of compartmentalization in the high concentrations range and across the depth of epidermis. In the low concentrations range using Calcium Green-1, cells with varying overall Ca-concentration appear, specifically at the SC/SG interface and partially persisting into the SC. Comparison of localized changes in Calcium concentrations and their regulation are especially important to assess functional consequences in barrier homeostasis and repair, differentiation, signaling, and cell adhesion, as well as various pathologic states, e.g., Darier Disease, Hailey-Hailey Disease, and Psoriasis. As extracellular and intracellular absolute Ca2+ concentrations in tissue are unknown, but can be assessed with FLIM, we believe that this method will contribute to elucidating basic physiology as well as pathology in epidermis.

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Netherton Syndrome: novel and recurrent mutations in SPINK5 and implications for screening and diagnosis

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Netherton Syndrome (NTS) is a congenital autosomal recessive ichthyosis often associated with atopic diathesis and caused by mutations in SPINK5 encoding the lympho-epithelial serine protease inhibitor, LEKTI. In this study, we examined the SPINK5 gene in 24 patients referred with a diagnosis of NTS and 7 patients with severe, generalized atopic dermatitis. In NTS patients, we identified 17 distinct mutations, 12 of which are novel, thus increasing the total number of distinct SPINK5 mutations to 51. Five mutations were recurrent and appeared to be more common in certain populations, which could facilitate future diagnostic testing. This is especially important, since the large number of intragenic polymorphisms, 29 in the coding sequence and 82 in flanking intronic sequences, disclosed so far, hamper mutation screening by dHPLC. In 12 of 15 patients with characteristic clinical features of NTS pathogenic mutations were detectable, while in 8 patients with uncertain clinical diagnosis only a frameshift deletion mutation and a novel missense mutation were identified. Together with our previously published data, the overall mutation detection rate in patients with NTS was 67% by direct DNA sequence analysis, suggesting that the remainder of mutations lie outside the coding sequence. Although no deleterious mutations were detected in 7 patients with atopic dermatitis, the allele frequency of Asn368 (0.428), a coding polymorphism previously associated with atopic eczema in the UK and Japan (allele frequency 0.5), was higher than among NTS patients (0.333). In addition, all patients with atopic dermatitis carried 2 to 5 other common sequence variants, including Arg267Gln, Asp386Asn, Val335Ala, Glu420Lys and Arg711Gln, further supporting that Asn368 alone or in combination with other polymorphisms could play a role in the pathogenesis of atopic dermatitis.

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Yin-Yang 1 negatively regulates the differentiation-specific expression of loricrin gene in undifferentiated keratinocytes

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Loricrin is a major component of the cornified cell envelope, a specialized structure that replaces the plasma membrane and contributes to the protective barrier function of the stratum corneum, and loricrin is expressed only in terminal differentiated keratinocytes. This cell differentiation-specific expression suggests specific suppression of the gene expression in undifferentiated keratinocytes as well as activation in differentiated keratinocytes. In an effort to locate negative regulatory sequences involved in suppression of loricrin gene expression in undifferentiated keratinocytes, we have identified a negative element in the intron 1 of loricrin gene. Deletion analysis in the intron 1 demonstrated that the region from +398 to +428 is responsible for the suppressive activity of the intron 1. Transcription factor database search identified the putative inverted binding motif for YY1 in this sequence. The point-mutated constructs with 2 bp mutation at the putative YY1-binding motif showed about 50% increase in reporter activity, indicating that YY1 negatively regulate the loricrin gene transcription in keratinocytes. Western blotting and immunohistochemical analysis indicated that YY1 was much more abundant in nuclear extracts of the cells grown in undifferentiating conditions (0.05 mM calcium) than in differentiating conditions (0.12 mM calcium). Luciferase assay demonstrated that overexpressed YY-1 suppressed the loricrin promoter activitiy. These findings suggest that YY1 contribute to specific expression of loricrin gene in differentiated keratinocytes by suppression of the gene transcription in undifferentiated keratinocytes.

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Locus heterogeneity in cutis laxa

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Cutis laxa (CL) is a heterogeneous group of disorders characterized by redundant, sagging and inelastic skin. X-linked recessive, autosomal dominant and autosomal recessive inheritance have been described for different forms of CL. The goal of our studies was to discover molecular defects in known loci and in candidate genes in an unselected cohort of CL families. Mutations in the elastin (ELN) fibulin-5 (FBLN5), lysyl oxidase (LOX) and lysyl oxidase-like (LOXL) genes were analyzed in probands from 25 CL families using denaturing high-performance liquid chromatography and direct DNA sequencing. The presence of putative disease alleles was investigated in at least 100 unrelated normal individuals and the segregation of these variants was studied in relatives of the probands. Six putative disease alleles were identified in ELN and two in FBLN5. Two of the ELN mutations segregated with CL in families in an autosomal dominant pattern. Two other ELN defects were identified in sporadic patients one of whom was shown to carry a *de novo* mutation. One additional sporadic patient carried two missense alleles in ELN and one missense allele in FBLN5. Fibroblasts from this individual and another patient carrying a FBLN5 mutation in a putative c-Myb factor-binding site showed dramatically reduced matrix deposition of fibulin-5. No molecular defects have been found in LOX and LOXL to date. We conclude that defects in both ELN and FBLN5 contribute to the molecular pathology of CL. *De novo* mutations in ELN and digenic inheritance of ELN and FBLN5 defects may cause sporadic CL. Multiple families with autosomal dominant CL lacked mutations in ELN and FBLN5 suggesting the presence of at least one additional major gene mutated in autosomal dominant CL.

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Cutis laxa caused by mutation in the tropoelastin gene

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Cutis laxa (CL) is a rare heterogeneous group of disorders characterized by loose inelastic skin that hangs in folds. Previously, a partial tandem duplication in the tropoelastin gene (ELN) was identified in a family with autosomal dominant cutis laxa (ADCL). Family members suffered from hernias and adult-onset obstructive pulmonary disease characterized by bronchioectasis and emphysema. Our goal was to identify other ADCL patients with mutations in the ELN gene and understand the underlying molecular mechanisms. Clinical evaluation of a second family with ADCL resulted in the diagnosis of inguinal hernia, thoracic aortic aneurysms and dissection with varying penetrance. Blood samples and skin biopsy was collected from family members and the ELN gene was screened for mutations by DNA sequencing and RT-PCR. Elastic (VVG) staining from the non-aneurysmal segments of the aorta from the proband showed fragmentation of the internal elastic lamina and thin, irregular elastic lamellae. Aneurysmal segments of the aorta lacked elastic fibers. Mutational analysis revealed a 25 base pair deletion in exon 30 (*2114-2138del*) potentially giving rise to a *de novo* protein at the 3 prime end of tropoelastin. Evaluation of ELN transcripts confirmed equal expression of both normal and mutant alleles. Mutant transcripts comprised 27% of the total. Moreover, the ratio of a naturally occurring splice variant lacking exons 30 and 32 increased to 13% in patient samples vs. less than 1% in normal control. We conlude that mutations in the tropoelastin gene may cause thoracic aortic aneurysms and dissections in autosomal dominant cutis laxa. The *2114-2138del* allele gives rise to the synthesis of stable mutant mRNA and alters the ratio of alternatively spliced forms or tropoelastin, therefore it may result in the synthesis of mutant tropoelastin and elastic fibers with compromised biochemical and biophysical properties.

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Novel cancer- and immortalization-related genes identified in malignant and genetically-engineered human keratinocytes using DNA microarrays

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Seeking to identify genes involved in neoplastic progression to squamous cell carcinoma (SCC), we used oligonucleotide microarrays containing coding sequences of 9896 genes to compare transcript levels among 23 human cell lines of oral and epidermal origin, including six derived from SCCs, three from premalignant dysplasias, five from normal epithelium, and versions of the latter engineered to express various proteins permitting evasion of senescence arrest mechanisms. A comparison of SCC cells and normal keratinocytes identified 64 genes with expression differences >3-fold and P values (t-test) <0.015. Quantitative PCR confirmed 30/30 genes tested from this set. The 64 genes included transcription factors, signal pathway proteins, cell cycle regulators, adhesion proteins, enzymes, and RNA-binding proteins. Interestingly, TERT-immortalized keratinocyte lines showed altered expression, compared with early passage primary cells, of 3 genes common to SCCs: increase of DHRS2 and decrease of ALEX2 and FL10097. Normal cells engineered to block p53 and either p16 or pRB function were found to overexpress 6 interferon-inducible genes, most of which were downregulated in SCCs. Using expression levels of the 64 gene SCC vs. normal set, a hierarchical cluster analysis of all cell lines grouped two premalignant lines with the SCCs, while most of the lines engineered to evade p53- and p16-/pRB-dependent mechanisms clustered with primary cells. 3 out of 5 TERT-immortalized lines clustered more closely with SCCs than with primary cells. Three dimensional clustering by principal component analysis yielded a tight grouping of SCC and premalignant cells, separate from all lines derived from normal cells. Thus, we have identified a set of genes that reliably distinguish neoplastic, immortalized, and normal keratinocytes, including candidates for previously undetected functions in neoplastic progression.

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Identification of a novel missense mutation of ATP2A2 in a Chinese family with Darier's

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The objective of this study is to detect the ATP2A2 gene mutations in Chinese Han patients with Darier's disease (DD) .DD is an autosomal dominant skin disorder characterized by multiple keratotic papules and abnormal keratinization. Mutations in the ATP2A2 gene encoding sarco/endoplasmic reticulum calcium pumping ATPase type 2 have been identified as the molecular basis of DD.The strategy for mutation search included briefly genomic DNA isolated from peripheral blood was subjected to polymerase chain reaction amplification, followed by direct automated sequecing. A novel missense mutation A→G was identified in exon 12, nucleotide 1704 of the ATP2A2 gene, which predicts to lead to the substitution of lysine by arginine at codon 514 (K514R). This mutation was not found in 7 normal individuals of this family and 100 unrelated population control and was not described in previous study. To sum up, in this study, we have reported the first three-generation of Chinese family with the co-occurrence of the severe classic "seborrheic" form of DD with neuropsychiatric disorders and identified and verified a nove missense mutations. This study will contribute to expand database on ATP2A2 in DD, and further illustrate the extensive diversity of mutational events that led to the different phenotypes of DD.

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Plectin is essential for sub-sarcolemmal-cytoskeletal interactions in striated muscle

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Epidermolysis bullosa simplex (EBS) associated with muscular dystrophy (MD, MIM#226670) is a disorder involving defects in certain isoforms of the cytoskeletal protein plectin expressed in skin and muscle. Immunoperoxidase staining was performed using previously reported EBS-MD patients skeletal muscle (n=3) together with control muscle (n=4) to determine the pathogenesis of muscular dystrophy. While plectin monoclonal antibodies 10F6 and HD1-121 both localized to the plasma membrane (PM) in control skeletal muscle, 10F6 also stained the center of type II muscle fibers. Conversely, HD1-121 stained the center of type I fibers. EBS-MD muscle demonstrated a loss of all membrane HD1-121 and 10F6 staining (in 3 cases) but retained some HD1-121 (in 1 case) and 10F6 staining (in 3 cases) within the center of selected muscle fibers. There was normal staining for dystrophin, β spectrin, vinculin and β1 integrin in all 3 cases of EBS-MD muscle. However, α actinin and desmin staining in 2 EBS-MD cases was disorganized and lacked the sharp, striated appearance of control muscle. Electron microscopy showed a widening of the space immediately beneath the PM with abnormal vacuolization and variation in the number, size and parallel organization of Z-lines in 2 cases. Postembedding immunogold electron microscopy of control muscle colocalized plectin and desmin to two main sites: filamentous bridges between Z-lines and to the inner surface of the PM, in contrast to dystrophin labeling that was restricted to focal densities on the membrane. We therefore conclude that multiple, distinct fiber-specific plectin isoforms play a role in linking cytoskeletal elements to Z-lines and more critically to the myocyte PM, analogous to epidermal hemidesmosome-cytoskeletal anchorage in skin.

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Identification of seven novel mutations in the *ADAR* gene among Chinese patients with dyschromatosis symmetrica hereditaria

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Dyschromatosis symmetrica hereditaria (DSH) is an autosomal dominant pigmentary genodermatosis characterized by hyperpigmented and hypopigmented macules of on the extremities and caused by the mutations in the RNA-Specific Adenosine Deaminase Gene (*ADAR*). Here we reported clinical and molecular findings of 6 Chinese multi-generation families and 2 sporadic patients with DSH. We found that the same mutation could lead to different phenotypes even in the same family and we did not establish a clear correlation between genotypes and phenotypes. Seven novel heterozygous mutations of *ADAR* were identified, which were c.2433_2434delAG (T811fs→841X), c.2197G>T (E733X), c.3286C>T (R1096X), c.2897G>T (C966F), c.2797C>T (Q933X), c.2375delT (L792fs→792X) and c.3202-1A>G (IVS12-1A>G) respectively. Our data add new variants to the repertoire of *ADAR* mutations in DSH.

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Refinement of dyschromatosis symmetrica hereditaria locus to a 9.4-cM interval at 1q21-q22 and a literature review of Chinese patients with this disorder

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Dyschromatosis symmetrica hereditaria (DSH) is a pigmentary genodermatosis characterized by hyperpigmented and hypopigmented macules of on the extremities, which maps to an 11.6-cM interval on chromosome 1q11-21 recently. So far, most cases of DSH have been reported in Japan and dermatologists in the world could think this disorder mainly occurs in Japan. In fact, there are 17 DSH families including 136 cases reported in China since 1980, but most of them are described in Chinese. Our purposes are to refine the previously mapped region and delineate the clinical and genetic features of Chinese DSH cases by a literature review of 136 cases reported in China. We performed genotyping and linkage analysis using polymorphic microsatellite markers at 1q11-q22 in two Chinese DSH families, and reviewed all of DSH cases reported in China since 1980. A cumulative maximum two-point lod score of 3.68 was produced with marker D1S506 at a recombination frequency of θ=0.00 in these two families. Haplotype analysis refined DSH locus to a 9.4-cM interval flanked by D1S2343 and D1S2635. This study confirms linkage of DSH to previously mapped region and refines DSH gene to a 9.4-cM interval at 1q21-q22. Likewise, the literature review indicates that DSH is not an uncommon disorder in China and the differences in the distribution of skin lesions could be related with race and environment.

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A refined locus for marie unna hereditary hypotrichosis1.1-cM: interval at 8p21.3

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The aim is to refine the MUHH locus to a narrow chromosome region and make the gene cloning feasible. We performed genotyping and two-point linkage analysis in a multi-generation Chinese family with 18 high-density polymorphic microsatellite markers spanning the previously mapped interval at 8p21. Significant evidence for linkage was observed in this region with a maximum two-point LOD score of 3.01 (θ=0) at D8S298 and D8S1725. Haplotype analysis localized the MUHH locus within the region defined by D8S282 and D8S1839. This region overlaps by 1.1cM with the previously reported MUHH region and it represents about 380-Kb physical distance. The HR gene, which is mutated in congenital atrichia, is critically close to the refined region but sequencing of the encoding region and intronic splice site revealed no causative mutations. This study provides a refined map location (1.1cM) for isolation of gene causing MUHH. These data also indicate the existence of a common MUHH locus at 8p21.3 between the Caucasian and Chinese families and suggest that MUHH and congenital atrichia could not be allelic disorders that are caused by the mutations in the HR gene and distinct molecular mechanisms underlie these two diseases.

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Molecular epidemiology of epidermolysis bullosa in Middle East populations

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Epidermolysis bullosa (EB) is a heterogeneous group of inherited mechanobullous skin disorders. Based upon ultrastructural criteria, EB is classified into simplex (EBS), junctional (JEB) and dystrophic (DEB) types, caused by mutations in 10 distinct genes. Although the genetics of these disorders has been extensively investigated in Europe and the US, little is known about the molecular epidemiology of EB in the Middle East, where EB is considered to be frequent. We established a collaborative network with the aim to investigate the clinical and molecular features of EB in the Middle East. We identified a total of 115 families diagnosed with EB over the years in 3 medical centers located in the Middle East. A total of 53 families gave their informed consent to participate to this study. Twenty-nine of those families could be unequivocally assigned to one of the major EB subtypes (48% EBS; 24% JEB; 28% DEB) based on pathological criteria. In each of these 29 families pathogenic mutations were identified, most of which are novel. In contrast with previously reported observations in studies performed in the US and Europe, we noticed: (1) An increased prevalence of recessive EBS cases; (2) An exceptionally high frequency of founder mutations; (3) A lack of correlation between location/type and phenotypic expression of K5/K14 mutations; (3) Unusual pathomechanisms including frameshift and hemizygous mutations in EBS; (4) A normal life span associated with PTC-causing mutations in laminin 5 genes in JEB; (5) No occurrence of previously reported hot spot mutations. In summary, our results suggest the existence of a unique spectrum of EB-causing mutations in Middle East populations and emphasize the need for implementation of a diagnostic strategy tailored to the specific features of EB in this region.

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Homozygosity mapping as a screening tool in the molecular diagnosis of congenital recessive ichthyoses

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Congenital recessive ichthyoses (CRI) are characterized by diffuse skin scaling and varying degrees of erythema. CRI have been mapped to at least 7 distinct chromosomal loci, significantly complicating molecular diagnostics. Unfortunately, no practical screening method prior to mutational analysis is yet available. An elevated rate of consanguineous unions underlies a high prevalence of CRI in Middle East populations. We attempted to exploit this peculiar demographic feature in order to facilitate the diagnosis of CRI in the Israeli population. We examined 63 individuals belonging to 12 consanguineous kindreds with CRI, including 19 patients. We developed screening panels comprising 57 microsatellite markers encompassing each of the 7 loci previously shown to be associated with CRI on 2q33-p35, 3p21, 12q11-q13, 14q11.2, 17p13.2-13.1, 19p12-q12, 19p13.2-13.1. Given the fact that all studied families were consanguineous, we assumed the existence of founder (homozygous) causative mutations in each kindred. We therefore searched for regions of homozygosity in each family for each of the 7 CRI loci (homozygosity mapping). We identified such regions in 9 out the 12 families, and identified by mutational analysis novel TGM1 mutations in those families that mapped to 14q11.2. Genotyping yielded uninformative results or ruled out linkage for the 3 remaining families, raising the possibility of additional CRI-associated loci. Results from a complete screen in one family were obtained in less than 3 days. In sum, homozygosity mapping represents an efficient, unexpensive and rapid screening diagnostic approach in consanguineous families with CRI; it can be used to direct subsequent mutational analysis as well as serve in prenatal diagnosis. It is amenable to semi-automation and could be applicable to additional recessive disorders characterized by genetic heterogeneity. The present study emphasizes the usefulness in human genetics of diagnostic strategies tailored to the demographic features of target populations.

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Sequence analysis of PSORS1 candidate genes: Only HLA-C and CDSN encode proteins unique to risk haplotypes

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Previous studies have narrowed the critical interval containing PSORS1, the psoriasis susceptibility locus on chromosome 6p21.3, to a ~200 kb region containing HLA-C and seven other genes (OTF3, TCF19, HCR, SPR1, SEEK1, STG and CDSN). In an effort to identify the PSORS1 gene, we have cloned and sequenced this region from ten chromosomes derived from five individuals, representing 2 risk and 8 non-risk haplotypes. In the expressed regions of these genes, two missense SNPs and one silent SNP in exon 2 of HLA-C are unique to risk among the 10 sequenced haplotypes. OTF3, TCF19, HCR genes do not have any variations unique to risk haplotypes. SPR1 did not have any protein alleles unique to risk, but has four different pair-wise combinations of mRNA variants unique to risk. All but one of these pairs involves untranslated regions (UTR) only. SEEK1 also does not have protein alleles unique to risk, but has one pairwise combination and four 4-way combinations of mRNA variants that are unique to risk. The pair-wise combination involves only UTR variations while the 4-way combinations involve missense mutations also. Two of the 4-way combinations shorten the predicted protein by 111 amino acids. CDSN has five different combinations of missense SNPs that are unique to risk, one of which is the previously reported TTC allele. The STG gene sequence differs between the two risk haplotypes (Cw6-B50 and Cw6-B57). There are two repeat units of an 11 amino acid segment on the Cw6-B50 haplotype and three units on the Cw6-B57 haplotype. Further analysis showed that both alleles are found on other risk and non-risk haplotypes, and is unlikely to be causal in psoriasis. Thus HLA-C and CDSN are the only genes that have predicted protein alleles unique to psoriasis risk haplotypes. The polymorphisms in these genes are being examined in a large cohort of approximately 3,000 individuals to discern the contribution of either or both genes to psoriasis susceptibility.

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Molecular chaperones involved in vitamin D receptor-mediated transcription in keratinocytes

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Vitamin D, via its active metabolite 1,25(OH)2D3, controls the proliferation and differentiation of keratinocytes through the vitamin D receptor (VDR) by directly regulating gene transcription. Using GST-VDR affinity beads, VDR binding protein complexes were purified from keratinocytes and identified by mass spectrometry (MS). As reported previously, the VDR binding complex from proliferating keratinocytes contained the coactivator DRIP205, whereas that from more differentiated cells included the coactivators SRC2 and 3. In addition to DRIP205, chaperone proteins were identified in the complex from prolifeating keratinocytes. Chaperone proteins are generally known to maintain the stability of nuclear receptors in cytoplasm, although their role in transcription is not fully elucidated. In these studies we evaluated the role of chaperone proteins in VDR stimulated transcription. Geldanamycin, which inhibits chaperone function by blocking ATP binding to heat shock protein 90 (Hsp90), was utilized. Effects on VDR transactivation were examined using a promoter reporter assay with constructs containing a vitamin D response element (VDRE). Inhibition of chaperone function by geldanamycin increased 1,25(OH)2D3 stimulated transactivation in a dose dependent manner, although the basal level of transcription was decreased. Direct binding of chaperone proteins to the VDRE was examined using chromatin immuno-precipitation. In response to 1,25(OH)2D3 stimulation, Hsp90 was recruited to VDRE sites in the 24 hydroxylase and keratin 1 promoters. These results suggest that chaperone proteins are involved in VDR mediated transcription presumably by mediating active assembly and disassembly of VDR-coactivator complexes on specific promoter sites. However their continued presence may block transcription following the assembly of the complex on the VDRE.

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Identification of differentially-expressed genes in atopic dermatitis

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Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterized by typically distributed eczematous skin lesions with lichenification, pruritic excoriations, severely dry skin, and a wide variety of pathophysiologic aspects. Many complicated factors, both genetic and environmental ones, increase the risk to manifest this disease. A lot of studies have been done to this diseases focusing on cytokine and T cell, however, little is known about molecular mechanism contributing to AD. To gain more insight on molecular event in AD, we used suppression subtractive hybridization (SSH). RNA was extracted from skin biopsy specimens from 8 AD patients as the tester against RNA from normal skin tissues as the driver. We obtained about 1000 clones from this experiment and selected 150 cDNA clones through dot blot analysis and sequenced them. Although these genes could be classified into named genes and unnamed genes, we chose several genes that seemed to be related with AD and confirmed their expression levels by RT-PCR analysis. These differentially expressed genes may provide significant insight for further understanding of genetic factors of AD.

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Spectrum of mutations in the *KIND1* gene, encoding the focal contact protein KINDLIN-1, in Kindler syndrome

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Kindler syndrome (OMIM 173650) is an uncommon autosomal recessive genodermatosis clinically characterised by initial trauma-induced blistering followed by variable photosensitivity, poikiloderma and an increased risk of mucocutaneous malignancy. The disorder was recently mapped to 20p12.3 and a few pathogenic mutations have been identified in a new gene, *KIND1*, which encodes the protein kindlin-1, a component of focal contacts in keratinocytes. In this study, we sequenced genomic DNA from 16 further affected individuals of various ethnic backgrounds (13 families) to expand the spectrum of pathogenic *KIND1* mutations. We identified 4 recurrent ancestral mutations: Pakistani (676insC), UK Caucasian (E304X), Omani (W616X) and Italian (958-1G>A). Haplotype analysis, using 9 intragenic polymorphisms, demonstrated common ancestral mutant alleles for each mutation, apart from one of the 6 Pakistani families in which the mutation 676insC (which occurs in a repeat of 7 cytosines) was present on a different genetic background. All mutations were homozygous, aside from the 3 UK Caucasian cases that were all compound heterozygotes (second allele mutations: L302X, 1161delA, 1909delA). Immunolabeling of skin, using a carboxyl-terminus anti-kindlin-1 antibody, showed complete absence or markedly reduced staining, which in normal skin was present at the dermal-epidermal junction and within the lower epidermis. Seventeen different loss-of-function *KIND1* mutations have now been elucidated and collectively they demonstrate the importance of kindlin-1 in maintaining epithelial integrity. Delineation of these new recurrent mutations is also relevant to optimizing mutation detection strategies in Kindler syndrome patients. Furthermore, the new anti-kindlin-1 antibody is a useful probe in establishing an accurate diagnosis of this syndrome.

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Defining a role for hairless by analytical comparison of differential gene expression

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Mutations in the hairless (hr) gene in mouse and humans results in the loss of hair and the integrity of the hair follicle. The hr protein has been suggested to act as a transcription factor with a critical role during catagen. hr is transcriptionally regulated by thyroid hormone in the brain, however, recent data from our laboratory indicates that it is not the major regulator of hr expression in the skin. Mouse models of other nuclear receptors such as knockouts of VDR and RXR suggest a possible regulatory pathway for hr, however, we have not found an interaction between VDR and RXR using multiple biochemical approaches. In order to better understand the function of hr, we took a global, data driven approach toward determining hairless target genes and the possible pathway(s) hr functions. We used Affymetrix microarrays to compare the differential gene expression profiles of wild type mouse day 2 epidermis and rhino (rhhr-/-) mouse day 2 epidermis. Using MAS5 and IBM Genes@Work software for supervised analysis, stringent parameters of two-fold or greater statistically significant values resulted in a list of 58 differentially expressed genes. 51 of these genes were found to be up-regulated by hr, while 7 genes were found to be down-regulated by hr. hr itself was found to be differentially regulated between these two populations, serving as an internal control. These genes can be organized into 9 main categories: extracellular matrix (12), cytoskeletal organization (4), cell cycle (3), signaling (4), proteolysis and peptidolysis (5), metabolism (4), transcriptional and translational regulation (5), unknown cDNA clones (5) and other (16). Validation of the array has been performed using Real-Time PCR, in situ hybridization, and immunoflourescence, confirming the expression of these genes in murine neonate epidermis. Using pathway building software, these differentially genes were analyzed for possible pathway or regulatory relationships to one another as well as to hr in order to place hr in the context of a regulatory pathway.

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Pathogenesis of hidrotic ectodermal dysplasia: the G11R mutation of Cx30 increases extracellular ATP levels due to abnormal hemichannel permeability

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The aim of this study was to analyse the functional consequences of the G11R mutation of human connexin30 (Cx30), which causes HED (MIM129500), a rare dominant genodermatosis. HeLa cells were transfected with plasmids driving the expression of the wild type (Cx30wt) and the G11R form (Cx30G11R) of the protein, either separately or in combination. The cellular localisation of both proteins was assessed by immunostaining and the dye transfer capacities of the resulting intercellular channels were studied by microinjection of Lucifer Yellow. We tested the capacity of Cx30wt and Cx30G11R to form connexin hemichannels, by measuring the extracellular ATP levels following transfection. Cells transfected with Cx30G11R alone showed an even distribution of the protein within the cytoplasm, with no obvious localisation at the plasma membrane. In contrast, when co-transfected with Cx30wt, Cx30G11R was expressed at the membrane interface between adjacent cells. Microinjection studies showed that the permeability to Lucifer Yellow of Cx30G11R intercellular channels was comparable to that of Cx30wt. These coupling characteristics were not modified in cells expressing both wild type and mutated forms of the protein. We also demonstrate that HeLa cells transfected with Cx30G11R either alone or in combination with Cx30wt formed functional connexin hemichannels, that induced ATP release into the extracellular medium, a finding not observed in cells expressing only Cx30wt. These results suggest that the epidermal manifestations of the G11R mutation are not due to loss of function of Cx30 intercellular channels between affected keratinocytes, but rather to an abnormal permeability or regulation of Cx30 hemichannels. Indeed, increased levels of extracellular ATP induce keratinocyte proliferation, which might explain the hyperkeratosis observed in the epidermis of HED patients.

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Hairless contains a novel nuclear matrix targeting signa and colocalizes with histone deacetylase 3 in nuclear speckles

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Hair follicle cycling is a highly regulated and dynamic cellular process consisting of phases of growth, regression and quiescence. The hairless (hr) gene encodes a nuclear factor that is highly expressed in the skin, where it appears to be an essential regulator during the regression in the catagen hair follicle. In hairless mice, as well as humans with congenital atrichia, the absence of hr protein initiates a premature and abnormal catagen due defects in the signaling required for hair follicle remodeling. Here, we report that hr protein is a nuclear protein that is tightly associated with the nuclear matrix scaffold. Using a series of deletion constructs of the mouse hr gene, we monitored the subcellular localization of the recombinant protein by in situ immunolocalization and biochemical fractionation after nuclear matrix extraction of transiently transfected cells. We identified a novel nuclear matrix-targeting signal (NMTS) in the hr protein and mapped the domain to amino acid residues 111 to 186 of the mouse hr sequence. Furthermore, we provide evidence that this region not only mediates the interaction of hr with components of the nuclear architecture, but also specifies the subnuclear location of the hr protein to nuclear domains containing deacetylase activity. The N-terminal region directs hr to a speckled nuclear pattern that co-localizes with the histone deacetylase 3 (HDAC), but not with HDAC1 or HDAC7. Based on our findings, we propose that hr protein is part of a specific multi-protein repressor complex that may be involved in chromatin remodeling.

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Injection of recombinant human type VII collagen restores type VII collagen expression and function in dystrophic epidermolysis bullosa *in vivo*

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Dystrophic epidermolysis bullosa (DEB) is a family of inherited mechano-bullous disorders caused by mutations in the type VII collagen gene. The lack of effective treatment for DEB provides an impetus to develop gene therapy strategies. Prior approaches to gene therapy included the stable integration of human type VII collagen sequences using both viral- and nonviral-based gene transfers. To develop a more simplified approach for the treatment of DEB, we evaluated protein-based therapy. In this study, we intradermally injected purified human recombinant type VII collagen into either immunodeficient or immunocompetent mice and then assessed whether the injected human protein would incorporate into the mouse basement membrane zone (BMZ). Skin biopsies were obtained every week after injection and subjected to immunostaining and immunoelectron microscopy using antibody specific for human type VII collagen. We found that the injected human type VII collagen transported and incorporated into the mouse BMZ and formed anchoring fibrils (AFs). The human type VII collagen and AFs were stably retained for at least 2 months (the last time point examined at this time) after a single intradermal injection. Further, intradermal injection of recombinant type VII collagen into intact RDEB skin transplanted onto immunodeficient mice also stably restored correctly localized type VII collagen expression at the BMZ. The restoration of type VII collagen reversed the RDEB disease features, including dermal-epidermal separation and AF defects. Our studies provide the first evidence for using protein therapy to correct a skin disease due to a gene defect in a structural protein. Since dermatologists have great experience and skill injecting type I collagen into human patients for the improvement of wrinkles and photoaging, this same therapeutic method using directly injected recombinant type VII collagen may provide a practical approach for restoring type VII collagen in DEB patients.

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Cloning of the breakpoints of a *de novo* inversion of chromosome 8 inv (8)(p11.2q22) in a patient with Ambras syndrome

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Ambras syndrome (AMS) is a unique form of universal congenital hypertrichosis. In patients with this syndrome the whole body is covered with fine, long hair except for the areas in which normally no hair grows. Minor facial dysmorphism and dental anomalies, including retarded first and second dentition and absence of teeth have been reported as well. Cytogenetic abnormalities of chromosome 8 have been reported in two isolated Ambras syndrome patients. The presence of a common breakpoint in 8q22 in both of these cases suggests the presence of a candidate gene(s) in this region of chromosome 8. In order to precisely determine the nature of the rearrangement in a case of Ambras syndrome, we have used Fluorescent In Situ Hybridisation (FISH) with YAC and BAC clones from 8q22-q24. We have generated a detailed physical map across the inversion breakpoint interval, cloned and sequenced inversion breakpoints in this patient, and identified transcripts in the vicinity of the breakpoints. Genomic sequence analysis of the breakpoint interval revealed that the inversion does not disrupt a gene and suggests that the phenotype is caused by a position effect. Interestingly, the breakpoints occurred within a Tigger1 element on the 8p arm, and the LINE1 element on the q arm. We are currently analysing expression of the genes that map in the vicinity of the inversion breakpoints. Identification of the AMS gene will provide new insights into the control of hair growth and cycling.

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Intradermal injection of lentiviral vectors corrects regenerated human dystrophic epidermolysis bullosa skin tissue *in vivo*

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Dystrophic epidermolysis bullosa (DEB) is an incurable blistering skin disease caused by mutations in the type VII collagen (COL VII) gene. These patients lack COL VII and anchoring fibrils, structures that attach the epidermis and dermis. An ex vivo approach for DEB requires surgical transplantation of gene-corrected cells with potential pain and scarring at the graft sites. Direct vector administration into skin would permit corrective and long-term gene transfer without the complications associated with ex vivo therapy. We previously developed a lentiviral vector expressing the full-length COL VII. In this study, we assessed the ability of lentiviral vectors to achieve COL VII gene transfer in vivo using a human skin tissue grafted onto immunodeficient mice. We generated and intradermally injected vesicular stomatitis virus envelop G pseudotyped lentiviral vectors encoding COL VII transgene into the regenerated DEB skin tissue grafted onto immunodeficient mice. Skin biopsies were obtained from grafts every week after injection and subjected to immunostaining and immunoelectron microscopy using an antibody specific for human COL VII. The intradermal injection of lentiviral vectors into RDEB skin corrected the histological subepidermal blistering and restored correctly localized COL VII expression at the BMZ, a pattern identical to skin regenerated from normal control cells. Further, COL VII expression at the BMZ was sustained for at least 2 months after a single vector injection. Immunoelectron microscopy showed restored formation of anchoring fibrils at the BMZ of RDEB skin, demonstrating correction of the major ultrastructural RDEB abnormality. We conclude that intradermal injection of lentiviral vectors can stably correct the abnormal RDEB dermal-epidermal separation and restore COL VII and anchoring fibrils at the BMZ in vivo. These data indicate that efficient and long-term in vivo gene transfer into skin via direct lentiviral vector injection may open new perspectives in the treatment of DEB.

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An identical mutation in the human XPC DNA repair gene in two patients with and without neurological symptoms

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Xeroderma pigmentosum (XP) is a rare, cancer-prone genetic disease with defective DNA repair. About 20% of XP patients have neurological abnormalities. Most XP patients with neurological symptoms are in complementation groups XP-A, XP-B, XP-D or XP-G. We now describe two newly diagnosed XP-C patients with markedly different clinical features but identical genetic defects. XP21BE, a 23y/o woman with marked freckling, had multiple angiokeratomas, squamous cell carcinomas and melanomas. She had sensorineural deafness and learning disabilities which have not been seen in other XP-C patients. XP329BE, an 8 y/o boy with marked freckling, had multiple basal cell carcinomas and a normal neurological examination. Both the patients' cells were hypersensitive to killing by UV. Host cell reactivation (HCR) of a UV-treated reporter gene plasmid co-transfected with a vector expressing wild-type *XPC* cDNA assigned XP21BE and XP329BE cells to XP-C. Using a real-time quantitative RT-PCR assay, both cells expressed low levels of *XPC* message. By sequence analysis, we identified a homozygous T107G mutation in *XPC* exon 1 in XP21BE cells that abolishes the ATG initiation codon. This identical homozygous mutation was also present in XP329BE. This allele was inactive since an *XPC* cDNA expression vector containing this mutation resulted in no increase in DNA repair activity in XP-C cells in a HCR assay in comparison to the empty vector control. We will examine microsatellite markers flanking the XPC gene on chromosome 3 to determine if there is a common ancestor for these two XP patients. These results indicate that this homozygous mutation identified in the *XPC* gene greatly reduces the DNA repair function of this gene and is associated with cancer susceptibility in both patients. The neurological symptoms in XP21BE and the differences in the pattern of skin tumors may be associated with other, presently unidentified, gene modifying effects.

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Acatalasemia: identification and characterization of a novel missense mutation

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Catalase is an important protein in the context of oxidative stress, disposing of hydrogen peroxide and therefore protecting cells from oxidant damage. Non-functional enzyme in acatalasemia leads to oral ulcerations and gangrene induced by hydrogen peroxide. In most cases literature does not provide clear-cut genotype-phenotype correlation in acatalasemic patients. Therefore we analysed the catalase gene in an affected family identified by the hydrogen peroxide in vitro blood test. Most polymorphisms were excluded to be pathogenic due to the pattern of inheritance. Two polymorphisms located in the promotor region have already been described to be non-pathogenic elsewhere. The only remaining polymorphism is a heterozygous missense mutation, located at position 96 of exon 3, consisting of a C to T substitution and as a consequence replacing an Arginine by a Tryptophan. Analyzing 30 nonacatalasemic individuals we could not detect this missense mutation. As the C to T substitution resulted in a tggt resembling a splice site sequence, exons 3 and 4 of the cDNA were cloned and analyzed for splicing variants. This analysis excluded a splice variation. Therefore, it came to our surprise that in the absence of stop codons and splicing variations we found a significant reduction of catalase-mRNA, suggesting instability of the RNA. Further studies will be necessary to elucidate the correlation between genotype and phenotype of mutated catalase in this family.

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A mouse model for epidermolytic hyperkeratosis that recapitulates the human disease course

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Epidermolytic hyperkeratosis (EHK) is an autosomal-dominant disorder of cornification due to point mutations in the suprabasal keratins K1 and K10. The onset of the disease phenotype is typically at birth and affected individuals present with erythroderma, blistering and peeling. Erythroderma and blistering diminish during the first year of life and hyperkeratoses develop, predominantly over the flexural areas of the extremities. We recently generated a mouse model for EHK by introducing a K10 point mutation, which is found in the majority of human cases of EHK, into the mouse germline. To overcome neonatal lethality, we established an inducible system, that allowed to induce the phenotype in a circumscribed area of the skin. Shortly after induction, erythroderma and blisters developed, resulting in scaling after the blisters had ruptured. With the onset of hair growth, scaling diminished and thick, brown hyperkeratoses started to form, predominantly in mechanical stressed areas, like the paws. These hyperkeratoses persisted, to date for over a year. Histological and immunofluoresence analysis confirmed blister formation in the suprabasal layers of the epidermis in newborn mice and vacuolization and a thickened stratum corneum in adult mice. Phenotypes of the mice closely resembled those obeserved in humans, both at the early neonatal blistering stage and later at the adult hyperkeratotic stage. This mouse model will be used to study the mechanisms that underlie the disease course in EHK and other genodermatoses.

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Regulation of the coproporphyrinogen oxidase gene promoter by C/EBP transcription factors: insights into enhanced ALA-photodynamic therapy

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Photodynamic therapy (PDT) is an emerging binary treatment for a number of epithelial cancers of skin and prostate. Aminolevulinic acid (ALA)-mediated PDT involves the administration of a precursor photosensitizer i.e. ALA, which is selectively accumulated and metabolized in target cells in the form of protoporphyrin IX (PPIX). The increased production of PPIX in differentiated cells is the result of increased expression of coproporphyrinogen oxidase (CPO), a heme-synthetic enzyme. Local irradiation with visible light induces tissue-specific cell death. We previously observed a strong correlation between increase in expression of CCAAT enhancer binding proteins (C/EBPs) and levels of CPO mRNA. This indicates the possibility of transcriptional regulation of CPO by C/EBPs. Sequence analysis of the DNA 1 kb upstream to the transcription start site of the CPO gene revealed 8 consensus C/EBP motifs. Nuclear extracts from COS-7 cells overexpressing C/EBP family members (LIP, LAP, LAP*, CHOP/gadd153 and C/EBPα) formed specific complexes with radiolabeled oligos on an electrophoretic mobility shift assay (EMSA). Competition, mutation and supershift assays defined the specificity of these complexes. A functional analysis was done by cloning 1 kb promoter region upstream of the luciferase (luc) gene, followed by transient transfection and reporter assays. Co-transfection of the luc-reporter with vectors expressing C/EBP family members resulted in up to 7-fold induction of the reporter. Despite considerable variation in the induction among different members of the C/EBP family, maximum induction was achieved by CHOP. A variety of agents including methotrexate induce the expression of CHOP. These results suggest that use of such agents to increase the level of CPO expression and thus PPIX prior to PDT will enhance ALA-PDT efficacy.

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Decolour pigmented rat skin by bombarding agouti signaingl protein cDNA -coated microscopic gold particles via the gene gun technique

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We developed a gene gun method for the transfer of human agouti signaling protein (ASP) cDNA to alter rat skin colour in vivo. Human ASP cDNA was cloned into a modified cytomegalovirus plasmid and delivered to the skin of Long-Evans (LE) rats by gene gun bombardment. Skin pigmentation, body weight and blood sugar of ASP cDNA transfected rats were recorded against the control group, which were injected with plasmids encoding for green fluorescent protein. The treated skin showed lighter skin colour after three days of ASP gene transfection. This depigmentation effect was most prominent on day 14 and the skin gradually returned to its original pigmentation by day 28. Successful transfection of ASP gene in skin and hair follicles, as well as downregulation of melanocortin-1 receptor (MC1R) and tyrosinase expression upon treatment was confirmed using immunohistochemistry and Western blot analysis. Body weight and blood sugar in the treated rats did not show statistically significant differences as compared to control groups. These observations demonstrate that gene transfer using gene gun method can induce high cutaneous ASP production and facilitate a switch from dark to fair colour without systemic pleiotropic effects. Such colour switch may be that ASP is acting in a paracrine fashion. In addition, this study verifies that ASP exerts its functions by acting as an independent ligand that down-regulates the melanocyte MC1R and tyrosinase protein in an in vivo system. Our result offers new, interesting insights about the effect of ASP on pigmentation, providing a novel approach to study the molecular mechanisms underlying skin melanogenesis.

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Cycloheximide regulates expression of the alternatively spliced forms of lysyl hydroxylase 2 in human kidney cells and skin fibroblasts

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The lysyl hydroxylases comprise a family of post-translational modifying enzymes in collagen biosynthesis. The hydroxylation of specific lysines is a crucial step in the synthesis of collagen crosslinks required for the integrity of connective tissue. Lysyl hydroxylase 2 (LH2) exists in two alternatively spliced forms; the major form (expressed ubiquitously) exists as a longer transcript with the inclusion of a 63bp exon (exon 13A) that is spliced out in the short form (expressed in human kidney, spleen, liver and placenta). These long and short transcripts may have specificity for hydroxylation of lysines in the telopeptide and helical collagen domains, respectively. Only the long form of LH2 is expressed in confluent human skin fibroblasts (HSF) whereas a lower cell density appears to favor the expression of the short LH2 transcript. Using semi-quantitative PCR, we have examined the regulation of this alternate splicing by cycloheximide (CHX) in human kidney cells (HEK293), in which both LH2 transcripts are expressed, and in confluent HSF. In HEK cells (in 10% FBS), CHX addition (10ug/ml) virtually eliminated the expression of the long LH2 transcript after 6h, whereas expression of the short transcript was slightly increased. Although the short form is not detected in untreated confluent HSF cells, administration of CHX to HSF (in 20% CS) resulted in preferential expression of the short LH2 transcript with a decrease in the long form. Decreasing the serum concentration to 1% in HEK and HSF did not change this result. This effect of CHX indicates that the splicing mechanism for inclusion of exon 13A in LH2 requires a newly-synthesised protein factor (that is suppressed by CHX). These regulation studies may have an important application in fibrotic diseases in light of a recent report (Van der Slot et al., JBC 2003:278:40967) showing that the long form of LH2 is over-expressed in scleroderma cells.

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Contrasting activities of the alternatively spliced forms of lysyl hydroxylase 2 may result from collagen domain-specific lysyl hydroxylation

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Lysyl hydroxylases catalyse the hydroxylation of specific lysines in collagen that are precursors for crosslink formation essential for the tensile strength of collagen. An alternatively spliced, longer form of lysyl hydroxylase2, isolated from skin, has been shown to be the major transcript of LH2 and is ubiquitously expressed. This longer transcript includes an additional 63bp exon, located between exons 13 and 14 of the originally-described shorter transcript of LH2 (isolated from kidney). To determine the function of the alternatively-spliced transcripts, we have expressed constructs for both forms as secretable recombinant enzymes in a baculoviral system. Their identity was confirmed by PCR, Northern and Western blots. Under assay conditions in which LH activity was measured by release of $^3H_20$ from a 4,5-^{3}H lysine-labeled underhydroxylated collagenous substrate, only trace activity was measured in the longer transcript whereas the short transcript had high activity comparable to that of recombinant LH1. This activity difference may be explained by a collagen domain-specific pattern of Lys hydroxylation by the alternate transcripts of LH2. If the short form of LH2 functions as a helical LH and the long form as a telopeptide LH, then based on the 15:1 ratio of available Lys hydroxylation sites in helical vs telopeptide domains of type I collagen, the relatively increased hydroxylation of a collagenous substrate by the short form is expected. The viability of these LH2 recombinants was confirmed in an alternative assay based on the hydroxylation-coupled decarboxylation of 2-oxo[1-^{14}C] glutarate using a synthetic trimer of Ile-Lys-Gly as substrate, which showed the long form of LH2 to be more active than the short form. The importance of this study is that the lysyl hydroxylation of specific collagen domains determines the pattern of collagen crosslinking which, when perturbed, can lead to fibrotic and connective tissue disorders such as Ehlers-Danlos syndrome.

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Mutations in SCN9A encoding a sodium channel alpha subunit in patients with primary erythermalgia

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Primary erythermalgia is a rare autosomal dominant disease characterized by intermittent burning pain with redness and heat in extremities. Previous study established the linkage of primary erythermalgia to a 7.94 cM interval on chromosome 2q, but the causative gene was not identified. We performed linkage analysis in a Chinese family with primary erythermalgia, and screened the mutations in the two candidate genes, SCN9A and GCA, in the family and a sporadic patient. Linkage analysis yielded a maximum lod score of 2.11 both for markers D2S2370 and D2S2330. Based on critical recombination events in 2 patients in the family, we further limited the genetic region to 5.98 cM between D2S2370 and D2S2345. We then identified two missense mutations in SCN9A in the family (T2573A) and the sporadic patient (T2543C). Our data suggest that mutations in SCN9A cause primary erythermalgia. SCN9A, encoding a voltage-gated sodium channel alpha subunit predominantly expressed in sensory and sympathetic neurons, may play an important role in nociception and vasomotor regulation.

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Compound heterozygosity for unique in-frame insertion and deletion mutations in the plectin gene in a mild case of epidermolysis bullosa with very late onset muscular dystrophy

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Epidermolysis bullosa with muscular dystrophy (EB-MD) is an autosomal recessive skin blistering disease characterized by tissue separation within the basal keratinocytes just above the hemidesmosomes. The skin blistering phenotype is accompanied by muscle weakness usually noted in childhood. A variety of mutations that result in premature termination codons in the plectin gene (PLEC1) have been identified in patients with childhood onset of EB-MD and low or undetectable expression of plectin. Plectin is a component of the hemidesmosomal inner plaque and is also expressed in the sarcolemma and the Z bands of skeletal muscle. The present patient is a 45-year-old female with a history of mild skin blistering and nail dystrophy since birth and a very recent complaint of upper extremity weakness. Staining of a skin biopsy with (HD-1) antibody revealed patchy discontinuous staining for plectin as well as focal weak staining for type XVII collagen (BPAG2) on the roof of microvesicles and within the basement membrane zone. Mutation detection performed by dHPLC separation of heteroduplexed PCR products spanning the entire coding region of PLEC1 followed by nucleotide sequencing revealed an in-frame 9-bp deletion in exon 22 (2677del9) resulting in the loss of 3 amino acids (delQEA) and a 36-bp insertion in exon 15 (1541ins36) resulting in the insertion of 12 amino acids (insVCVYPLHERLVA). Both of these mutations are in the B4 integrin binding domain within the amino terminal globular domain of the protein. The late onset of muscle weakness in the 5th decade of life and very mild skin findings in this patient can be attributed to the in-frame nature of the mutations, allowing the expression of modified yet partly functional plectin protein. In addition, the position of these mutations yields information regarding the functional significance of these protein segments in the epidermis and muscle.

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Recurrent and *de novo* dominant COL7A1 mutations in dystrophic forms of epidermolysis bullosa

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Dystrophic Epidermolysis Bullosa (DEB) is an inherited mucocutaneous blistering disorder characterized by scarring and dystrophic nails. In DEB patients dermo-epidermal tissue separation occurs below the basement membrane, and reduced or absent immunostaining for type VII collagen and alterations in anchoring fibrils are diagnostic for this disorder. DEB can manifest with a spectrum of severity extending from the severe mutilating recessively inherited Hallopeau-Siemens variant to very mild dominant (DDEB) forms, depending upon the types and combinations of the mutations and their consequences at the mRNA and/or protein levels. Over the past decade, molecular analyses by several laboratories have identified a number of mutations spanning the COL7A1 gene in patients with DEB. The DebRA Molecular Diagnostics Laboratory at Jefferson Medical College has performed mutation detection analyses on 319 patients with biopsy confirmed DEB and disclosed 303 mutations in 204 patients. Twenty-eight patients were determined to have autosomal dominant forms of DEB with eleven different autosomal dominant glycine substitution mutations identified to date. Six different recurrent dominant mutations have been identified (G2034W, G2043R, G2064R, G2079E, G2079R, G2713D) in 21 of 28 patients, with G2043R being the most prevalent (9 of 28). Eight DDEB patients had confirmed de novo mutations implying a reduced recurrence risk in subsequent offspring of the same parents. Based on these data, we estimate the probability of a new mutation to be at least 2.6% (8/303) for all mutations in COL7A1 and possibly as high as 29% (8/28) for dominant forms of DEB. In addition, the presence of recurrent mutations allows simple and rapid mutation screening in DDEB. These findings underscore the utility of mutation detection in the determination of recurrence risk in families with DEB especially when no previous family history is noted.

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Recurrent ITGB4 mutations in Hispanic patients with epidermolysis bullosa and pyloric atresia

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Epidermolysis bullosa with pyloric atresia (EB-PA) is a rare, frequently lethal, inherited disorder with neonatal onset of blistering skin associated with pyloric atresia. Most of the cases have been attributed to mutations in the α6 (ITGA6) and β4 (ITGB4) integrin genes and recently a few cases due to plectin (PLEC1) mutations have been reported. Dermo-epidermal tissue separation occurs in the lamina lucida in most cases. In patients affected with EB-PA, immunostaining for α6β4 integrin, a transmembrane attachment protein with signaling function, is markedly reduced or absent. Our laboratory has identified 53 mutations in 36 EB-PA patients. Among them, two patients carried mutations in the ITGA6 gene and 34 in the ITGB4 gene, and 12 patients were homozygotes for the same mutation and 24 patients compound heterozygotes. The recurrent mutation C61Y, a result of a G to A transition at position 182 (TGC->TAC) in the ITGB4 gene was identified in three of five patients of Hispanic ethnic origin on five of the ten alleles. C61Y was not identified in patients of other ethnic backgrounds in our cohort. This result emphasizes the usefulness of recognizing the ethnic background for mutation detection, and facilitates the mutation analysis in Hispanic patients with EB-PA. Furthermore, our findings suggest that C61Y is a founder mutation in this population.

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Novel and recurrent mutations in the keratin 5 and 14 genes in epidermolysis bullosa simplex: implications for genetic counseling

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Epidermolysis bullosa simplex (EBS) is an inherited mechanobullous skin disorder in which the level of tissue separation is within the epidermal keratinocyte layer. EBS mutations have been disclosed in the keratin 5 (KRT5) and 14 (KRT14) genes with both autosomal recessive and, more commonly, autosomal dominant inheritance patterns. EBS patients can exhibit a phenotypic spectrum from the severe, sometimes lethal Dowling-Meara form to the milder Weber-Cockayne form, depending upon the amino acid substituted and its location in the protein. Using automated DNA sequencing of selected exons we have identified mutations in eighteen EBS patients with biopsy proven disease. Of these, nine patients had recurrent mutations that have been previously reported in EBS patients in the literature (N123S (3), R125C (3), R125H, M119T in KRT14 and P25L in KRT5). Nine patients had novel mutations, six of which were missense (I467M, I183F, E477K, I161S, S482R, S523G in KRT5 and Q120P in KRT14), while one nonsense mutation (C512X) and one in frame 3 bp deletion (delI183) were identified in KRT5. Of the eighteen patients analyzed, thirteen patients (72%) had de novo mutations where neither parent was a carrier. R125C and N123S (both in KRT14) were found as de novo events in three patients each and represented the most common type of de novo mutations in our patient population. Of the eighteen families studied, eight prenatal tests have been requested and correct outcome was predicted in six cases with one pregnancy ongoing and one lost to follow up. Of the eight prenatal tests performed, in five the affected family member was a parent of the pregnancy, and of these, two affected fetuses were predicted. No elective terminations were reported and the most common reason given for the prenatal test was to prepare for an affected child. This data underscores the high new mutation rate in EBS families seeking genetic testing, and the use of prenatal testing in EBS families to receive genetic counseling and prepare for an affected child rather than prevention.

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dHPLC screening detects novel and recurrent mutations in pseudoxanthoma elasticum

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Pseudoxanthoma elasticum (PXE) is a heritable connective tissue disorder manifesting with progressive calcification of elastic fibers in skin, retina and the cardiovascular system. Mutations have recently been identified in the ABCC6 gene, which encodes the MRP6 protein, whose function is as yet unknown. Our laboratory has been developing a systematic strategy for screening all 31 exons and immediate flanking intronic regions of the ABCC6 gene for disease causing mutations in PXE patients using dHPLC separation of heteroduplexed PCR products, followed by nucleotide sequencing. Previously, two recurrent mutations were identified, R1141X and del ex 23-29 in approximately 20% and 11% of PXE patients, respectively. Thus the current mutation detection strategy begins with restriction enzyme digests designed to detect these two common mutations, followed by dHPLC screening and DNA sequencing. Using this approach we have analyzed eight patients for mutations in ABCC6. Eleven mutations were disclosed on sixteen alleles in these patients. Six mutations have been previously reported: R1141X on five alleles and T1301I on one allele. The five novel mutations include; three missense (R807Q, R1418P, and L639P), one nonsense (R964X), and one deletion (3427delC) mutation. The remaining five mutations have not been identified to date and may represent alterations in the regulatory region of ABCC6 or intronic sequences beyond the regions examined by the current strategy. The detection rate in this analysis is 69% (11/16 alleles) consistent with published reports (72%) on detection of ABCC6 mutations using dHPLC screening.

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Targeted disruption of *Spink5* in mice results in abnormal desquamation and impaired hair formation reminiscent of Netherton syndrome, in addition to severe skin fragility

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SPINK5, encoding the putative multi-domain serine protease inhibitor LEKTI, is the defective gene in the severe autosomal recessive ichthyosiform skin condition, Netherton syndrome. LEKTI is a marker of epithelial differentiation, strongly expressed in the granular layer of the epidermis. To investigate its role *in vivo*, we have genetically engineered mice with a targeted disruption of *Spink5*. The targeted locus was confirmed by Southern blot hybridization. Loss of expression of Lekti was demonstrated by absence of immunostaining in the epidermis of the knockout mice. Homozygous mutant mice displayed an oedematous skin with loss of elasticity and died shortly after birth. The animals presented with superficial peeling and suffered from marked cutaneous fragility responsible for deep skin detachment after mild trauma. These lesions were predominant on the limbs, the tail and the trunk. Hair development was impaired, resulting in the absence of erupted vibrissae. Ultrastructural examination of the skin showed detachment of the stratum corneum from the granular layer where acantholysis was observed. These mice displayed a defect in skin barrier function as measured by penetration of external dyes and rapid decrease of body weight. At the histological and ultrastructural level, the dermis of these animals revealed localized oedema and cleavage below hair follicle bulbs. Our study provides the first *in vivo* evidence for a role of *Spink5* in the desquamation of the epidermis through the regulation of cell-cell adhesion between terminally differentiated keratinocytes. These results also demonstrate that *Spink5* is essential for hair development and surprisingly, disclose a critical role of Lekti in dermal integrity. The *Spink5* knockout mice provide an animal model to study the role of *Spink5* in desquamation and hair development, which are abnormal in Netherton syndrome.

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Treatment of human skin with retinoic acid strongly induces aldehyde dehydrogenase 1A3

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Retinoids are widely used for topical and systemic treatment of skin disease. In order to study the effect of retinoic acid, which serves as ligand for nuclear retinoic acid receptor and activates transcription, we treated human keratinocytes in a skin equivalent model with 10-6 M all-trans-retinoic acid (ATRA) and compared the expression profile with an untreated control sample by hybridization of an 11040 spot DNA array. Out of 194 ATRA induced genes (cut off 1,8-fold regulation) aldehyde dehydrogenase 1A3 (ALDH1A3) former named aldehyde dehydrogenase 6 (ALDH6) or retinaldehyde dehydrogenase 3 (RALDH3) showed highest regulation. When human keratinocytes were treated with ATRA a rapid induction of this gene could be seen with a maximum of 30-fold up-regulation as tested by real time PCR. This strong induction was verified by Northern blot and in situ hybridization of skin equivalents. Interestingly all other class I family members, ALDH1A1, ALDH1A2 and ALDH1B1 and the tested class 2 and 3 members ALDH2 and ALDH3A2 were not induced by retinoic acid. The effect of retinoids in activating ALDH1A3 is very likely restricted to epithelial cells as retinoid-treatment of fibroblasts under the same conditions resulted in no up-regulation of its expression. Since we could show in addition that ALDH1A3 is also strongly induced by 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) (30-fold induction), we suggest that ALDH1A3 has a general detoxifying function in addition to its role in the retinoic acid synthesis pathway.

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Molecular cloning and characterization of alternatively spliced S100A15-isoforms overexpressed in psoriatic skin

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In an effort to identify psoriasis-associated genes, we compared gene expression in normal and psoriatic skin using differential display RT-PCR technique. Sequence analysis of a 650 bp cDNA fragment (clone 110) that was highly upregulated in lesional skin revealed homology to a non-coding cDNA (NICE-2). Initial sequence analysis showed high homology to a noncoding cDNA. By subsequent cDNA cloning of psoriatic skin, a corresponding coding region was identified which is present in two alternatively spliced mRNA-isoforms. The splice variants detected are about 0.5 kb and 4.4 kb in length and differ in their untranslated regions. Both isoforms share a coding region predicting a protein of 101 amino acids that shares high homology to the S100A7 protein. Analysis of the deduced amino acid sequence revealed two potential EF-hand motifs as Ca2+- binding domains. By sequence comparison, we have mapped the novel gene, named S100A15, within the epidermal differentiation complex (chromosome 1q21), where other members of the S100 family are clustered. Analysis of genomic DNA revealed that the S100A15 gene is split into three exons and two introns spanning a total of about 7 kb. Increased expression of the mRNA-splice variants in psoriatic skin was confirmed by RT-PCR and Northern blot analysis. In-situ hybridization revealed a markedly increased number of mRNA molecules in the basal and suprabasal epidermal layers of psoriatic skin when compared to healthy skin tissue. Our data on expression and distribution of this gene suggest an involvment of the S100A15 in epidermal proliferation and differentiation and might be important for the pathogenesis of psoriasis and other diseases.

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Identification and characterization of the transcriptional control elements that regulate the expression of ΔNp63 in keratinocytes

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p63, a homolog of the tumor suppressor p53, plays an important role in maintaining the progenitor cell populations in stratified epithelium such as those in the epidermis of the skin. Indeed, p63-/- mice exhibit a profound block in development of skin epidermis including abnormal stratification and failure of keratinocytes to express certain differentiation markers. Interestingly, the p63 gene gives rise to multiple functionally distinct protein isoforms, including ΔNp63, which lacks the N-terminal transactivation domain and is synthesized from an internal promoter. ΔNp63 can function as a dominant-negative and hence, it is likely that the biological activity of p63 is governed in part by the relative amounts of these isoforms. Therefore, we have initiated a detailed analysis of the transcriptional control mechanisms that govern the expression of ΔNp63 in keratinocytes. In order to identify the cis-elements that regulate ΔNp63, we have analyzed the chromatin conformation of the ΔNp63 promoter by DNAse I hypersensitive site mapping. These experiments demonstrate that the promoter region of ΔNp63 as well as an upstream segment, are in open chromatin states in keratinocytes. We have performed transient transfection assays with several 5' serially deleted constructs containing various segments of the ΔNp63 promoter. These studies have identified a 120 bp fragment that harbors most of the transcriptional activity of the ΔNp63 promoter. Interestingly, this segment shows remarkable sequence conservation between several species suggesting its functional importance. Additional studies of this element by gel shift and mutation analysis have revealed critical roles for CCAAT-box binding factor (CBF/NF-Y), Sp1/Sp3 family of proteins, and GATA-like proteins. Our data provide novel insights into the regulatory mechanisms that govern the expression of ΔNp63 and support the notion that the expression of individual p53 family members and their isoforms is controlled by unique and distinct pathways.

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Transcriptional regulation of ABCC6 gene expression: identification of novel *cis*-elements, role of Sp1, and cytokine modulation of the promoter activity

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ABCC6, a member of the ATP-binding cassette family of genes, encodes MRP6, a putative transmembrane transporter expressed primarily in the liver and to a significantly lower extent in other tissues. Mutations in ABCC6 result in pseudoxanthoma elasticum, a multi-system heritable connective tissue disorder with variable phenotypic expression. To examine the transcriptional regulation and tissue-specific expression of this gene, we cloned 2.6 kb of human ABCC6 promoter and developed a series of 5'-deletion constructs linked to luciferase reporter gene. Transient transfections in a number of cultured cell lines of diverse origin identified a specific NF-κB-like sequence (-235/-226) which conferred high level of expression in both human (HepG2) and murine (MLE-10) cells, inferring liver specificity. The human promoter was more active in human cells in comparison to mouse cells, also suggesting species specificity. Testing of selected cytokines revealed that TGF-β upregulated, while TNF-α and interferon-γ downregulated the promoter activity in HepG2 cells. The responsiveness to TGF-β was shown to reside primarily within an Sp1/Sp3 cognate binding site at -58 to -49. The expression of the ABCC6 promoter was also shown to be markedly enhanced by Sp1 protein, as demonstrated by co-transfection of ABCC6 promoter-luciferase constructs and an Sp1 expression vector in *Drosophila* SL2 cells which are devoid of endogenous Sp1. Collectively, the results indicate that human ABCC6 displays tissue- and species-specific gene expression which can be modulated by proinflammatory cytokines. These findings may have implications for phenotypic expression of heritable and acquired diseases involving abnormality in the ABCC6 gene.

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A novel nonsense mutation in Kindler syndrome

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Kindler syndrome (KS) is a rare autosomal recessive genodermatosis characterized by skin atrophy and blistering, photosensitivity and poikiloderma. Recently, mutations in a new gene, designated as kindlin-1 (KIND1), were reported in patients with KS. Kindlin-1 protein is expressed in keratinocytes and is implicated in linking the actin cytoskeleton to the plasma membrane. Here we report a novel homozygous nonsense mutation in the KIND1 gene in a patient previously thought to have epidermolysis bullosa simplex with mottled pigmentation (EBS-MP). When the clinical diagnosis was reconsidered and the kindlin-1 gene sequenced, a homozygous nonsense mutation E304X, was identified. Phenotypic characterization by electron microscopy and immunofluorescence analysis of the dermal-epidermal junction revealed subnuclear splitting and vacuolisation of basal keratinocytes, pigmentary incontinence and an inhomogenous staining pattern of basement membrane proteins. From the biological point of view, the similarity of KS and EBS-MP is intriguing. In both disorders the linkage between the cytoskeleton and the cell membrane is impaired. In KS, a linker protein is functionally defective, in EBS-MP a cytoskeletal component, keratin 5. Kindlin-1 is thought to mediate the connection between the actin cytoskeleton and the cell membrane, and keratin 5 is an essential component of the keratin filaments which are attached to the cell membrane by hemidesmosomal proteins, such as plectin or BP230. Several precedents exist for similar phenotypes caused by mutations in genes encoding for functionally related molecules in multiprotein complexes.

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Identification of two keratinocyte stem cell regulatory loci implicated in skin carcinogenesis

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Several converging lines of evidence suggest that the cellular targets for carcinogens and tumor promoters in the cutaneous epithelium are keratinocyte stem cells, including in vitro clonogenic cells with high proliferative potential. These observations indicate that the identification of the mechanism regulating keratinocyte stem cell frequency and proliferative activity is critical for understanding the mechanism of skin carcinogenesis. To address this problem and to look toward the identification of stem cell regulatory genes, we investigated the clonogenic activity of epidermal stem cells isolated from two mouse strains C57BL/6 and BALB/c, and their genetic crosses. We established that mouse skin have at least two subpopulations of epidermal stem cells able to produce small (less then 2 mm2) and large (more then 2 mm2) colonies in vitro. We found that these two subpopulations were regulated by different genes: Ksc1 that is situated in the central region of mouse chromosome 9, and Ksc2 - in the proximal region of mouse chromosome 4. That Ksc1 and several minor loci regulating frequency of small colonies may be involved in the regulating the susceptibility to skin carcinogenesis follows from our data. When we sequenced the Ksc1 region we found several polymorphic genes that might influence frequency and proliferative activity of epidermal stem cells. To confirm the involvement of the genes in the regulation of our phenotype we took evaluated the influence of these genes on the clonogenic and proliferative activity of epidermal stem cells isolated from C57BL/6 and BALB/c mice. Investigation of the genes regulating the frequency and proliferative potential of keratinocyte stem cells may play an important role in the development of stem cell gene therapy and may help to establish a new view on mechanisms of skin tumor promotion.

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Procollagen VII self-assembly and its perturbations in dystrophic epidermolysis bullosa

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The purpose of the present study was to determine the molecular basis of the collagen VII-collagen VII dimer formation and to study how single amino acid substitutions found in patients with DEB alter the process of collagen VII self-assembly. In order to accomplish our aims, we engineered mini mouse procollagen VII variants consisting of NC1 and NC2 domains and a shortened triple-helix in which the C-terminal region encompassing Cys-2625 was either preserved or was substituted with the region encompassing Cys-1448 derived from the N-terminal part of the triple helical domain. To study the effects of mutations on the formation of collagen VII dimers, the G2575R, R2622Q and G2623C mutant procollagens were analyzed. Genetically engineered DNA constructs encoding recombinant procollagen VII variants were expressed in 293 cells. The formation of collagen VII dimers was analyzed by solid-state binding assays or by the use of an optical biosensor. The physicochemical properties of the interacting regions were determined by homologous molecular modeling. As evidenced by biochemical and EM assays, the mini procollagen VII variants were secreted from cells as intact monomers. Collagen VII-collagen VII binding assays indicate that procollagen VII self-assembly depends on site-specific interactions between the NC2 domain and the triple helical region adjacent to the Cys-2625, and that this process is promoted by the cleavage of the NC2 by procollagen C-proteinase. Thus, the molecular mechanism for collagen VII-collagen VII dimer formation involves site-specific interaction between the NC2 domain and the region adjacent to Cys-2625 of another procollagen VII molecule. We postulate that the correct structure of this region is critical for accurate collagen VII self-assembly and that single-base substitutions in COL7A1 may alter this structure leading to an alteration of dimer formation.

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Consequences of GABEB mutations on the assembly and stability of the BP180 triple helix

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BP180 (type XVII collagen) is a key component of the hemidesmosome and a member of the collagen protein family. BP180 is a transmembrane protein with a long carboxy-terminal collagenous domain. The purpose of this study was to investigate the structural consequences of the following BP180 mutations associated with the GABEB phenotype: a) an arginine to glutamine substitution (R1303Q) in non-collagen domain 4 (NC4); b) a 2-bp deletion (4003delTC) in the coding region of NC4 which results in a frame-shift and premature termination codon; c) a 2-bp insertion in the coding region of COL3 (delTC-insGG) that restores the frame-shift of 4003delTC. While most known GABEB-associated BP180 mutations are of the null type, the R1303Q mutant isoform has been found to be stably expressed in the patients' skin. Also previously published, the 4003delTC protein is not detectable in the skin of GABEB patients; however, in one such patient, there is focal expression of delTC-insGG in a pattern indicative of revertant mosaicism. In this mutant protein, the stretch of 25 amino acids spanning the two mutation sites are substituted with amino acids encoded by the shifted reading frame. To carry out this study, recombinant forms of the BP180 extracellular domain containing these GABEB-associated mutations were generated using a mammalian expression system. These proteins were structurally compared with the wild type protein (sec180e). Cross-link analysis showed that, like sec180e, all three of the mutant proteins formed stable homo-trimers. By gel filtration, the three mutant trimers had Stoke's radii indistinguishable from that of sec180e. The three mutant recombinant proteins were shown to be resistant to proteolysis, and each of the melting temperatures fell within the range of 40-46°C. Taken together, these results indicate that protein structure and stability is not significantly affected by any of these mutations, but rather suggest that the resulting GABEB phenotype is due to alterations in protein function.

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CD95/FAS enhancer mutations in mycosis fungoides: a potential mechanism for the acquisition of tumor clone resistance to apoptosis

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Early MF has many features suggesting it may be predominantly a lymphoaccumulative disorder rather than a lymphoproliferative disorder. FAS plays a central role in T-cell apoptosis. To determine the status of FAS genes in MF, we analyzed its 9 exons and selected promoter regions in 12 cases (stages IB-IVA). Using a combination of single stranded conformational polymorphism analysis, DNA elution, restriction enzyme digestion, PCR and nucleotide sequencing, we detected FAS mutations in 9/12 cases (75%) including six with enhancer mutations, one with enhancer and exon 9 mutation, one with exon 3 and 9 mutation and one with exon 4 mutation. Thus while only 25% of cases contained coding mutations, a much larger 58% harbored enhancer mutations. We then used genomic PCR and nucleotide sequencing to assess the enhancer region in additional MF cases. 3/5 showed mutations. Thus the overall prevalence of enhancer mutations was 10/17 (59%). Some of these mutations fell within knownl transcription factor binding sites including GAS (−869) and GABP (−857, −856, −827). A previously reported polymorphism at −670 within a GAS binding site, was observed in 4/17 cases (24%). The abnormal FAS proteins resulting from the coding mutations are predicted to act in a dominant negative manner to impair apoptosis. The enhancer mutations were clustered in "hot spots" where two or more cases had mutations: −856(2),−810(2), −691(4) and −626 (3). Because enhancer mutations typically result in diminished function, we hypothesize that these mutations reduce FAS transcription. This has been proven previously for some GABP and AP1 sites in the FAS enhancer. Thus the majority of MF cases are likely to have impaired apoptosis due to reduced and/or structurally abnormal FAS. The enhancer mutations are particular interest because they provide a novel genetic mechanism by which a MF tumor clone could acquire resistance to apoptosis via somatic mutations in FAS transcriptional regulatory elements that do not affect FAS protein structure but do down-regulate its synthesis.

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Type 2 segmental Hailey-Hailey disease originating from early loss of heterozygosity: molecular confirmation of a novel genetic concept

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Hailey-Hailey disease (HHD) (OMIM 169600) is an autosomal dominant trait characterized by erythematous and oozing skin lesions preponderantly involving the body folds in a symmetrical fashion (diffuse phenotype). In the present unusual case, however, unilateral segmental areas along the lines of Blaschko showed a rather severe involvement superimposed on the ordinary symmetrical phenotype. This observation, next to similar ones in various other dominantly inherited cutaneous disorders, prompted one of us to postulate a novel genetic concept for autosomal dominant skin diseases in which two different forms of segmental involvement can be distinguished. The type 1 manifestation reflects heterozygosity for a de novo postzygotic somatic mutation occurring at an early stage of embryogenesis thus representing a true cutaneous mosaicism. The cutaneous lesions within the affected segments reveal a degree of severity that is similar to the clinical expression of the diffuse phenotype caused by a germline mutation. In contrast, the type 2 segmental manifestation would result from loss of heterozygosity (LOH) occurring in a heterozygous embryo at an early developmental stage, giving rise to an area of homozygous or hemizygous tissue clinically showing rather pronounced segmental lesions. Accordingly, the linear lesions of the aforementioned patient would represent a type 2 segmental manifestation of HHD. Using PCR based techniques, we initially detected a splice site mutation in the germline giving rise to the ordinary symmetrical phenotype. Upon studying whole skin fragments and primary as well as cultured keratinocytes from the segmental lesions by haplotype analysis, real-time PCR and DNA sequencing we were then able to demonstrate LOH. Our data for the first time confirm the hypothesis for type 2 segmental skin diseases on the cellular and molecular level.

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The ATP2C1 golgi ATPase is required for Ras-driven human epidermal neoplasia

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Physiologic calcium levels regulate epidermal adhesion and differentiation and are particularly important for the formation of intercellular junctions, including desmosomes. Although it is known that desmosomal protein levels correlate with the malignant potential of epithelial neoplasias, and that decreased cell adhesion is implicated in tumor invasion and dissemination, the role of calcium homeostasis and calcium-dependent adhesion in carcinogenesis has not been clearly demonstrated. Mutations in ATP2C1, which regulates calcium uptake into the golgi, result in altered intracellular calcium regulation and Hailey-Hailey disease, a dominantly inherited blistering disease characterized by disrupted keratinocyte adhesion, as evidenced by epidermal acantholysis. In order to study the effects of disrupted calcium homeostasis and calcium-regulated adhesion in epidermal tumorigenesis, we compared the neoplastic potential of primary human Hailey-Hailey keratinocytes to a panel of cells from normal patients. To do this, we co-expressed Ras with the NF-kB inhibitor IkBa in an approach established to produce invasive epidermal neoplasia indistinguishable from squamous cell carcinoma (SCC) when human tissue grafted is to immune-deficient mice. While it led to invasive carcinoma after 2-4 weeks in normal human epidermis regenerated on scid mice as previously reported, Ras-IkBa co-expression in APT2C1 mutant epidermal tissue from Hailey-Hailey patients (n=3 unrelated patients, with triplicate independent grafts) showed acantholysis and blistering, but no neoplastic invasion or even hyperplasia over that time period. Moreover, the elevated mitotic index observed in response to Ras-IkBa in ATP2C1 wild-type tissue was absent in ATP2C1 mutant tissue. These studies indicate that disordered calcium homeostasis and calcium-regulated adhesion can inhibit epidermal tumorigenesis and identify disruption of calcium regulation as a potential therapeutic target in epidermal neoplasia.

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Peroxisome proliferator activated receptor coregulator isolation from epidermal keratinocytes

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Peroxisome proliferator (PP) control of epidermal keratinocyte gene expression and differentiation is mediated through PP activated receptors (PPARs), members of the nuclear receptor (NR) superfamily of ligand-activated transcription factors. NR function is also governed by coregulator proteins that contribute to either activation or repression of NR-mediated gene transcription. To better understand PPAR function in keratinocytes, we sought to identify coregulator proteins present in these cells, define the basis for their interaction with PPAR, and examine their activating or repressing effect on PPAR function. We screened a human keratinocyte cDNA library using the carboxyl portion of human PPARalpha as bait in a yeast two-hybrid assay. The screen yielded known PPAR-interacting proteins such as TRAP220, SMRT and RXR. In addition, a new candidate coregulator was represented nine times among the positive clones. The partial cDNA clones from the screen and the full-length version interact with PPARs in an isoform-specific fashion. The new coregulator contains LXXLL (L, leucine; X, any amino acid) domains known in other NR coregulators to mediate interaction with receptors' AF-2 domain. Site directed mutations of the LXXLL or AF-2 domains significantly decrease interaction of the new coregulator and all three PPARs. In transient transfections, coexpression of the coregulator clone affects PPAR function at defined PP response elements. Subcellular localization of the endogenous coregulator protein varies with keratinocyte culture conditions. In epidermis, its distribution is consistent with a differentiation-associated expression pattern. Given these results and its interaction with PPARs, we expect this newly identified nuclear receptor coregulator may play a role in regulating epidermal keratinocyte response to peroxisome proliferators.

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Upregulation of the involucrin gene as a response to increased Ca2+ concentration is impaired in Hailey-Hailey keratinocytes

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Benign familial chronic pemphigus (morbus Hailey-Hailey, HHD) is a rare hereditary condition characterized by development of blisters at sites of friction and in the intertriginous areas. Mutations in the *ATP2C1* gene have been verified in the background of the disease. Normal and HHD keratinocytes were cultured in 0.07 mM Ca2+, then increased to 1.2 mM Ca2+ to stimulate the synthesis of involucrin, a major cytoplasmic protein precursor of the cell envelope and a well established marker of early keratinocyte differentiation. Involucrin protein levels were determined using Western blotting. HHD keratinocytes synthesized less involucrin than did normal keratinocytes, while filaggrin synthesis levels were comparable. This decoupling of Ca2+-induced signaling also has been reported when intracellular Ca2+ stores were depleted with Thapsigargin. In order to investigate the mechanism of impaired involucrin protein synthesis, we tested whether the Ca2+ -induced rise in involucrin mRNA was blunted in HHD keratinocytes. Involucrin mRNA levels were compared with SYBR Green quantitative PCR. The increase in the involucrin mRNA copy number as a response to increased Ca2+ concentartion was higher in healthy adult keratinocytes than in HHD keratinocytes. We conclude that the higher baseline cytoplasmic Ca2+ level and the alterations in the Ca2+ signaling as a consequence of the Golgi Ca2+ ATPase dysfunction results in an impairment of the early keratinocyte differentiation. This impairment may be due to a decrease in the AP-1 promoter activity.

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Developing a mouse model of pachyonychia congenita

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We have previously generated mice with a null mutation in the keratin 6a and 6b genes (MK6a/b-/- mice). Most of these mice died due to lesions on the dorsal surface of the tongue, which severely affected feeding. An analysis of these mice also led to the discovery of a third MK6 gene, which was termed MK6hf. Expression of this protein most likely compensated for the loss of MK6a and MK6b in skin appendages, in particular hair follicles and nails, and thereby prevented a gross phenotype in these tissues. To analyze the function of MK6hf in skin appendages, we introduced a dominant-negative mutation into this gene using mouse embryonic stem cell technology. The mutation, a deletion of asparagine residue 159 in MK6hf, corresponds to the deletion of N171 in the human K6a gene in Pachyonychia Congenita (PC-1) patients. Transfection experiments demonstrated that expression of this mutant MK6hf protein leads to a collapse of the intermediate filament cytoskeleton of cultured epithelial cells. Mutant mice developed a severe hair loss and nail atrophy phenotype similar to PC patients. On the basis of these results, we conclude that MK6hf is essential for maintaining structural integrity of hair follicles and nails. We have also generated an inducible mouse model, which allows focal activation of the mutant MK6hf allele. This model should be useful for studies directed toward understanding the pathogenesis of PC and for assessment of novel therapeutic approaches for this disorder.

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A novel function for $p53^{w/w}$ in DCoH/HNF-1α transcription and its regulation by H_2O_2

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Recently, it has been shown that the human epidermis holds the capacity for DCoH/HNF-1α transcription in melanocytes and keratinocytes. In this context, tyrosinase was recognised as a new target gene. Using the *in vivo* model vitiligo, it has been demonstrated that 10^{-6} M concentration of hydrogen peroxide (H_2O_2) upregulated DCoH/HNF-1α. Since $p53^{w/w}$ is also upregulated by 10^{-6} M concentration of H_2O_2, we here asked the question, whether $p53^{w/w}$ could possibly regulate HNF-1αtranscription. Analysis of the p53 DNA-binding sequence yielded the putative binding site in the HNF-1α gene promoter. Using electrophoretic mobility shift assay, we were able to demonstrate for the first time the binding of $p53^{w/w}$ to the HNF-1α gene promoter. This result indicates that $p53^{w/w}$ controls DCoH/HNF-1α transcription, including tyrosinase. Moreover, this result also supports the importance of H_2O_2 in the regulation of this transcription pathway.

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Tetracycline-regulated transactivators driven by the involucrin promoter to achieve epidermal conditional gene expression

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To achieve conditional gene expression in the differentiated layers of the epidermis, we generated transgenic mice in which the tetracycline-regulated transactivator proteins, tTA and rtTA, are expressed from the involucrin promoter. Interaction with tetracycline turns off or on the tTA and rtTA molecules, respectively, allowing for regulation of downstream target genes during development and postnatally. These transactivator lines were crossed with reporter mice driving LacZ expression from a tetracycline response element to analyze the specificity and levels of target gene expression. Quantitative b-galactosidase experiments demonstrate a 30 fold level of induction when the lines are induced specifically in epithelial tissues. Immunohistochemistry results illustrate that the b-galactosidase staining follows that of the endogenous involucrin promoter. We characterize the expression of these lines during development showing that induction initiates at embryonic day 14.5 with expression over the entire epidermal surface by day 16.5. Together with other driver lines, expressing the tetracycline transactivators in the mitotically active layers of the epidermis, these mice will allow investigators to specifically modulate the expression of their target genes to specific stages of epidermal differentiation.

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Identification of a recurrent frameshift mutation in the hairless gene in a patient with atrichia with papular lesions

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Identification of mutations in the hairless genes in patients with atrichia with papular lesions (APL) has proven of chief importance because it provides a basis for the differential diagnosis between APL and alopecia universalis. The definition of the diagnosis criteria of APL (Zlotogorski et al, 2002) has triggered the identification of a large number of APL patients among those suspected to suffer from alopecia universalis, and treated accordingly with consistent negative outcomes. This has resulted in an increasing number of hairless mutations in both consanguineous and non-consanguineous APL families. We report the identification of a homozygous mutation, 3435delC, in an APL patient of Arab-Palestinian descent. The proband is a 23-year-old female with generalized scalp and body alopecia and absent eyelashes and eyebrows. Soon after birth, she lost all her scalp hair, which never regrew. At present, she has a papular rash on her arms and legs that started at 3 years of age. The proband belongs to a consanguineous family (first cousin parents) and no history of hair loss (both parents and her two sisters are unaffected). In order to confirm the diagnosis of APL and to identify the specific mutation, we sequenced the hairless gene. We first excluded the presence of the mutation 2147delC, previously described in five Arab Palestinian families (Zlotogorski et al, 1998). Sequencing of the remaining exons in the hairless gene revealed a homozygous frameshift mutation, 3434delC, in exon 18. Interestingly, the same mutation was previously identified in an Arab-Israeli family (Sprecher et al, 1999). As is the case for the 2147delC mutation, the data presented here suggest that the 3434delC mutation most likely represents a founder mutation in this geographical region. In summary, these findings extend the body of evidence implicating hairless gene mutations in the pathogenesis of APL.

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Stable intergration of telomerase in human fibroblasts permits non-malignant growth transformation *in vitro* that maintains transgene expression *in vivo*

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Genetically modified human fibroblasts (GMFb) lose integrated transgene expression (TGE) in nude mice in vivo. TGE/S in vivo is modulated by the ECM surrounding the GMFB and the inherent life-span of the GMFb; inherent life-span = greater effect. GMFb cloned for TGE in a nylon matrix in vivo are gone by 12 weeks unless transduced with human telomerase (hTERT) via a retroviral vector (rv). These senescence protected GMFb were cloned and assessed for survival in vitro via passage. One of the hTERT clones underwent a second transformation, > passage 50, past the passage limit of normal fibroblasts, < 25 . This GMFb grows more rapidly than other clones transduced with hTERT. These growth modified GMFb were transduced with lacZ.rv and seeded into a nylon matrix that had been matured in vivo, transplanted to nude mice, recovered at regular intervals for 16 weeks, and assessed for TGE/S. The GMFb that have undergone the growth transition have TGE/S that far exceeds that of all other GMFb sources tested. Two clones that did not undergo growth transformation process displayed TGE/S of normal transduced GMFb. The experiment was repeated with GMFb encoded with human transferrin (hTf). This construct shows detectable blood levels as long as the GMFb.hTf are viable. At 4 weeks hTf levels from non-growth modified cells was 4 x less than growth modified cells. By 16 weeks there was a 50 x difference, respective deterioration and increased levels in the non-growth modified and growth modified constructs. In contrast to immortalized 3T3 cells carrying hTf, which form tumors in vivo by week 8, no tumors were seen in the growth modified constructs in this 20 week experiment. Microarray analysis of message of two senescence protected growth modified fibroblasts vs two cell lines from the same source that have not undergone growth modification are substantive. It appears that over-expression of hTERT permits further non-malignant growth modification and that transgene survival and expression is preserved in vivo by these cells.

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Expression profiling of genes regulated by keratinocyte growth factor in immortalized human keratinocytes

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Keratinocytes growth factor (KGF) a member of the fibroblast growth factor (FGF-7) family, and is strongly expressed at the wound edge after injury. A keratinocytes model system with KGF under TCN regulation will help us better understand gene expression profile in wound healing process. The aim of this study was to identify possible gene targets for regulation by KGF in immortalized human keratinocytes (IMKc). In this study, IMKC were transduced with retroviral vectors of RetroTet-Art system carrying human KGF cDNA. RT-PCR and real time PCR analysis show regulatable KGF expression. To identify candidate and novel gene expression patterns, we compared the gene expression profile of IMKc-hKGF(/-) cells using Affimytrix chip (Human Genome U133 Plus 2.0) carrying 47,000 gene transcripts. Samples with and without doxycycline were run in duplicate and showed identical clustering pattern. We identified by SAM analysis 42 up-regulated and 2 down-regulated genes with a significant differential regulation ($p<0.05$). This study validated the expression patterns for known genes like Activin, Nrf2 and Cox-2. A plausible model of gene interaction (by PathwayAssist) is put forth for KGF mediated response in keratinocytes. Most notable were the cascade of cytokines (vascular endothelial growth factor,tumor necrosis factor SF10, epidermal growth factor receptor, and diptheria toxin receptor) as they work in a network-fashion. The effect of KGF was also noticeable for genes involved in adhesion and extracellular matrix (integrin B5, collagen type XVIII A1, plasminogen activator urokinase and matrix metalloproteinase 14). This up-regulated network of novel gene transcripts could play possible role in understanding the molecular mechanisms of gene regulation in cell proliferation and /or re-epithelialization in wound healing process.

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The R683W common mutation occurs in xeroderma pigmentosum group D patients with and without neurological symptoms

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Xeroderma pigmentosum (XP) is an autosomal recessive disease with markedly increased susceptibility for sun-induced skin cancer in association with defective DNA repair. There are 7 XP nucleotide excision repair (NER) genes (*XPA-XPG*) and an XP variant (*pol-eta*). *XPD* is a helicase involved in NER and also in transcription as a member of the basal transcription factor complex, TFIIH. Surprisingly, defects in the *XPD* gene result in 6 different clinical phenotypes: XP, XP with neurological disease, the XP/Cockayne syndrome complex, trichothiodystrophy (TTD) (a disorder with brittle hair without increased cancer susceptibility), XP/TTD and cerebro-ocular-facial-skeletal (COFS) syndrome. The molecular basis for this phenotypic heterogeneity is not known. We analyzed cells from patients with reduced post-UV cell survival and used a plasmid host cell reactivation (HCR) assay to assign 22 cell lines to XP complementation group D. Of these there were 18 XP, 2 XP/CS, 1 TTD, and 1 XP/TTD patients. We found the same R683W mutation in exon 22 of the XPD gene in 14 (78%) of the 18 XP but not in any of the TTD, XP/TTD or XP/CS patients. The R683W mutation was homozygous or hemizygous in one and heterozygous in 13 XP patients. XP neurological disease is progressive and may not be manifest in children. We focused on the 7 adult XP-D patients who were compound heterozygotes for R683W and another allele; 5 had severe neurological disease and 2 had a normal neurological examination. We plan to test the contribution of the second allele to the genotype-phenotype correlation by using site directed mutagenesis to create *XPD* cDNA sequences containing these mutant alleles and test their DNA repair activity in the HCR assay.

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The hairless promoter is differentially regulated by thyroid hormone in keratinocytes and neuroblastoma cells

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The hair cycle is an extraordinarily complex process relying on spatially and temporally coordinated intercellular signaling, cell division and death, cell migration, and gene expression. The hairless gene, whose mutation is responsible for the hairless phenotype in mice and atrichia with papular lesions in humans, is expressed with hair cycle-dependent kinetics. A thyroid hormone responsive element previously identified in the rat hairless promoter confers T3 responsiveness to promoters in front of which it is placed. However, prior studies have not focused on the hairless promoter itself. We have cloned the hairless promoter, and here we show that hr promoter activity is transactivated by T3 in neuroblastoma cells, but not keratinocytes. Therefore, while T3 can have a significant role in neuronal cell type-specific expression of hairless, upregulation in skin is T3 independent. The inhibition of promoter activity by TR and T3 in keratinocytes is nonspecific and TRE independent. Furthermore, hr is subject to cell type-specific negative autoregulation, inhibiting activity of its promoter in keratinocytes but not neuroblastoma cells. These findings illustrate a molecular distinction between the disparate phenotypes caused by thyroid hormone dysregulation and hairless gene mutation.

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Microarray analysis of gene expression between human CD45RA+ and CD45RO+, CD4+ T cells

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Memory T cells induce a greater immune response on re-encounter with an antigen than naive T cells. Memory T cells express a higher level of cytokines and effector functions. In the human CD4+ T cell subset, the genes that are differentially expressed in memory T cells are not well identified. CD4+ T cells can be separated by CD45RA+ for the naive subset and CD45RO+ for the memory subset. Using these markers, cells were separated for analysis of differential gene expression between naive and memory T cells. CD45RA+ and CD45RO+ CD4+ cells were purified by magnetic separation to greater than 95% purity. These were stimulated so that differences in genetic response between unactivated and activated naive, and unactivated and activated memory population could be examined. Highly purified total RNA was isolated and high density oligonucleotide microarray (Affymetrix) was used to analyze for gene expression differences between naive and memory T cell subsets. This study revealed genes that were: 1) differentially expressed between naive and memory T cells at rest, 2) differentially induced between unstimulated and stimulated cells of the same cell type, and 3) differentially expressed between the stimulated naive and stimulated memory T cells. For example, a gene known to be expressed higher in the naive population, such as CCR7, was identified in the naive population. After stimulation, the memory cells showed increased cytokine expression for IL-2, IL-4 and IFN-gamma in comparison to the stimulated naive cells, indicating the memory cells functioned as expected for the memory subset. Overall, the study showed that there were a greater number of genes expressed higher in the memory subset than in the naive subset. A subset of the differentially expressed genes identified by microarray were confirmed additionally by RT-PCR. The differences in gene expression should provide insight into the molecular differences between naive and memory T cells and should lead to understanding of signals and factors that regulate the development of memory T cells.

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Apoptotic signaling pathways in Darier's disease keratinocytes

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Darier's disease (DD) is a rare autosomal dominant disease caused by a mutation of the ATP2A2 gene, encoding the SERCA2 Ca2+ ATPase. The ATP2A2 is localized to the endoplasmic reticulum of cells and is important in Ca2+ -induced Ca2+ signaling, which controls essential keratinocyte functions such as differentiation, motility and cell-to-cell adhesion. Previously, we have shown that Golgi Ca2+ stores, controlled by a similar Ca2+ ATP'ase ATP2C1, are essential for normal Ca2+ signaling and that mutations in the ATP2C1 cause the similar acantholytic disorder Hailey Hailey disease. However the distinctive feature of Darier's disease is apoptotic modification of cells carrying DD mutation. To further investigate this feature we compared the levels of apoptosis-associated proteins, such as caspase 2, BAK, BAX and PARP in healthy human keratinocytes and DD keratinocytes in cell culture. Here we report that the levels of PARP and caspase 2 proteins are elevated in DD keratinocytes, and the cleavage of both molecules is distinct from that of normal keratinocytes. The levels of BAK and BAX do not show significant changes in this disease. We have also investigated the role of ATP2C1 Golgi Ca2+ ATPase in regulating Ca2+ in DD keratinocytes. Our findings suggest that the levels of ATP2C1 are upregulated in DD cells. We are further investigating the possible translocation of ATP2C1 to the ER in order to compensate for the loss f ATP2A2 function as the consequence of DD mutation. To locate ATP2C1 within the cellular compartments we employed the method of Opti-Prep gradient with slight modifications. The gradient allows the separation of the ER and Golgi, detected by SDS-PAGE analysis of the fractions, and allows for localization of molecules associated with specific organelles.

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The wnt pathway effector, Lef-1, regulates human *hairless* gene expression

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The hairless gene encodes a putative zinc-finger transcription factor whose expression is predominately limited to the developing brain and to skin, where it is expressed in a hair-cycle dependent manner. The mechanisms coordinating the kinetics of hr gene expression remain largely undefined. Recently, we showed that a previously identified thyroid hormone responsive element in the human hr gene is responsible for transactivation of hr gene expression by thyroid hormone in the neuroblastoma cell line, SY5Y, but not in primary human keratinocytes or the HaCaT human keratinocyte cell line, thereby repudiating a role for T3 in the hairless pathway in skin. Scanning of the promoter sequence for alternative DNA consensus binding sites revealed the presence of 9 putative lef-1 binding sites downstream of the TRE which have a 1bp deviation the canonical lef-1 binding site and one putative binding site 386bp upstream of the hr mRNA cap site that is a perfect match. To test the hypothesis that hr is a wnt target gene, expression constructs of the wnt pathway genes wnt1, beta-catenin, and lef-1 were cotransfected into neuroblastoma cells with hr promoter reporter constructs or a luciferase reporter driven by 7 upstream concatenated lef-1 binding sites. Here, we show that hr expression is transactivated by co-expression of wnt pathway genes along with hr-luciferase reporter genes in SY5Y cells. Furthermore, we show that upregulation of hr expression is lef-1 dose dependent. Finally, we demonstrate that T3-dependent and lef-1-dependent transactivation are not synergistic. These findings identify a second pathway in which the hr gene is a downstream effector of an extracellular signal.

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Expression of bone morphogenetic protein-5 in epidermis and hair follicles of the mouse

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Bone Morphogenetic Proteins (BMP) and their receptors (BMPR) are members of the Transforming Growth Factor-Beta family of signaling molecules that play an essential role in a variety of morphogenetic processes. Although BMP-2 and -4 expression is known to function in the patterning and cycling of hair follicles, BMP-5 has been understudied. Therefore, the purpose of this investigation is to characterize BMP-5 messenger RNA and protein expression in mouse skin. RT-PCR and Western blot analysis were used to detect BMP-5 transcripts and protein, respectively. Immunofluorescence was performed to localize the expression of BMP-5 and BMPR-1A in mouse skin. We detected BMP-5 messenger RNA and protein in freshly harvested epidermal cells including keratinocytes from the hair follicle bulge isolated by fluorescence activated cell sorting with antibodies directed against alpha-6 integrin and CD34. Immunofluorescence microscopy revealed that BMP-5 and its receptor localized to the basal layer of the epidermis and outer root sheath of the hair follicles, including the bulge region throughout the hair growth cycle. These expression studies enable further functional analyses of BMP-5 both in vivo and in vitro. We conclude that, like BMP-2 and -4, BMP-5 may play a role in epidermal growth and morphogenesis. The significance of this work is that identifying genes responsible for the homeostasis, proliferation, and differentiation of keratinocyte stem cells would make an important contribution to cancer research and therapy, as well as tissue engineering and replacement.

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Inhibition of adenine nucleotide translocator function in the skin of transgenic mice

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The adenine nucleotide translocators (ANTs) exchange ADP for ATP across the inner mitochondrial membrane and regulate apoptosis through the mitochondrial permeability transition pore (mtPTP). Mice have two ANT isoforms: ANT1 found in heart, muscle, and brain and ANT2 found in all tissues except muscle. To examine the role of ANT in the skin, we selectively ablated *Ant2* in the epidermis of transgenic mice. The *Ant2* gene was targeted in murine embryonic stem cells using the Cre/Lox strategy, and these cells were injected to generate transgenic mice harboring the floxed *Ant2* allele. These mice were mated to mice expressing Cre recombinase under the control of the keratin 5 promoter, which targets the transgene expression to basal keratinocytes. This Cre recombinase is activated in the presence of 4-hydroxy tamoxifen (OHT). We induced the Cre-mediated excision of the *Ant2* gene using topical OHT dissolved in two different vehicles: ethanol-sunflower oil (ETS) and dimethyl sulphoxide (DMSO). Topical OHT was applied to shaved back skin of homozygous and hemizygous *Ant2* floxed/Cre mice once daily for 5 days to activate Cre recombinase. We observed cutaneous inflammatory changes both at the OHT administration site as well as at remote sites, especially in the ears. One to two weeks following administration of the OHT-ETS preparation, the skin showed interface dermatitis and focal epithelial necrosis, followed by keratinocyte hyperproliferation, and focal micro-abscess formation. We also observed severe weight loss and death in mice treated with OHT dissolved in DMSO 8 to 10 days after drug application. Although topical OHT treatment was localized to mouse back skin, the presence of weight loss, death, and inflammation at sites remote from drug application indicates either a systemic absorption of drug or a systemic response triggered by local administration of drug. Whether these changes are a result of energy depletion or of alterations of apoptosis through mtPTP is currently under investigation.

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Analysis of gene expression in primary T cells by radio-frequency (RF) electroporation

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Efficient DNA transfer into primary cells is essential for success in gene therapy, dissecting the function of gene products, and analyzing the mechanisms of gene regulation in normal cells. Expression of exogenous DNA in primary lymphocytes has been approached by transduction using viral vectors and by DNA transfection. Viruses have the advantage of efficient gene transfer, but construction of viruses is inefficient and laborius, thereby rendering high thruput analysis prohibitive. Liposome mediated DNA transfer has been valuable for many cell types, but has not been successful for primary peripheral blood cells. We describe the optimization of transfection method into primary T cells using RF electroporation, whereby electrical parameters are precisely controlled. This approach permits precise control of voltage, pulse duration, number of pulses, frequency and modulation of waveform for electroporation. Peripheral blood leukocytes (PBL) were isolated, prepared and the cells were electroporated after 20 to 24 hours. The green fluorescent protein (GFP) reporter gene was used to monitor expression of transgene. Using this approach, transfection efficiencies of up to 40% of live cells was achieved with PBL as quantitated by flow cytometry. Expression of GFP was rapid and could be measured as early as 6 hours after electroporation. These conditions were used to transfect reporter plasmids for analysis of luciferase expression to measure promoter activity in T cells. To determine whether all T cell subsets were transfected with equal efficiencies, CD4 and CD8 cells were separated by magnetic sorting and purified cells were transfected. We found that for T cells, the CD4 subset was transfected with a higher efficiency than the CD8 subset. Furthermore, we were able to transfect PBL from patients with mycosis fungoides. The ability to successfully electroporate and analyze gene expression in primary T cells should lead to direct understanding of mechanisms of gene expression in normal and abnormal T cells.

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Reporter gene transfer into primary keratinocytes with a parvovirus vector

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Parvoviruses are small, non-enveloped viruses which contain a 5 kb single strand DNA genome. We have previously described vectors based on LuIII, an autonomous parvovirus of the rodent group, able to infect human cells. The rodent autonomous parvoviruses preferentially replicate in malignantly transformed cells and they have generally been considered only poorly able to infect normal cells (e.g. fibroblasts) in primary culture. Surprisingly, we have observed efficient transduction of human primary keratinocytes (KCs) by LuIII vectors carrying luciferase (LUC) or enhanced green fluorescent protein (eGFP) reporter genes. Previously, we observed high levels of LUC expression after transduction of several human melanoma and other malignant cell lines but observed much lower expression in diploid fibroblasts and other primary cell cultures. However, each of several independent isolates of foreskin KCs showed high expression of LUC and eGFP (up to approx. 30% of the cells, depending on viral titer) after transduction with these vectors (using parvoviral or CMV promoters). Frequent green fluorescent cells were seen in the transduced KC cultures for at least 2 weeks. Experiments in progress with tetracycline-inducible LuIII-eGFP vectors should determine whether the transduced KCs support long-term transcription from the vectors (as opposed to persistence of the stable eGFP reporter protein). In contrast with primary cultures, we observed that immortalized lines of human KCs (HaCaT and IMKc) were transduced only relatively inefficiently by LuIII vectors. The reasons for this difference are under investigation. Our results suggest that autonomous parvovirus vectors may be useful for ex vivo, and possibly in vivo, transduction of KCs in skin for gene transfer and gene therapy applications. We thank Gary Bellus and Desiree Kelly (UCHSC) for primary cultures and Prit Gill (Univ. Utah) for IMKc cells.

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p16 mutational status influences responses to T-oligo-simulated DNA damage

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Cyclin-dependent kinase inhibitor 2A (p16) germline mutations are associated with a hereditary predisposition to melanoma. A primary function of p16 is the induction of cellular senescence, but the molecular mechanisms by which p16 mutations lead to melanoma susceptibility are poorly understood. It has been shown that treatment with a DNA oligonucleotide homologous to the telomere 3' overhang sequence TTAGGG (T-oligo) triggers DNA damage responses in normal neonatal fibroblasts, including an S-phase arrest and induction of senescence. The purpose of our investigation was to determine whether adult fibroblasts, derived from heterozygous carriers and non-carriers of a p16 mutation, respond to T-oligo in a similar fashion. Carrier and non-carrier fibroblasts were synchronized prior to treatment with either 40 μM T-oligo or diluent control. Cells were harvested at 12, 24, and 48 hours, stained with propidium iodide and analyzed for DNA content by flow cytometry. Portions of the same cell populations were evaluated for senescence by staining for senescence-associated β-galactosidase (S.A. β-Gal) activity. Carrier and non-carrier fibroblasts both demonstrated S-phase arrest of the cell cycle within 1-2 days, and also showed S.A. β-Gal staining by 48 hours. In contrast, neonatal cells require 5 days to enter senescence. These data suggest that adult fibroblasts may respond more quickly to telomere-based DNA damage triggers than neonatal fibroblasts. In addition, it appears that the p16 mutation carriers re-enter the cell cycle more quickly and display a less striking senescent phenotype than non-carriers. These data support the hypothesis that inheritance of a p16 mutation leads to a diminished capacity to maintain S-phase arrest and enter a senescent state in response to DNA damage. Over time, this propensity could potentially lead to an accumulation of un-repaired DNA damage that may help to explain why mutations in p16 result in a predisposition toward melanoma.

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Single chain, variable fragment antibodies to the membrane bound cytochrome P450 CYP2B19 reveal preferential expression in stratified squamous epithelia at the environmental interface

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Mouse Cyp2b19 encodes a keratinocyte-specific arachidonic acid monooxygenase expressed in differentiated skin keratinocytes. We developed CYP2B19-specific recombinant antibodies to prove that CYP2B19 is present in epidermal keratinocytes and thus likely responsible for endogenous epoxyeicosatrienoic acid formation. CYP2B19-specific scFv antibodies interacted with keratinocyte proteins (55 kDa), which were upregulated during in vitro differentiation. The specificity of these binding interactions visualized on Western immunoblots was corroborated by the ability of CYP2B19-specific scFv to capture native CYP2B19 in keratinocyte lysates, determined by mass spectrometry. On Western immunoblots, CYP2B19-specific scFv antibodies also interacted with oral keratinocyte proteins (55 kDa), the levels of which varied with the extent of epithelial keratinization in different regions of the oral cavity. No interactions were detected with liver or eleven recombinant CYP proteins. Cutaneous and oral epithelia interface the environment and function to protect against injury and dehydration. Preferential expression in these stratified squamous epithelia implies functions for CYP2B19 catalysis in the type of epithelial barrier formed at these sites.

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The human K1 gene is regulated by cooperative interactions between multiple regulatory elements

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One of the earliest events associated with keratinocyte differentiation is expression of keratin 1 (K1). In an attempt to identify regulatory elements required for expression of the K1 gene, we initially prepared a series of reporter constructs consisting of regions flanking the human K1 (HK1) gene. These constructs were transfected into primary mouse keratinocytes, which were induced to differentiate by increasing the calcium concentration of the medium. Site-directed mutagenesis experiments, coupled with gel-retardation assays, identified a key binding site for AP-2 in the proximal region of the HK1 promoter. In addition, critical binding sites for Sp1, NFκB and AP1 were found at distal locations in both the 5' flanking (Sp1 and NFκB) and 3 ' flanking (AP1) regions. Mutations in any of these binding sites caused significant effects on HK1 reporter activity, suggesting cooperative interactions between both proximal and distal elements. To determine the contribution of these regulatory elements to both temporal and spatial expression of the HK1 gene in the epidermis, we have initiated *in vivo* studies in transgenic mice. Previous studies have shown that the expression of short transgenes can be influenced by sequences surrounding the site of integration. To minimize positional effects, we used a large BAC clone, containing the HK1 gene surrounded by 87 kb of 5' sequence and 117 kb of 3' sequence. A lacZ reporter gene was inserted into this clone under the control of the HK1 promoter. Site-directed mutagenesis was then performed for these transcription factor binding sites using ET recombination and transgenic mice were then generated with each BAC construct. The effects of these mutations on tissue- and differentiation-specific expression have been assessed by β-gal staining of tissue sections, and the effects on expression levels have been assessed by β-gal assays on skin extracts. Our *in vivo* results confirm that the HK1 gene is regulated by complex cooperative interactions between multiple regulatory elements.

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A novel dominant missense mutation of GJB6 (Cx30) causing KID syndrome with mild cutaneous phenotype

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Dominant mutations of the connexin genes GJB2 (Cx26) and GJB6 (Cx30) have been associated with a broad spectrum of clinical phenotypes involving ectodermal stratifying epithelia. Cx26 mutations may cause Keratitis-Ichthyosis-Deafness (KID) syndrome, Vohwinkel syndrome, and hearing loss associated with palmoplantar keratoderma or mucocutaneous erythema and hyperkeratosis. Cx30 mutations are responsible for Clouston syndrome, pachyonychia congenita-like nail dystrophy and, in one case, KID syndrome with atrichia. In this study, we report an eight-year-old boy with congenital profound sensorineural hearing loss, palmoplantar keratoderma and symmetric erythrokeratodermic plaques of the face, features characteristic of KID syndrome. Hair, nails, mucous membranes and the anterior eyes were not involved. The localized facial plaques nearly vanished under topical treatment with tacrolimus 0.1% ointment.

In contrast to other KID syndrome patients, no pathogenic mutation was identified in GJB2. However, the patient harbored a heterozygous 176G>T transversion in GJB6 resulting in a glycine to valine substitution at codon 59 of Cx30 (G59V). This previously unreported mutation was not detected in either parent or in 112 control alleles. It affects a highly conserved residue in the second extracellular domain that is invariably present in mammalian connexins. Replacement of the orthologous residue in Cx26 with alanine has been shown to cause autosomal dominant hearing loss and palmoplantar keratoderma. Our finding is the second report of a GJB6 mutation resulting in a KID phenotype, which further underscores the genetic heterogeneity of this ectodermal dysplasia. Consequently, we propose screening of multiple epidermal connexin genes, such as Cx26, Cx30, Cx31, and Cx30.3 in patients presenting with hearing loss and cutaneous findings.

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Autosomal dominant inheritance in pseudoxanthoma elasticum revisited

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Pseudoxanthoma elasticum has long been considered the prototypic connective tissue disorder, which occurs sporadically or follows either an autosomal dominant or autosomal recessive mode of transmission. Mutations in the ABCC6 gene, encoding MRP6, an ATP dependent efflux pump in liver and kidneys, have been causally linked to all types of PXE. Furthermore, molecular genetic analysis of our patient cohort has previously established the inheritance pattern as autosomal recessive. However, several clinical investigators have reported dominant inheritance in families with affected individuals in two generations. Also, obligatory heterozygous carriers may have a limited phenotype, often reflected only by positive histopathology at predilection sites or by asymptomatic eye findings. Within our cohort of over 70 PXE families, we have ascertained 7 families that meet these criteria. Using microsatellite markers and SNPs spanning 3 cM around the ABCC6 locus, we have haplotyped these families and sequenced the entire coding region of ABCC6 in affected individuals and presumed carriers with limited phenotype. All affected individuals and those with limited phenotype harbored two mutated alleles, most mutations being private and novel. In two of these families, consanguinity in previous generations was detected through haplotype analysis. In the other families, affected individuals in two generations carried two sets of affected alleles, with a total of three different mutations in each family. Collectively, these data support pseudodominant inheritance in these selected families and further emphasize that PXE is an autosomal recessive disease that requires mutations in both alleles to express even minimal clinical findings. Phenotypic variations between generations may be attributed to the consequences of mutations (e.g. missense vs. loss of function) at the level of the encoded protein. Furthermore, our findings suggest a much higher prevalence of PXE than previously predicted, thus impacting on genetic counseling and disease prevention.

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Gene therapy approaches for epidermolysis bullosa simplex

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The Dowling-Meara variant of epidermolysis bullosa simplex (EBS-DM) is a severe blistering disease inherited in an autosomal-dominant fashion. No effective therapeutic treatment is available for EBS making gene therapy the only corrective option for these patients. We previously generated a transgenic mouse model that mimics EBS-DM at the genetic level. In this model, the expression of the mutant K14 allele (mtK14) can be restricted to a small area of the skin. Focal activation of the mtK14 gene by a topically applied inducer results in formation of blisters that heal after a few weeks and never reappear. We demonstrated that the mtK14 gene is activated in epidermal stem cells, and that the defective EBS stem cells are replaced by normal ones that migrate from the untreated areas surrounding the blister. This explains the absence of mosaic forms of EBS and suggests that if EBS stem cells were removed from a patient, genetically corrected and then returned to a blistered area, they would have a selective growth advantage over defective EBS stem cells. In addition, we observed that mice expressing the mtK14 allele at levels approximately 50% of wild type K14 (wtK14) fail to exhibit a skin phenotype, suggesting that as long as the wtK14:mtK14 ratio is above a threshold; the skin will have a normal appearance and be fully functional. Thus, successful gene therapy may not require correction or complete suppression of the mutant allele. We have developed a ribozyme that specifically cleaves the mtK14 message in vitro and in vivo. We have also developed a lentiviral vector carrying the wtK14 gene, and demonstrated that it effectively transduces keratinocytes which retain long-term expression of the transgene in tissue culture and up to 4 weeks after grafting onto nude mice. Current studies are directed toward determining if the EBS-DM phenotype can be corrected by altering the ratio of wtK14 to mtK14.

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First study on BRAF mutation in the serum of melanoma patients

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The BRAF protooncogene encodes a RAS-regulated kinase that mediates cell growth and malignant transformation. A high frequency of activating mutations in BRAF has been identified in nevi and primary melanoma samples, suggesting involvement of the Ras-RAF-MAPK pathway in melanocytic tumorigenesis. None of the 25 original research reports on BRAF and melanoma published before January 2004 have addressed the issue of potential BRAF gene mutations in the serum. In order to establish whether there would be circulating tumor cells that express mutated BRAF, we purified genomic DNA via standard phenol-chloroform extraction followed by ethanol precipitation from the serum of 45 patients with cutaneous melanoma (greater than 10 individuals for each AJCC stage) and 19 control patients suffering from benign inflammatory dermatologic diseases. An approximately 100 base-pair oligonucleotide was PCR amplified using primers specific for BRAF exon 15. Amplification products were evaluated using the colorimetric assay Mutector IM (Trim-Gen Corp, Sparks, MD), a technology achieving sequencing like accuracy. Mutations in BRAF exon 15 were detected in 1 of 45 melanoma patients (AJCC stage IA) and 2 of 19 control patients, one with atopic eczema and another one with erysipelas. We conclude that the prevalence of activating BRAF mutations found in the serum of melanoma patients appears to be low according to our assay. We are therefore in the process of applying a more sensitive gap ligase chain reaction to an extended collection of serum samples. Our observation may have implications for the clinical use of raf kinase inhibitors and also suggests that mutated BRAF gene may be related to a fraction of chronic inflammatory states such as atopic eczema and erysipelas.

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Genotype-phenotype correlation in 62 patients with pseudoxanthoma elasticum

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Pseudoxanthoma elasticum (PXE) is a rare heritable connective tissue disorder affecting skin, eyes and cardiovascular system. Clinical manifestations are protean, displaying both inter- and intrafamilial phenotypic variation. Mutations in the ABCC6 gene on the short arm of chromosome 16 are pathogenic and to date there is no evidence of locus heterogeneity for this autosomal recessive disease. In the following study, we have ascertained a cohort of 62 PXE families of mostly Northern European extraction. The coding region as well as exon intron borders of all affected individuals was subjected to direct sequencing. We have discovered over 10 distinct polymorphisms within the coding region of ABCC6, and have identified pathogenic mutations on 107 alleles (89%). The spectrum of mutations resembled those of previous reports with two recurrent mutations, R1141X and deletion exon 23-29, and the majority constituting private mutations. Amongst those, we have identified 25 novel sequence aberrations. Mutations were plotted against the clinical phenotype that was graded by disease severity in case of skin and specific diagnoses in case of eye and cardiovascular disease. In addition, exonic polymorphisms were scrutinized in the same manner in order to determine a disease modifying effect. Compound heterozygotes for two missense mutations had milder disease in all clinical aspects, often being diagnosed only through biopsy. However, no obvious genotype-phenotype correlation was detected despite our extensive clinical and genetic data. In conclusion, PXE may be significantly altered by environmental factors as intrafamilial variability suggests and secondly, a larger cohort may have to be examined to detect the most discrete genotype phenotype correlation.

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Selective hydrolysis of RAR activator tazarotene by human skin carboxylesterases

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Tazarotene is widely used for skin disorders such as plaque psoriasis and acne vulgaris. Upon absorption, this acetylenic retinoid undergoes rapid hydrolysis in the skin and results in the formation of tazarotenic acid. The hydrolytic metabolite interacts with the retinoic acid receptors (RAR) and exerts its therapeutic activity. In human skin, there are multiple forms of carboxylesterases. The aim of this study was to determine whether these carboxylesterases contribute similarly to the hydrolysis of tazarotene. Cells were transfected with a cDNA encoding a carboxylesterase and lysates were analyzed for the ability to hydrolyze tazarotene. Among three carboxylesterases assayed, HCE1 and HCE3 comparably hydrolyzed this ester whereas HCE2 showed little activity (~40 versus 6%). In the skin, HCE1 was abundantly expressed compared with HCE2 and HCE3, suggesting that tazarotene is predominately activated by HCE1 in the skin. A chimeric carboxylesterase containing three quarter sequence of HCE3 showed a comparable activity of the parent enzyme, suggesting that the N-terminal sequence determines the overall activity toward tazarotene. HCE2, compared with HCE1 and 3, has a 15-amino acid deletion in the so-called acyl pocket. To determine whether this sequence is actually responsible for higher hydrolytic activity of HCE1 and 3, a deletion mutant prepared with HCE1 exhibited an ~95% decrease in the hydrolysis of tazarotene, suggesting that the acyl pocket plays an important role in interacting with tazarotene (Supported by ES07965).

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Constitutive phosphorylated Smad3 interacts with Sp1 and p300/CBP in scleroderma fibroblasts

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Systemic sclerosis (SSc) is characterized by the excessive extracellular matrix (ECM) deposition in the skin, lung, or other organs, and numerous previous reports indicated the significance of autocrine TGF-β loop in the pathogenesis of SSc. In this study, we focused on the TGF-β signaling to understand the pathogenesis of SSc. Constitutive increased Smad3 phosphorylation was detected in SSc fibroblasts compared with normal fibroblasts. Increased interaction of Smad3 with Sp1 as well as p300 was also detected in SSc fibroblasts. The overexpression of Smad3 caused up to 5-fold increase in COL1A2 promoter activity in normal fibroblasts, while Smad3 caused a little increase in COL1A2 promoter activity in SSc fibroblasts. However, neither Smad2 nor Smad4 caused significant effects in COL1A2 promoter activity in normal fibroblasts or SSc fibroblasts. The overexpression of Sp1 caused further increase in COL1A2 promoter activity stimulated by TGF-β in normal fibroblasts, but did not change COL1A2 promoter activity in the presence of TGF-β in SSc fibroblasts. The combinatory overexpression of Smad3 and Sp1 enhanced TGF-β response significantly in normal fibroblasts as well as SSc fibroblasts. These results suggest that constitutive phosphorylated Smad3 interacts with Sp1 and p300/CBP in SSc fibroblasts and that SSc fibroblasts are less sensitive to exogenous TGF-β stimulation.

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Histamine enhances the production of granulocyte-macrophage colony-stimulating factor via protein kinase Cα and extracellular signal-regulated kinase in human keratinocytes

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The production of granulocyte-macrophage colony-stimulating factor (GM-CSF) in keratinocytes is highly related to the chronicity of atopic dermatitis. Histamine released from mast cells contributes to the cross-talk between mast cells and keratinocytes in skin inflammation. We examined the in vitro effects of histamine on GM-CSF production in human keratinocytes. Histamine increased GM-CSF secretion and mRNA level. Both GM-CSF mRNA stability and promoter activity were enhanced by histamine. The elements homologous to activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) on the promoter were responsible for the activation by histamine. Histamine enhanced transcriptional activity and DNA binding of AP-1 and NF-κB. In histamine-treated keratinocytes, AP-1 composition shifted from c-Jun homodimers to c-Fos/c-Jun heterodimers, and transient expression of c-Fos protein was revealed. Histamine rapidly induced the phosphorylation and degradation of inhibitory κB. Conventional protein kinase Cα translocated to membrane in histamine-treated keratinocytes. Histamine-induced increases of GM-CSF secretion, promoter activity, and mRNA stability were completely abolished by H1 antagonist pyrilamine and conventional protein kinase C inhibitor Gö6976, and partially suppressed by PD98059 which inhibits the activation of extracellular signal-regulated kinase, downstream of protein kinase C. Gö6976 and PD98059 suppressed histamine-induced c-Fos expression and enhancement of DNA binding and transcriptional activity of AP-1. Gö6976 completely and PD98059 partially suppressed histamine-induced enhancement of NF-κB transcriptional activity. Histamine-induced phosphorylation and degradation of inhibitory κB was suppressed by Gö6976, but not by PD98059. These results suggest that histamine may enhance GM-CSF production at transcriptional and posttranscriptional levels via H1 receptor, protein kinase Cα and extracellular signal-regulated kinase.

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Mechanical pressure-induced phosphorylation of p38 and c-jun NH_2-terminal kinase in epithelial cells

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Cells within human skin are permanently targeted by mechanical forces of different qualities (e.g. stretching and pressure). In contrast to stretching the underlying molecular signaling pathways which are involved in transduction of mechanical pressure are still enigmatic. In the present in vitro attempt epithelial cells were mechanically stimulated by teflon weights that were placed into the culture dishes. Cells were analysed after different time intervals using immunohistochemical methods, SDS-PAGE and Western blotting. Mechanical pressure applied for a maximum time of 20 min showed a peak phosphorylation for the stress-kinases p38 and c-jun NH_2-terminal kinase-1 (JNK1) between 5 and 10 min. In order to further dissect the signaling cascade, upstream and downstream regulators of p38 were examined. Downstream of p38 the phosphorylation of the small heat shock protein 27 (HSP27) was shown in response to pressure application. The pressure mediated phosphorylation of HSP27 could be blocked by p38 specific inhibitors. Additionally, we examined the suppression of pressure-induced p38 phosphorylation after blocking of protein kinase C by calphostin C. Furthermore, the involvement of small G proteins was investigated in the mechanical induced signaling cascade. Inhibitor studies dealing with toxin B as well as transient transfection experiments using dominant-negative constructs of Rac showed no effects on the pressure-induced p38 phosphorylation. Respectively, the pharmacologic inhibition of Src kinase circumvented the pressure-induced phosphorylation of JNK1 but not that of p38. These findings indicate that both stress-kinases are independently phosphorylated by distinct signal transduction pathways. Our experimental results indicate mechanical pressure as a new type of cellular stress which yields in p38 and JNK phosphorylation with implications to (patho)physiological conditions as acanthosis or the Koebner phenomena as well as tumor biology.

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Differential regulation of keratinocyte chemokinesis and chemotaxis through distinct nicotinic receptor subtypes

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Nicotinergic agents can act as both chemokines and chemoattractants for cell migration. Epidermal keratinocytes (KCs) both synthesize acetylcholine (ACh) and use it as a paracrine and autocrine regulator of cell motility. To gain a mechanistic insight into nicotinergic control of KC motility, we determined types of nicotinic ACh receptors (nAChRs) and signaling pathways regulating KC chemokinesis and chemotaxis, using respective modifications of the agarose gel KC outgrowth assay. Random migration of KCs was significantly ($p<0.05$) inhibited by hemicholinum 3, a metabolic inhibitor of ACh synthesis, as well as α-conotoxins MII and AuIB preferentially blocking α3-containing nAChRs. The use of nAChR subunit selective antisense oligonucleotides (AsOs) and knockout mice demonstrated pivotal role for α3β2 channel in mediating ACh-dependent chemokinesis. Signaling pathways downstream of α3β2 included activation of the protein kinase C isoform δ, and RhoA-dependent events. Chemotaxis induced by nicotinergic agents was most pronounced toward the concentration gradient of choline, a potent agonist of α7 nAChR. The α7-preferring antagonist α-bungarotoxin significantly ($p<0.05$) diminished KC chemotaxis, further suggesting a central role for α7 nAChR. This hypothesis was confirmed in experiments with anti-α7 AsOs, and α7 knock out mice. The signaling pathway mediating α7-dependent KC chemotaxis included elevation of intracellular calcium, activation of calcium/calmodulin-dependent protein kinase II and conventional isoforms of PKC, as well as engagement of Rac/Cdc42. Redistribution of α7 immunoreactivity to the leading edge of KCs upon exposure to choline preceded that of α3. The obtained results for the first time identify distinct nAChR types regulating chemokinesis and chemotaxis of KCs, and define signaling pathways mediating each function, which has clinical implications for wound healing and control of cancer metastases.

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Activation of store-operated channels in human epidermal keratinocytes requires phospholipase C γ1 and the inositol 1,4,5-trisphosphate receptor

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Store-operated calcium entry depicts the movement of extracelluar Ca^{2+} into cells through plasma membrane Ca^{2+} channels activated by depletion of intracellular Ca^{2+} stores. The members of the canonical subfamily of transient receptor potential channels (TRPC) have long been implicated as the molecular bases for store-operated channels (SOC). Here we characterize the regulation of native SOC and the expression of endogenous TRPC in human epidermal keratinocytes. Calcium entry in response to store depletion with thapsigargin was reversibly blocked by the membrane-permeable inositol 1,4,5-trisphosphate receptor (IP3R) inhibitor, 2-aminoethoxydiphenyl borane, and suppressed by the diacylglycerol analogue, 1-oleoyl-2-acetyl-sn-glycerol. Inhibition of phospholipase C (PLC) with U73122 or transfection of a PLCγ1 antisense cDNA construct completely blocked SOC activity. These findings indicate a requirement for IP3R and PLC, especially PLCγ1, in the activation of SOC. RT-PCR and immunoblotting analyses showed that TRPC1, 3, 4, 5 and 6 are expressed in keratinocytes. Knockdown of the level of endogenous TRPC1 or TRPC4 by antisense cDNA transfection inhibited SOC function, demonstrating their involvement in mediating store-operated calcium entry. Immunoprecipitation with antibodies against PLCγ1 and IP3R as well as an agarose-conjugated peptide containing the SH2SH2SH3 domain of PLCγ1 demonstrated the interaction of PLCγ1 and IP3R with TRPC1, but not with TRPC4. The association of TRPC1 with PLCγ1 and IP3R decreased in keratinocytes with higher intracellular Ca^{2+}, coinciding with a down-regulation in SOC activity. Our results demonstrate that the activation of SOC in keratinocytes depends, at least partly, on the interaction of TRPC with PLCγ1 and the IP3 receptor.

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ERK-independent uPA-stimulated p70 S6 kinase activation is triggered by overexpression of ganglioside GM3

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Overexpression of GM3, the predominant ganglioside of keratinocyte membranes, inhibits ligand-dependent and ligand-independent activation of epidermal growth factor receptor, leading to suppression of Ras/extracellular regulated kinase (ERK) signaling and, thus, to inhibition of cell proliferation. Paradoxically, we have recently shown that overexpression of GM3 facilitates proliferation in the presence of urokinase-type plasminogen activator (uPA). We hypothesized that overexpression of GM3 triggers ERK-independent signaling as the mechanism to stimulate cell proliferation in the presence of uPA. Using human squamous carcinoma (SCC12) cells with modulated ganglioside expression, we have found that GM3 promotes uPA-induced p70 S6 kinase activation. In contrast, depletion of GM3 by either overexpression of sialidase or treatment with PPPP leads to suppression of p70 S6 kinase activation. Inhibition of of p70 S6 kinase, but not Ras/ERK signaling, suppresses the stimulation of cell proliferation by GM3. In these GM3-overexpressing SCC12 cells, p70 S6 kinase phosphorylation is stimulated at the Thr-389, Thr-421/Ser-424, and Ser-411 sites of p70 S6 kinase, and is associated with activation of phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC)-zeta. Phosphorylation of the Thr-389 site requires PI3K activation, of the Ser-411 site PKC-zeta activation, and of the Thr-421/Ser-424 site both PI3K and PKC-zeta activations. These studies show GM3 to be a novel trigger of p70 S6 kinase activation in the presence of uPA via a mechanism that is independent of ERK signaling, yet leads to epithelial cell proliferation through stimulation of PI3K and PKC-zeta. Furthermore, the data regarding site-specific activation implicate PKC-zeta and PI3K as intermediaries in ERK-independent p70 S6 kinase phosphorylation.

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Tenascin-C up-regulation by transforming growth factor-beta in human dermal fibroblasts involves Smad3, Sp1, and Ets1

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In cultured human dermal fibroblasts, transforming Growth Factor (TGF)-beta induced the mRNA expression of tenascin-C (TN-C). The molecular mechanism(s) underlying this process are not presently understood. In this study, we performed serial 5' deletion and a transient transfection analysis to define a region in the TN-C promoter mediating the inducible responsiveness to TGF-beta. This region contains an atypical nucleotide recognition element for the Smad family of transcriptional regulators. A DNA affinity precipitation assay revealed that Smad2/3 bound to this site in a transient and specific manner. Overexpression of Smad3 or Smad4 activated the TN-C promoter activity and superinduced TN-C promoter activity stimulated by TGF-beta. Moreover, simultaneous co-transfection of Smad3 and Smad4 activated the TN-C promoter activity in a synergistic manner. Mutation of the Smad binding sites, the Ets binding sites or Sp1/3 binding sites in the TN-C promoter abrogated the TGF-beta/Smad-inducible promoter activity. Immunoprecipitation analysis revealed that Smad3, Sp1 and Ets1 form a transcriptionally active complex. Furthermore, the interaction between Smads and CBP/p300 in TGF-beta signaling was confirmed. These findings demonstrate the existence of a novel, functional binding element in the proximal region of the TN-C promoter mediating responsiveness to TGF-beta involving Smad3/4, Sp1, Ets1, and CBP/p300.

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Cholesterol depletion alters involucrin gene expression in epidermal keratinocytes through activation of p38

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Cholesterol has been recently suggested to regulate the early steps of keratinocyte differentiation through lipid rafts. In many cell types, depletion of cholesterol activates signaling proteins like EGFR, HER2 or ERK known to affect cell differentiation. In this study, we explored the effects of cholesterol depletion on the phenotype of cultured keratinocytes, using a treatment with methyl-beta-cyclodextrin (MbCD) to extract cholesterol and a treatment with lovastatin to inhibit cholesterol neosynthesis. Analysis of the expression of differentiation marker genes in early-differentiating confluent cultures reveals that cholesterol depletion induces downregulation of keratin 14 (K14) and keratin 10 (K10) and upregulation of involucrin. MbCD treatment induces phosphorylation of EGFR, HER2 and ERK, but not HER3. Inhibition of EGFR with PD153035 impairs the MbCD-induced phosphorylation of EGFR, HER2 and ERK, but does not impair the alteration of K14, K10 or involucrin gene expression, indicating that other signaling proteins regulate this phenomenon. p38 has been suggested to regulate the expression of involucrin during keratinocyte differentiation. We found that MbCD treatment induces a prolonged phosphorylation of p38 and that the inhibition of p38 with PD169316 impairs the upregulation of involucrin mRNAs, which might suggest that cholesterol depletion alters involucrin gene expression through activation of p38.

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Constitutive activation of Stat3 in keratinocytes is essential for development of psoriasis

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Psoriasis is a common skin disease characterized by epidermal hyperplasia with inflammation and angiogenesis. Although the pathogenesis remains elusive, it is known that re-epithelizing processes can recapitulate aspects of psoriasis. Previous studies have shown that Stat3 in keratinocytes is required for wound healing. In fact, psoriatic lesions from a majority of patients (19/21) demonstrated activated Stat3 in the epidermis, while Stat3 was inactive in other non-psoriatic inflammatory dermatoses with accompanying epidermal hyperplasia. Transgenic mice whose keratinocytes expressed a constitutively active Stat3 (K5.Stat3C) developed spontaneous psoriasiform lesions in the tails as early as two weeks of age. Spontaneous, hyperkeratotic lesions spread to the dorsum and hind feet in some mice. Furthermore, full thickness wounding, tape-stripping or topical treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA) induced this phenotype resembling human psoriasis. Keratinocytes from K5.Stat3C mice exhibited upregulation of VEGF, TGFα, cyclin D1, I.κBα, and ICAM-1, which likely contributed to the development of psoriasiform lesions. Targeted inhibition of Stat3 by topical treatment with a decoy oligonucleotide hindered the development of psoriasiform lesions. We conclude that constitutive activation of Stat3 in keratinocytes is essential for development of psoriasis, and that targeting Stat3 may be of potential therapeutic benefit in the treatment of this disease

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Characteristics of whole-cell patch clamping in cultured human keratinocytes

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Previous experiments using patch clamping in keratinocytes have revealed the presence of ionic channels. Recent analyses in these cells with in-vitro Ca2+ imaging and flow cytometry have demonstrated the functionality of ionic glutamate N-methyl D-aspartate receptors (NMDARs), which was dependent on the confluency of the cultures. However, no data are present on the whole-cell current characteristicss in human keratinocytes exposed to NMDA. In order to analyze NMDA-mediated currents, we performed whole cell patch clamping on cultured human keratinocytes with exposure to the specific agonist. Bath application of 100 µM NMDA evoked prominent current responses (peak current 150 pA & duration 1500 ms) in confluently grown keratinocytes voltage-clamped at +40 mV. Nonconfluently grown keratinocytes with gigaohm seals demonstrated no current responses to NMDA. The patchability index (the ratio of the number of cells that had gigaohm seals over the total number of cells that were attempted to be patched clamped), also widely differed between keratinocytes grown in a confluent and nonconfluent cultures. The patchability index was 2/57 (0.03%) for confluent cells and 6/35 (17.14%) for nonconfluently grown keratinocytes. Upon examining the differential infrared contrast images while patch clamping, confluently grown cells had a distinct relief pattern of the nucleus and cytoplasmic structures. However, the nonconfluent cells had a smooth relief pattern. In summary, confluently grown keratinocytes demonstrate prominent NMDA-mediated currents, while no NMDA-evoked currents are seen in nonconfluent cells. Furthermore, it is much more difficult to create gigaohm seals in confluent keratinocytes as compared to nonconfluent keratinocytes. This difficulty in patch clamping may arise from the fact that a confluent grown keratinocyte may have greater surface tension across its cell membrane.

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The p75 neurotrophin receptor signals apoptosis in a subpopulation of human keratinocytes

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The p75 neurotrophin receptor (p75NTR) belongs to the TNF-receptor superfamily and signals apoptosis in many cell settings. Human keratinocytes express both p75NTR and the high affinity neurotrophin receptor trk. While the NT nerve growth factor protects keratinocytes from apoptosis through trk, nothing is known on the role of p75NTR. The aim of the present study was to evaluate the expression and function of p75NTR in human keratinocytes. p75NTR was detected in a subpopulation of basal keratinocytes by single and double immunostaining. In particular, p75NTR staining did not overlap the expression of transglutaminase and keratin 10 (suprabasal) nor the expression of MIB (proliferating basal keratinocytes). p75NTR staining was negative in psoriatic lesions. p75NTR was more expressed in confluent (23.5%) than in subconfluent cells (5.7%), while it was absent in stratified cells, as shown by western blotting and FACS. Addition of Ca++ increased percentage of subconfluent keratinocytes expressing p75NTR (44.1%). Moreover, p75NTR was expressed in young transit amplifying cells while it was nearly absent in stem keratinocytes. Brain-derived neurotrophic factor (BDNF) and neurotrophin-4, which signal only through p75, induced keratinocyte apoptosis, as shown by MTT assay and TUNEL ($p<0.05$). This effect was partially blocked by anti-p75 antibody. The trk inhibitor K252 induced a higher rate of apoptosis in keratinocytes retrovirally transfected with p75cDNA than in mock-transfected cells ($p<0.01$). These results show that p75NTR signals apoptosis in keratinocytes. It would also appear that apoptosis induced by inhbition of trk is favored by a strong p75 expression. As trk is overexpressed in psoriasis, the absence of p75NTR expression could allow the lower rate of cell death observed in this dermatosis.

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Effect of NGF on matrix metalloproteinases and tissue inhibitors of metalloproteinases produced by human dermal fibroblasts and myofibroblasts

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Neurotrophin (NT) family comprises a group of functionally and structurally related proteins that play a key role in the development and survival of neurons. NT family includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5. All NT are produced by keratinocytes, which also express all NT receptors, except for trkB. NT exert proliferative and antiapoptotic effects on keratinocytes. Little is known however on the expression and function of NT in other skin cells. The aim of the present study was, at first, to evaluate the expression and function of NT and their receptors in human dermal fibroblasts (DF) and their differentiated type myofibroblast (MF). DF were cultivated in DMEM with 5% FBS. MF were obtained in vitro after treating DF with TGFβ1 (1 ng/ml) for 6 days. Both DF and MF synthesize and release all NT, as shown by RT-PCR and ELISA. In particular, DM express higher amounts of NT, as compared to DF (NGF, NT-3, BDNF $p<0.05$; NT-4 $p<0.01$). In addition, DF and MF express all NT receptors except for trkC. The NT low-affinity receptor p75 is more expressed in MF than in DF, as shown by western blot. NGF reduces production of collagen I and III by these cells ($p<0.05$). Moreover, DF release higher levels of matrix metalloproteinases-3 (MMP-3), MMP-10, MMP-13 and their inhibitors (TIMP-1) than MF ($p<0.05$), as shown by a proteome ELISA. NGF decreases significantly MMP-2 secretion in both DF and MF ($p<0.01$) since MMP-9 was only decreased in MF ($p<0.01$). On the other hand, NGF increases MMP3 in DF ($p<0.05$) and decreases it in MF ($p<0.01$). These results indicate that fibroblast and myofibroblast could participate in the complex NT network at the skin level either as a target or as a source for NT. In addition, NT are involved in the extracellular matrix remodelling and repair, thus playing a key role in both fibrotic processes and skin aging.

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Cell cycle arrest by CDK inhibitor CYC 202(R-roscovitine) in normal human keratinocytes is followed by induction of p38 MAPK phosphorylation and subsequent involucrin expression

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The aim of our study was to explore the antiproliferative effect of the CDK inhibitor CYC 202(R-roscovitine) on normal cultured human keratinocytes. We examined the effect of concentrations between 0,1mM and 200mM of this cell cycle inhibitor on cell viability, DNA synthesis, as well as the induction of differentiation and signal transduction in highly proliferative subconfluent keratinocytes and in growth-arrested confluent keratinocytes in autocrine cultures. Proliferative keratinocytes were more sensitive to this compound in term of cell viability than growth-arrested keratinocytes. In subconfluent conditions, CYC 202 inhibits cell proliferation in a dose-dependent manner, which is followed by induction of p38 MAPK phosphorylation. Phosphorylation of p38 MAPKis strongest at 20 mM CYC 202, when cell viability is lower and the activity of the other MAP kinases ERK 1 and 2 is inhibited. Because the natural occurence of growth arrest in normal epidermal keratinocytes is linked to the induction of cell differentiation, we analysed also the phenotype after CYC202 treatment. Prolonged inhibition of the cell cycle in subconfluent keratinocytes resulted in downregulation of the early differentiation marker keratin10 and in upregulation of late differentiation marker involucrin. The connection between p38 kinase activity and the expression of involucrin has been already suggested. We observe a similar link as a result of the inhibition of cell cycle progression in proliferating keratinocytes by the potent CDK inhibitor CYC202.

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Proliferation regulatory signal between SMAD 3 and MAPK p42/44

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Smad3 and MAPK p42/44 proteins are both transduction signals for TGF-β related factors. There are no definite answers about the possible crosstalk between these two proteins. We have previously reported that Smad3 null mice display accelerated healing of incisional wounds. Using this novel model for delayed wound closure, we studied the effect of wounding in Smad3 null and wild type mice. Full-thickness wounds measuring 0.3 by 1 cm were made down to fascia on the dorsal aspect of the mouse tail in Smad3 K-O mice and control littermates, approximately 1cm distal to the body of the animal. The wounds were left to heal by secondary intention and were assessed histologically by computerized planimetry for wound closure at various times after wounding. The wounds in wild-type mice displayed delayed healing, with full closure occurring between 14 and 25 days after wounding. Wounds in Smad3 null mice healed 30% faster ($p<0.01$). By immunostaining with ki67 a marker for proliferation, Smad3 null animals showed increased proliferation of dermal wound cells. By thymidine uptake assay, cultured dermal fibroblasts from Smad3 null mice showed increased baseline DNA synthesis and interestingly enhanced response to TGF-β1 compared to dermal fibroblasts from wild-type mice. Using Western blotting, we found that MAPK p42/44 is significantly more phosphorylated in fibroblasts cultured from Smad 3 K-O mice at baseline and in response to TGF-β1, suggesting that MAPK overcompensation together with loss of Smad3 may be involved in the modulation of faster healing. These results point to crosstalk between Smad 3 and MAPK p42/44, and that these two transduction proteins may control fibroblast proliferation and the response to injury.

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Fatty acids, thiazolidinediones, and VP16-PPARγ activate PPARγ in human keratinocytes and inhibit keratinocyte cell growth *in vitro*

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Human keratinocytes (HKC) grow vigorously when rendered essential fatty acid (EFA) deficient and are growth arrested by the EFAs 18:2 (linoleic acid) and 20:4 (arachidonic acid), n-6. Because fatty acids and derivatives are ligands for peroxisome proliferator-activated receptor γ (PPARγ), we hypothesize that this nuclear hormone receptor regulates HKC proliferation. Neonatal HKC express PPARγ mRNA and protein which increase in response to EFAs. HKC were then transfected with a PPAR-dependent promoter gene construct, FATP3x-tk-luciferase, containing three direct repeats of the PPAR response element subcloned upstream of the thymidine kinase promotor. Both ciglitazone (CIG, 10 µM) and troglitazone (TRO, 10 µM) activated transcription of the FATP3x-tk-luciferase reporter gene (3.5- vs. 6-fold, respectively), while the saturated fatty acid 16:0 (15, 25 and 35 µM), and the EFAs 20:4 and 18:2 (both at 5, 15 and 25 µM) also resulted in increased luceriferase activity. HKC were also transfected with a constitutively active form of PPARγ, VP16-PPARγ. Transfection with VP16-PPARγ induced a marked increase in PPRE-dependent luciferase activity when compared to control treated cells. EFA-supplemented media or CIG (0.1 to 10 µM) inhibited HKC DNA synthesis 50% (^{3}H-Tdr uptake), with BADGE (0.5 & 1.0 µM), a PPARγ inhibitor, reversing the inhibition. VP16-PPARγ also inhibited the growth of HKC by approximately 60%. In summary, we demonstrate that PPARγ is constitutively expressed and is functionally active in HKC. The EFAs 18:2, 20:4 and the synthetic PPARγ ligands, CIG and TRO, inhibit HKC growth. In addition, similar inhibition of HKC proliferation was achieved using a constitutively-active PPARγ. These data suggest that PPARγ is an important regulator of HKC proliferation and may serve as a potential therapeutic target in proliferative skin disorders.

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The role of telomere-based signaling in the management of metastatic carcinoma

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Telomeres, tandem repeats of TTAGGG that cap chromosomes, are central to senescence and apoptosis. Critical telomere shortening during aging leads to irreversible cell cycle arrest, and experimental disruption of the telomere loop induces apoptosis or senescence, depending on the cell type. To mimic telomere loop disruption, we provided an 11-base DNA oligonucleotide homologous to the telomere 3' overhang sequence (T-oligo) to a metastatic carcinoma cell line, PA-1, and compared its effect to a diluent and complementary oligonucleotide (negative control). Within 4 days, T-oligo (40uM) inhibited cell growth by >15 fold compared with diluent ($p<0.001$), as determined by cell yield. In addition, within 48 hrs, T-oligo induced S phase arrest of 43±2% of cells as compared to 14±.01% of diluent (FACScan analysis), and most T-oligo treated cells eventually became apoptotic (TUNEL). Within 7 days, the few remaining cells displayed large, spread morphology and positive staining for the senescence associated β-galactosidase activity. To determine T-oligo mediated signaling pathways, cells were stimulated as above, total cellular proteins were isolated for western blotting at different intervals up to 96 hrs after stimulation. Within 24 hrs, T-oligo induced the level of the tumor suppressor protein p53 as well as the level of active p53 (serine 15 phosphorylated), with a maximum of 300% and >30 fold induction respectively, within 72 hrs. Interestingly, within 96 hrs, T-oligo also induced >250% the level of the p53 homolog protein, p73. Furthermore, within 24 hrs, T-oligo induced phosphorylation of p95/Nbs1, a protein known to mediate S phase arrest in response to ATM kinase activation. We conclude that similar to DNA damage, T-oligo induces its effects on metastatic carcinoma cells via activation of at least three ATM kinase dependent proteins (p53, p73 and p95/Nbs1), leading to cell cycle arrest followed by apoptosis or senescence. Treatment with T-oligo may be a promising novel therapy for metastatic carcinoma.

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Differential regulation of karyopherin alpha 2 expression by TGF-β1 and IFN-γ in normal human epidermal keratinocytes

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To investigate the role of nuclear transport regulation, we studied the expression pattern of karyopherin a (KPNA) subtypes 1, 2, 3, 4 and 5, in normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF). Northern blot analysis demonstrated that both NHEK and NHDF expressed all subtypes of KPNAs. In both cell types, KPNA2, 3 and 4 seemed to be the major subtypes expressed, while KPNA 1 and 5 were minor in terms of relative mRNA expression. TGF-β1 (5 ng/ml) stimulation of NHEK resulted in the selective down-regulation of KPNA2 mRNA expression, and a 70% decrease in expression was observed after 24 hours. Conversely, IFN-γ (100 U/ml) stimulation of NHEK cells specifically up-regulated KPNA2 expression. Maximal induction, about 2.5 times higher than initial expression levels, was observed after 6 hours,and expression gradually declined to approximately 20% of the initial expression level, suggesting dual regulation of KPNA2 by IFN-γ. Nearly identical data was obtained when protein levels were assessed by Western blotting. Addition of cycloheximide, antagonized the effects of both TGF-β1 and IFN-γ on KPNA2 mRNA expression, indicating the requirement for ongoing protein synthesis. Because no specific changes were seen in NHDF, these observations suggest a tissue specific regulatory function for KPNA2 in NHEK. No other KPNAs were modulated by these cytokines. Our data suggest that KPNA2 may play important roles in the signal transduction pathways related to epidermal proliferation and differentiation.

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Inhibition of dermal fibroblast proliferation by phosphatidylethanolamine-binding protein (PEBP): repression of PEBP correlates with TGF-b-induced fibroblast proliferation

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Phosphatidylethanolamine-binding protein (PEBP) is a basic protein that has been identified as a novel Raf-kinase inhibitor. Transforming growth factor b (TGF-b) is a multifunctional cytokine that has proliferative effects on fibroblasts. However, while the most attention has been concentrated on clarifying the mechanism whereby TGF-b inhibits cell growth, far less attention has been given to elucidating the mechanism of growth stimulation by TGF-b. In this study, we revealed that overexpression of PEBP significantly inhibits serum-induced cell growth of normal human dermal fibroblasts (NHDF), and TGF-b1 significantly promote the proliferation of the NHDF, while both PEBP mRNA and protein expression were reduced indicating a co-relation between enhancement of cell growth of NHDF and reduction of PEBP expression by TGF-b1. TGF-b1 induced the phosphorylation of ERK1/2 in a transient manner, and the activation of ERK1/2 was not observed at the later time points. We propose that reduced PEBP expression may make fibroblasts susceptible to growth factors or cytokines. It will be of significance to investigate the molecular status of PEBP in a certain proliferative disease, such as keloid, in which TGF-b has been reported to play a pathogenic role for hyperproliferation of dermal fibroblasts.

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Srcasm modulates EGF signaling and proliferation in human keratinocytes

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Srcasm (Sm) is a recently identified molecule containing a VHS membrane binding domain and several tyrosine motifs which interact with Grb2, PI-3 kinase, and Src-family kinases. To further characterize the role of Sm in keratinocyte biology, studies using adenoviruses (Ad-Sm or Ad-GFP) were performed on human primary keratinocytes. Under standard growth conditions with EGF, Sm is tyrosine phophorylated in human keratinocytes. Growth factor depletion of keratinocytes for 24 hours decreased Sm tyrosine phosphorylation to near undectable levels, while EGF stimulation rapidly promotes Sm tyrosine phosphorylation within 5 minutes. The EGF-dependent tyrosine phosphorylation of Sm is inhibited by pretreating keratinocytes with AG112, an inhibitor of EGF receptor kinase activity, and PP2, a Src-kinase inhibitor. The appropriate control compounds do not significantly alter the EGF-induced phosphorylation of Srcasm. Immunoprecipitation of the EGF receptor from keratinocyte lysates also immunoprecipitated Sm, suggesting that these two molecules may be part of the same molecular complex. Sm activates Src-kinases in a dose-dependent manner, and Sm preferentially activates Src and Fyn but not Yes. The ability of Sm to activate Src-kinases is inhibited by PP2, supporting the hypothesis that Sm phosphorylation at Y457 by Src kinases is important for Sm-dependent activation of Src-kinases. Sm also potentiates the EGF-dependent activation of Src-kinases in keratinocytes. Under standard culture conditions, higher Sm levels increased the amount of activated p44/42 MAP kinase in keratinocytes; subsequent EGF stimulation led to a rapid decrease in levels of activated p44/42 within Sm-infected cells. Increased levels of Sm induced decreased BrdU incorporation in primary keratinocytes, and were associated with an increased percentage of cells in G2/M and a decreased percentage of cells in S phase. These results demonstrate that Sm may modulate growth-regulatory pathways in human keratinocytes and play a role in epidermal homeostasis.

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Effects of mechanical stimuli on mitogen-activated protein kinases phosphorylation

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Human skin is exposed to mechanical stimuli such as stretching and consecutive relaxation by muscles induced forces. Epithelial cells were mechanically stimulated in an *in vitro* stretching device including silicon rubber chambers. We established three different models (A, B, C) in order to study the effect of stretching and relaxation on phosphorylation of ERK1/2, p38 and c-jun NH_2-terminal kinase (JNK1/2). In model (A) cell relaxation was studied by withdrawal of stretch. In model (B) cells were slowly stretched for about 3 hours and then immmediately relaxed. In model (C) a break of about 4 hours between stretching and relaxation was inserted. Based on the relaxation time protein lysates were generated and analysed by SDS-PAGE and Western-blotting. In model (A) maximum phosphorylation of ERK1/2 appeared after 10 min while JNK1/2 peaked after 20 min. In contrast to this almost no phosphorylation of p38 was detected. In model (B) equal phosphorylation of ERK1/2, p38 and JNK1/2 was measured showing a biphasic activation peaking after 5 min and 30 min. In model (C) maximum phosphorylation of ERK1/2 appeared after 10 min while JNK1/2 peaked at 15 min after relaxation. No phosphorylation of p38 was detected in model (C). Our experimental results showed that different stretch-relaxation regimes lead to distinct phosphorylation patterns of ERK1/2, p38 and JNK1/2. These findings indicate that MAPK-signaling is differentally regulated by mechanical stimuli and may have implication for the molecular understand of abdominal skin reduction after pregnancy.

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Calcium induced keratinocyte differentiation requires *src* and *fyn* activation of phosphatidylinositol 3-kinase

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We have previously demonstrated that phospholipase C-γ1 (PLC-γ1) is required for calcium-induced keratinocyte differentiation. In the present study, we investigated whether the activation of PLC-γ1 by non-receptor kinases such as *src* and *fyn* plays a role in mediating calcium-induced keratinocyte differentiation. We found that treatment of cells with a *src* family inhibitor PP2 or the expression of the combination of dominant negative *src* and *fyn* prevented the activation of PLC-γ1 as well as the subsequent induction of the keratinocyte differentiation markers involucrin and transglutaminase by calcium. The activation of PLC-γ1 by calcium was seen as early as 5 minutes after calcium administration. The increase in PLC-γ1 activity was accompanied by an increase in the enzymatic activities of c-*src* and *fyn* within minutes, and a subsequent increase in c-*src* protein level within hours. However, unlike the activation of PLC-γ1 by epidermal growth factor, calcium activation of PLC-γ1 was not a result of tyrosine phosphorylation. To determine whether calcium activates PLC-γ1 through a non-phosphorylation mechanism such as phosphatidylinositol trisphosphate (PIP_3), we examined the effects of calcium on phosphatidylinositol 3-kinase (PI3K), the enzyme that produces PIP_3 from PIP_2 in the membrane. In particular, we determined whether calcium induced the phosphorylation of the p85α regulatory subunit of PI3K, and found that it did. The phosphorylation of p85α paralleled the increase in PI3K activity as expected. The induction of phosphorylation of p85α was blocked by the *src* family inhibitor PP2 or the expression of the combination of dominant negative *src* and *fyn*. Inhibiting PI3K with PI3K inhibitors LY294002 and wortmannin blocked calcium activation of PLC-γ1 as well as the induction of keratinocyte differentiation markers involucrin and transglutaminase. These data indicate that calcium activates PLC-γ1 via increased PIP_3 mediated by c-*src* and *fyn* activated PI3K, and this activation is required for calcium-induced keratinocyte differentiation.

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Mouse keratinocytes are relatively resistant to UV-light and chemotherapy-induced apoptosis compared to human keratinocytes

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Despite widespread use of transgenic mouse models to study carcinogenesis, few investigators directly compare proliferating mouse keratinocytes (mKCs) to human (hKCs). Since many insights into regulation of apoptosis and pathogenesis of skin cancer have derived from animal models, we characterized apoptotic responses of mKCs versus hKCs using stimuli implicated in either the cause of cancer (UV-light), or treatment of cancer (adriamycin). mKCs were obtained from CELLnTEC (Bern) and grown in serum-free medium (CnT-02) yielding dividing cells with doubling time of 24 hrs. hKCs were grown in serum-free medium (KGM, Clonetics), which had similar morphology and growth curve to mKCs. However, apoptotic responses of these proliferating cell types were different. When hKCs are exposed to UV-light over a dose range of 5 to 100 mJ/cm^2 there is progressive increase in apoptotic cells (24 - 72 hrs) as assessed by annexin staining, or PI staining and DNA content analysis by FACS with Sub-G_0 levels. At 50 mJ/cm^2 over 75% of all hKCs are apoptotic by 48 hrs. By contrast, mKCs are almost completely resistant to apoptosis using UV doses of 15 and 30 mJ/cm^2, and even at 50 mJ/cm^2 less than 25% of cells are apoptotic after 48 hrs. When KCs were treated with DNA damaging agent-adriamycin (0.1 µg/ml; 48 hrs), hKCs were approximately 2 fold more susceptible to apoptosis compared to mKCs. To determine if medium influenced results, murine KCs were grown in KGM, but this did not enhance their apoptotic responses. To probe the mechanism underlying these unexpected results, we focused on the tumor suppressor gene, p53, as a well-known regulator of apoptosis in response to UV-light and DNA damage. hKCs contained higher levels of p53 by Western blot analysis as well as by promoter reporter assays for several target genes including p21 and GADD45 when compared to mKCs. These results indicate caution is warranted when extrapolating apoptotic responses from mice to human skin, since cultured mKCs are significantly more resistant to apoptosis induced by either UV-light or adriamycin.

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UV irradiation activates epidermal growth factor receptor via reactive oxygen-mediated inhibition of receptor type protein tyrosine phosphatase

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Ultraviolet (UV) irradiation rapidly activates epidermal growth factor receptor (EGFR) protein tyrosine kinase (PTK) activity, which drives UV-induced signaling in human keratinocytes (KC). UV irradiation generates reactive oxygen species (ROS) in cells. All protein tyrosine phosphatases (PTP) are highly susceptible to inhibition by ROS due to redox-sensitive catalytic cysteine residues. We investigated the contribution of ROS-mediated PTP inhibition to UV-induced EGFR activation in KC. Membrane fractions prepared from KC were supplemented with ATP and magnesium, and EGFR activation was measured by tyrosine autophosphorylation, using Western blot. Addition of EGF activated EGFR 5-fold ($p<0.05$) in the membrane fraction. Addition of hydrogen peroxide activated EGFR 12-fold ($p<0.05$), and concurrently decreased phosphatase activity 2-fold ($p<0.05$). Addition of PTP inhibitors orthovandate or vanadate also activated EGFR 18-fold and 25-fold, respectively ($p<0.05$), providing evidence that inhibition of membrane-associated PTPs results in EGFR activation. Specific EGFR kinase inhibitor PD169540 blocked stimulation of EGFR tyrosine phosphorylation by both hydrogen peroxide and vanadate, demonstrating that EGFR tyrosine phosphorylation was dependent on EGFR tyrosine kinase activity. To further examine the ability of ROS to activate EGFR and inhibit PTPs, isolated KC membranes were irradiated with visible light in the presence of rose bengal, which generates singlet oxygen. Irradiation activated EGFR 20-fold, and coincidently inhibited membrane-associated PTP activity 3-fold ($p<0.05$). Removal of non-integral membrane proteins by 0.5M NaCl extraction of the membranes did not alter ROS induction or EGFR activation. Our data support the concept that UV generated ROS inhibits integral membrane receptor type PTP activity resulting in increased EGFR tyrosine phosphorylation in KC. Receptor type PTPs are a novel target for antioxidant protection against UV-induced skin damage.

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Activation of β-catenin and c-myc inhibits epithelialization in chronic wounds

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Lack of understanding of molecular pathogenesis of impaired healing in chronic ulcers leads to a serious health problem that contributes to excessive limb amputations and mortality. Using biopsies from patients with chronic wounds, skin organ culture and primary keratinocytes in culture we identified that β-catenin and its downstream target, c-myc, play important role in development of chronic wounds. In contrast to normal epidermis, we observed significant nuclearization of β-catenin and elevated c-myc expression at the non-healing wound edge of patients with chronic ulcers. In vitro studies indicated that activation and stabilization of nuclear β-catenin inhibits wound healing and keratinocyte migration by: blocking EGF response and inducing c-myc. Using Affymetrix large scale microarrays we found that β-catenin downstream target, c-myc, is induced in skin by an inhibitor of wound healing (glucocorticoids) and repressed in the initial phase of normal wound healing, 4 to 48hr, whereas it becomes de-repressed at 96hr post wounding. Therefore, the activation of β-catenin/c-myc pathway(s) contributes to impaired healing by inhibiting of keratinocyte migration and altering keratinocyte differentiation. The presence of activated β-catenin and c-myc in the epidermis of chronic wounds may serve as molecular markers of impaired healing and future targets for therapeutic intervention. While β-catenin signaling has been implicated in epithelial development and oncogenesis its role in wound healing has never been postulated. This further illustrates the importance of tissue context specificity, because β-catenin in the context of malignant tissue promotes invasion whereas in the context of a wound environment does the opposite.

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Telomere overhang-induced DNA damage signals involve activation of the Fanconi anemia/BRCA pathway

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Ubiquitylation of the protein FANCD2, which is mutated in the Fanconi anemia (FA) complementation group D2, marks the activation of the recently described FA/BRCA DNA damage response pathway, known to be induced by ionizing radiation and DNA crosslinking agents. This pathway involves gene products of various complementation groups of FA, a cancer-prone chromosome breakage disorder, and BRCA1 and BRCA2, which are mutated in familial breast cancer. Upon ubiquitylation, FANCD2 forms nuclear foci with BRCA1, NBS1, MRE11, and Rad50, and activates DNA repair through homologous recombination and DNA end-joining. Until now, the FA/BRCA DNA damage response pathway has not been clearly linked to the well-characterized DNA damage responses mediated via p53 and its downstream effectors. Since previous reports from our laboratory demonstrate that oligonucleotides homologous to the telomere 3′-overhang sequence (T-oligos) activate p53-mediated DNA damage responses, we studied the effect of T-oligos on the FA/BRCA pathway and its relation to p53 responses. As early as 12 hours after adding an 11-base T-oligo to cultured primary human fibroblasts, but not after adding complementary oligonucleotides, a prominent shift was observed from FANCD2-S-isoforms (non-ubiquitylated) to FANCD2-L-isoforms (ubiquitylated) in Western blots, indicating an activation of the FA/BRCA pathway by the T-oligos. Maximum activation was observed at 24 hours, with degradation of FANCD2 on days 2, 3, and 4. Phosphorylation of FANCD2, which is part of a separate, ATM-dependent pathway, was not observed with the T-oligo, as there were no band shifts and no reactivity with a phospho-specific FANCD2 antibody. Activation (serine-15 phosphorylation) and induction of p53 by T-oligo was significantly abrogated in primary fibroblasts from FA patients. This indicates that p53-mediated responses and the FA/BRCA responses, which have been considered to be part of two independent damage signaling pathways, actually act in concert, and suggest that activation of the FA/BRCA pathway has effects upstream of p53 activation.

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O-glycosylation of epidermal catenins: cloning and characterization of the fruit fly and murine O-GlcNAc transferases

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O-glycosylation at serine and threonine residues has been shown to modify and regulate a variety of intracellular proteins. We describe the cloning and characterization of the *Drosophila melanogaster* and *Mus muscularis* O-glycosyltransferases and their expression in transgenic organisms and cell lines. Both enzymes are structurally similar. The *Drosophila* OGT (dOGT) cDNA and the murine (mOGT) cDNA encode proteins whose predicted amino acid sequences are 73% identical to each other and 80% and 83% identical to human OGT, respectively. The protein expressed from the dOGT transgene has an intracellular distribution localizing to both the cell cytoplasm and nucleus. *In vivo* expression of the dOGT transgene results in increased O-glycosylation of intracellular proteins. O-glycosylation of mammalian β-catenin and the *Drosophila* homolog Armadillo were observed. The high degree of similarity between the fly and mammalian OGTs implies a fundamentally important regulatory role.

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Interleukin-1β induce *c-fos* gene expression through a mechanism involving ERK/p38 mitogen-activated protein kinases-MSK1-CREB activation

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Interleukin-1β (IL-1β) plays a central role in acute and chronic inflammation. At the transcriptional level, IL-1β promotes expression of a variety of immediate-early-response genes like *c-fos*. In turn, immediate-early-response genes influence the expression of secondary response genes contributing to the phenotypic response of the cell to proinflammatory stimuli. It is known that stimulus-dependent gene transcription of *c-fos*, is mediated via several cis-acting elements, including at least three cAMP response elements and a serum response element. However, the precise mechanisms by which IL-1β induce *c-fos* gene expression remain unclear. Here we show that inhibition of either ERK or p38 mitogen-activated protein kinases (MAPK) abolished IL-1β-induced *c-fos* gene expression in human HaCaT keratinocytes as revealed by northern blotting and by use of a *c-fos*-promoter-containing reporter construct. We further provide evidence that the p38-MAPK- and ERK-activated kinase MSK-1 is required for full *c-fos* gene transcription, as well as for the phosphorylation of cAMP response element-binding protein (CREB). In contrast, the adenylate cyclase activator forskolin-, or exogenous prostaglandin E2-induced *c-fos* gene transcription was barely detectable in HaCaT keratinocytes, and indomethacin, an inhibitor of cyclo-oxygenase-1/2, only marginally abolished IL-1β-induced CREB phophorylation and *c-fos* gene expression. In conclusion, these results show that MSK1 is an important p38-MAPK- and ERK-activated mediator of IL-1β-stimulated *c-fos* induction. In addition, they indicate that MSK1 could act through CREB-phosphorylation to archieve c-fos promoter activation, while IL-1β-induced prostaglandin secretion may only marginally contribute to *c-fos* gene expression. Thus, these studies provide additional insights into the complex molecular regulation of the prototypical immediate-early-response gene *c-fos*.

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Urokinase-type plasminogen activator expression by sphingosylphosphorylcholine in cultured human dermal fibroblasts and its role in wound model

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Sphingosylphosphorylcholine (SPC) is a bioactive sphingolipid metabolite that can enhance wound healing. Although SPC is known to act as a mitogen in wound healing, the precise mechanism underlying the acceleration of wound healing remains to be elucidated. Plasminogen activator system is well known to affect wound healing by regulating the fibrinolytic environment of the wounded area. In the previous study, we demonstrated the SPC markedly induced the expression of plasminogen activator inhibitor-1 (PAI-1) in human dermal fibroblasts, at both the transcriptional and translational levels. In this study, we further examined the effect of SPC on urokinase-type plasminogen activator (uPA) expression in human dermal fibroblast cultured in vitro. ELISA showed that SPC significantly induced the release of uPA from human dermal fibroblasts. Consitent with this, Northern blot analysis indicated that SPC markedly increased the mRNA level for uPA. In in vitro wound model, SPC markedly enhanced the proliferation and migration of fibroblast in a time- and dose-dependent manner, leading to fast closure of wounded area. In addition, SPC-stimulated wound closure was significantly attenuated by neutralization with anti-uPA antibody. These results suggest that SPC could enhance the wound healing process through the induction of uPA, with the stimulatory effect on cell migration.

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Insulin like growth factor-II-mediated cyclooxygenase-2 gene expression in human keratinocytes through extracellular signal-regulated kinase pathway

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Psoriasis is a chronic disease characterized by abnormal epidermal proliferation and inflammation. IGF-II is increased in psoriatic lesions, especially in the serum or blister fluid of psoriasis patients. To examine the relationship between IGF-II and the inflammatory response involved in psoriasis, we monitored inflammatory factor COX-2 expression in the IGF-II treated human keratinocytes and explored the IGF-II signaling pathways with respect to the expression of COX-2. IGF-II induced COX-2 mRNA and COX-2 protein level in the primary normal keratinocytes and HaCaT. The upregulation of COX-2 expression by IGF-II was reduced by pretreatment with inhibitors of tyrosine kinase, Src and PI3-kinase. The ERK inhibitor, PD98059, also reduced the increased expression of COX-2 genes by IGF-II, whereas the p38 inhibitor, SB203580, did not. To further examine the roles of these two MAPKs in IGF-II-induced COX-2 expression, we performed COX-2 promoter analysis using dominant negative plasmids of MEK1 and p38. Although IGF-II increased COX-2 promoter activity approximately 2.5-fold, this increase was blocked by cotransfection with dominant negative MEK1 mutant. However, dominant negative p38 mutant did not block the IGF-II-induced COX-2 promoter activity. In addition, inhibition of ERK with PD98059 reduced the increases of IGF-II-induced PGE2 synthesis or cell proliferation. These results suggest that IGF-II induces COX-2 expression through the tyrosine kinase-Src-ERK and tyrosine-kinase-PI3-kinase pathways, but not via the p38 MAPK pathway.

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Delineating the role of PKCα in skin proliferation, differentiation and wound healing utilizing PKCα null mice

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PKCα, a classical PKC isoform, is implicated in proliferation and differentiation of keratinocytes in vitro and in the wound healing process in vivo. We have further delineated the role of PKCα isoform in skin, by utilizing PKCα knockout (KOα) mice. The KOα mice are born viable and are fertile. Furthermore, skin gross appearance and morphology is normal. Mouse keratinocytes isolated from wild type (WT) and KOα mice were characterized in vitro. Both WT and KOα keratinocytes attached normally to the culture dish. In addition, proliferation of KOα keratinocytes as measured by thymidine incorporation was similar to WT cells. Next, we studied the differentiation potential of WT and KOα cells by elevating calcium concentrations in the growth medium. In comparison to WT cells, differentiating KOα keratinocytes expressed lower levels of spinous markers keratin 1 and 10 with increased expression of Loricrin, a granular differentiation marker. Overexpression (OE) of PKCα confirmed these results. While OE of PKCα dramatically induced expression of spinous differentiation markers Keratin 1 (K1) and Keratin 10 (K10), granular markers expression was abolished. We have next subjected WT and KOα mice to wound healing studies. Full skin incisions were made on the back of mice and wounds were followed for 7 days. In comparison to WT mice, wounds of KOα mice induced a marked hyperpalstic response associated with improved epidermal closure. Improved wound healing parameters were studied by immunohistochemical analysis of serial sections at the wound gap, including expression of markers of proliferation (PCNA), basal layer formation (K14), differentiation (K1, K10, Loricrin and Filaggrin), migration (K6) and wound strength (bursting chamber). Altogether, these results suggest PKCα as a main regulator of keratinocyte spinous differentiation as well as inhibitor of granular differentiation in vitro. Furthermore, PKCα has an inhibitory role in the epidermal wound healing processes in vivo.

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Protein kinase Cδ serves as a divergence point in down stream insulin signaling in skin keratinocytes

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Impaired insulin signaling as occurs in diabetes results in development of skin complications. We have previously characterized the biological actions of insulin in skin utilizing mouse keratinocytes and identified a novel role of PKCδ in mediating insulin induced keratinocyte proliferation. Binding of insulin to the insulin receptor (IR) induces tyrosine phosphorylation and activation of the receptor. This results in recruitment and activation of the insulin receptor substrate (IRS) family of proteins represented mainly by IRS1 and IRS2. IRS proteins become platforms for other signaling molecules, such as PI3K (Phosphoinositide 3-Kinase). We have further characterized the down stream elements associated with insulin stimulation in primary murine keratinocytes. In Insulin treated keratinocytes IR, IRS1 and IRS2 are phosphorylated and activated in a dose and time dependent manner. In keratinocytes, insulin induces PKCδ association with IR. Overexpressing PKCδ induces PKCδ-IR association, coupled to activation of IR. Whereas inhibition of PKCδ activity by overexpressing D/NPKCδ abolishes PKCδ-IR association and enhances IR activation. In addition, PKCδ associates with IRS2, but not with IRS1. Furthermore, overexpressing WTPKCδ, inhibits IRS2 phosphorylation and induces IRS1 phosphorylation suggesting an inhibitory role for PKCδ in mediating IRS2 signaling pathway. In concordance with these results inhibiting PKCδ by overexpressing D/NPKCδ, reduces PKCδ-IRS2 association, induces IRS2 phosphorylation while IRS1 phosphorylation is reduced. We also demonstrate that overexpression of WTPKCδ inhibits IRS2-PI3K association while D/NPKCδ increases IRS2-PI3K association. In conclusion: Activation of PKCδ drives insulin signaling toward an IRS1 dependent pathway while blocking PKCδ activity leads to IRS2-PI3K dependent pathway. These results suggest PKCδ activation as a divergence point in mediating down stream insulin effects in keratinocyte physiology.

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Disruption of lipid rafts causes apoptotic cell death in HaCaT keratinocytes

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Lipid rafts are cholesterol-enriched microdomains in plasma membranes. The functional activity of many membrane proteins, including death and growth factor receptors, depends on their insertion in lipid rafts. We have previously demonstrated presence of lipid rafts in keratinocytes and shown that lipid rafts are involved in the control of keratinocyte proliferation and metabolic activity. In this work we investigated the effect of lipid raft disruption on HaCaT keratinocyte survival. Lipid rafts could be disrupted or rearranged with cholesterol targeting detergents: methyl-β-cyclodextrin and filipin III. Moreover, cholesterol oxidation by a specific oxidase or blocking of cholesterol synthesis by mevastatin had a similar effect on lipid rafts. All cholesterol modifying substances caused cell death in a concentration-dependent manner. More detailed studies on the effects of cyclodextrin revealed apoptotic cell death in concentrations >0.5 % (w/v). The molecular mechanism of apoptosis precipitated by raft disruption awaits further studies.

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STAT3 regulates proliferation and differentiation in primary keratinocytes

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STAT3 is a member of a family of Signal Transducers and Activators of Transcription associated with diverse biological actions. In previous studies we identified STAT3 as a downstream mediator of insulin and PKCδ signaling in keratinocyte proliferation. Both tyrosine phosphorylation (tyr 705) and serine phosphorylation (ser 727) were shown to be critical in regulation of STAT3 activation state and the functional consequences associated with induction of transcription. In the present study we have investigated the contribution of STAT3 expression including the significance of its serine and tyrosine phosphorylation sites in keratinocyte proliferation and differentiation. Experiment was performed in primary murine keratinocyte cultures utilizing wild-type STAT3 (WTSTAT3), STAT3 tyrosine inactive mutant (DNSTAT3) and STAT3 serine 727 inactive mutant (SmS) recombinant adenoviruses. Differentiation was induced by elevating calcium concentrations in the growth medium. Overexpression of WTSTAT3 induced proliferation as analyzed by thymidine incorporation and expression of PCNA, a nuclear marker of cell proliferation. Moreover, overexpression of WTSTAT3 inhibited expression of calcium induced spinous differentiation markers (Keratin 1 and Keratin 10) as well as inhibited expression of granular differentiation markers (Loricrin and Filaggrin). In concordance with these results overexpression of DNSTAT3 increased levels of both spinous differentiation markers (Keratin 1 and Keratin 10) as well as granular differentiation markers (Loricrin and Filaggrin). Interestingly, serine mutant STAT3 increased spinous differentiation markers with no changes observed in the levels of Loricrin and Filaggrin expression. Both tyrosine and serine mutants did not affect keratinocyte proliferation. Altogether these results suggest an active role for STAT3 in mediating keratinocyte proliferation and initiating the differentiation program in keratinocyte physiology.

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The inhibition of mastocytosis mast cell growth using KIT kinase inhibitors

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The autoactivating (D816V) point mutation in the KIT receptor is associated with adult mastocytosis. One strategy for treating adult mastocytosis is to inhibit the signaling pathways of autoactivated KIT with protein kinase inhibitors. Our previous studies using Jak3 and MAPK inhibitors (Whi-P131 and UO126) on human mastocytosis mast cell lines (HMC-1 and RMT) have shown that JAK3/STAT3 is the predominate signaling pathway in KIT initiated mast cell growth. Addition of the JAK3 specific inhibitor Whi-p131 (20µM) to cell culture for 3 days reduced the growth of mastocytosis mast cell line HMC-1 to 38±7%, as compared to cells in medium alone. To potentiate this finding, PKC inhibitors (Safingol and GÖ6976),PI3K inhibitor (Wortmannin) and STAT3 inhibitor (AG490) were added to mast cell cultures with or without Whi-P131. The presence of Safingnol (5µM), Wortmannin (5µM) and GÖ6976 (5µM) in mast cell culture for 3 days inhibited HMC-1 growth by 80±7%, 36±5% and 65±3% respectively. Although, a high concentration of AG490 (50µM) did not inhibit mast cell growth, a combination of AG490 (50µM) with Whi-P131 (20µM) did enhance the growth inhibitory effect of Whi-P131 on HMC-1 to 78±11%. The JAK3/STAT3 pathway involves the activation of JAK3 followed by the activation of STAT3 dimmers, leading to gene expression and cell proliferation. It appears that a combination of the JAK3 and STAT3 specific inhibitors, Whi-P131 and AG490 are more effective than each individual drug used alone in the inhibition of mastocytosis mast cell growth. Moreover, when Whi-P131, AG490 and a combination of the two inhibitors were added to normal neonatal skin cell culture controls for 3 days, the growth inhibitory effect was less prevalent, 15±4%, 16±4% and 34±4% respectively. These results further confirm that the JAK3/STAT3 pathway is important in the transduction of the mutated (D816V) KIT receptor, but not in normal KIT receptors. Thus, targeting mastocytosis mast cells expressing mutated KIT with a combination of JAK3 and STAT3 specific inhibitors may prove to be useful in the treatment of adult mastocytosis.

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JNK activation cooperates with oncogenic Ras to induce invasive human epidermal neoplasia

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The c-jun N-terminal kinase (JNK) MAP kinase cascade acts in parallel with the NF-kB pathway downstream of TNFR1 to regulate morphogenesis in a variety of tissues. In human epidermis, only 2 genetic alterations - NF-kB blockade along with oncogenic Ras - are sufficient to trigger invasive neoplasia. In mediating these effects, NF-kB inhibition both bypasses Ras-triggered CDK4 down-regulation and cellular senescence to permit uncontrolled proliferation as well as enables tumor growth independent of exogenously added matrix. We recently observed that epidermal NF-kB blockade leads to JNK activation and that this augmented JNK activity is essential for the increased proliferation seen in this setting. These data raised the possibility that JNK induction represents the major functional effect of inhibiting epidermal NF-kB function and that activation of the JNK cascade might be sufficient to replace NF-kB blockade as an enabler of Ras-driven epidermal neoplasia. To address this, we examined the ability of simultaneous activation of Ras and JNK pathway elements to drive tumorigenesis in human epidermal tissue grafted to immune-deficient mice. To induce JNK cascade function, both constitutively active JNK isoforms and active MKK7, the JNK pathway MAPKK activator immediately upstream of JNK proteins, were used. JNK cascade activation recapitulated all the effects of NF-kB blockade, with Ras-JNK pathway tumors indistinguishable from spontaneous human squamous cell carcinoma (SCC). Tumorigenesis via both Ras-JNK induction as well as Ras-NF-kB blockade was dependent on intact AP1 function because a dominant-negative c-jun mutant abrogated tumorigenesis, consistent with a broad role for JNK-AP1 in Ras-driven human epidermal neoplasia. In spontaneous human SCCs, clear evidence for JNK activation was also detected in 75% of samples examined (n=12). These data indicate that JNK cascade induction enables Ras-driven human epidermal tumorigenesis and identifies this pathway for further investigation as a possible therapeutic target in human SCC.

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Potential downstream targets of over-expressing RhoC in human melanomas

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Over-expression of RhoC in various human cancers has been correlated with metastatic stage of tumor progression and poor prognosis of cancer patients in most cases. RhoC has also been identified as one of the genes that enhance metastasis in human melanoma cell line A375, and mouse B16 melanoma cell line (Clark et al. 2000, Nature). The purpose of this work was to understand how RhoC over-expression contributes to human melanoma metastasis.

We have established stable transfectants over-expressing RhoC, using a human radial growth phase melanoma cell line, WM35. To identify potential downstream targets of over-expression of RhoC in WM35 cells in a global way, we performed Affymatrix microarray assays using human U133A chip. We identified 73 potential genes as downstream targets of RhoC over-expression. Some of these genes are tumor progression markers, and many have well-defined functions in migration, detachment, and invasion of cells. Unexpectedly, we also found significant changes in some genes involved in immune surveillance. Our preliminary FACS analysis confirmed that over-expression of RhoC down-regulated these genes involved in immune surveillance. These data suggest that RhoC over-expression might promote melanoma metastasis by modulating important functional characteristics of migration, detachment and invasion, and also by enhancing tumor immune escape. These studies identified a potential novel mechanism by which RhoC over-expression might promote melanoma metastasis.

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A potential role for the aquaporin-3/phospholipase D2 signaling module in keratinocyte differentiation

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Aquaporin-3 (AQP3) is a member of the aquaporin family of water channels; however, it belongs to the subfamily of aquaglyceroporins that also transport glycerol. In fact, AQP3 efficiently transports glycerol but is quite inefficient in water transport. We recently demonstrated that AQP3 is associated with phospholipase D2 (PLD2) in caveolin-rich membrane microdomains. PLD is known to catalyze both a hydrolysis reaction to produce phosphatidic acid and also, in the presence of primary alcohols, a transphosphatidylation reaction to generate phosphatidylalcohols. We have also shown that PLD2 can utilize the physiological primary alcohol glycerol to form phosphatidylglycerol (PG) via this transphosphatidylation reaction. We hypothesized that AQP3 provides glycerol to PLD2 to produce PG and that this signal acts to induce keratinocyte differentiation. Since the transport activity of AQP3 is inhibited at low pH in other systems, we determined the effect of low pH on radiolabeled glycerol transport and PG production in keratinocytes. Both glycerol uptake and PG synthesis were inhibited at low pH (pH 4). We also performed co-transfection experiments in which an empty vector or AQP3 was transiently introduced into keratinocytes simultaneously with reporter constructs composed of keratinocyte differentiation or proliferation promoters directing expression of a luciferase reporter gene. Overexpression of AQP3 decreased the promoter activity of the proliferative marker keratin 5 and increased the promoter activities of the differentiative markers, keratin 10 and involucrin. In addition, low concentrations of added glycerol (0.02 to 0.1% volume:volume) inhibited DNA synthesis. Higher concentrations (0.1 to 1%) also decreased DNA synthesis, whereas the same concentrations of xylitol, as an osmotic control, had little or no effect. Thus, our results support the idea that in epidermal keratinocytes AQP3 supplies PLD2 with glycerol for synthesizing PG and that this signaling module may mediate induction of keratinocyte differentiation.

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Blockade of ERK-dependent autocrine keratinocyte proliferation by Src family iinase inhibitors and calcium

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c-Src potentiates proliferation, survival, and invasiveness in response to activation of ErbB1 in human mammary carcinoma cells overexpressing c-Src and ErbB1. A key mediator of these events is activation of Tyr 845 of ErbB1 by c-Src, with subsequent activation of STAT5. Here we demonstrate that normal human keratinocytes (NHK) express c-Src and c-Yes at levels comparable to mammary carcinoma cells, and that Tyr 845 undergoes phosphorylation in response to high concentrations of EGF (10-100 ng/ml). Low concentrations of either of two Src family kinase (SFK) inhibitors (PP1 or PD173952) markedly reduced EGF-stimulated Tyr 845 phosphorylation. Both inhibitors also blocked autocrine proliferation of NHK in a way that could not be overcome by EGF. However, autocrine growth of NHK is driven by very low concentrations of ErbB ligand(s) (< 1 ng/ml), suggesting that Tyr 845 phosphorylation may not be relevant to the process of autocrine NHK growth. Interestingly, the MEK activation inhibitor U0126 also inhibited autocrine NHK growth in a way that could not be overcome by EGF. Moreover, PP1 and PD173952 potently inhibited ErbB-mediated ERK phosphorylation under autocrine conditions and in response to low concentrations of EGF (1ng/ml). Increasing the calcium concentration from 0.1 to 0.2 mM dramatically inhibited autocrine ERK activation and autocrine proliferation of NHK. However, unlike the effects of SFK inhibitors, the effects of calcium on proliferation were readily reversed by EGF. Taken together, these observations suggest that calcium acts upstream of ErbB1 to inhibit autocrine ERK activation, which in turn inhibits proliferation. In contrast, SFKs appear to act downstream of ErbB1, coupling activation of ErbB1 to activation of ERK. By promoting phosphorylation of ErbB1 Tyr 845, overexpression of ErbB1 and c-Src may allow carcinoma cells to rewire this highly calcium-sensitive, ERK-dependent pathway of autocrine proliferation to a STAT-dependent pathway that is calcium-resistant.

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Cytokeratin expression in co-culture of bone marrow cells and skin fibroblasts

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We have placed lineage negative (Lin-) selected bone marrow cells obtained from C57BL/6 mice over adherent feeder cells derived from skin in order to the determine the effect of these feeder cells on bone marrow cells. The adherent feeder cells were primary fibroblast cultures derived from human neonatal foreskin. Both early and middle to late passage feeder cultures were used. Feeder cells also consisted of early passage neonatal foreskin fibroblasts that were previously transduced with a retroviral vector conferring high level constitutive expression of either Tranforming Growth Factor beta-1 or Epidermal Growth Factor. FACS analysis was performed on the adherent and non-adherent cells in culture at 2 and 5 days. Cells were analyzed for expression of cytokeratin, a marker for epidermal cell differentiation. Cytokeratin expression was detected in the non-adherent cells. No detectable expression of cytokeratin was noted in the (adherent) feeder cells. The retroviral transduced feeder co-cultures yielded a higher percentage of cytokeratin expression in the non-adherent cells. These studies indicate that skin fibroblast cultures may have the ability to alter the phenotype of bone marrow derived cells.

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Increased apoptosis in cultured fibroblasts from different aged donors

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Aging is characterized by loss of cells in the whole organism. The cause of this decrease in somatic cell number is unknown. Since somatic cells accumulate damage as we age, one hypothesis for cell loss is that they undergo programmed cell death. Fibroblasts from different aged donors were investigated to determine the levels of programmed cell death in the cultures. Caspase-3 activity is a marker of mitochondrial directed programmed cell death or apoptosis. Using tow different assay, ELISA and flow cytometric, we observed increased caspase-3 activity in fibroblast from different aged donors. Mitochondria membrane potential also was lower in the higher donor ages. In addition oxygen consumption decreased with donor age and reactive oxygen species increased with donor age. These data suggest that the mitochondria function appeared to be defective which occurs during apoptosis. The increased levels of reactive oxygen species resulted in increased oxidative damage to DNA and RNA in the form of 8-oxoguanine. The levels of 8-oxoguanine also increased with donor age. The fibroblasts from increasing donor age were senescencing as demonstrated by increase beta-galactosidase staining with donor age. Therefore in aging cells we observed increased apoptosis

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Overexpression of Smad7 in keratinocytes accelerates cutaneous wound healing

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Transforming growth factor-beta (TGF-beta) has both positive and negative effects on cutaneous wound healing. Smad7 acts as a major downstream antagonist of TGF-beta signaling in keratinocytes and its role in wound healing has not been defined. We have established a Smad7 transgenic mouse line using a keratin 5 (K5) promoter (K5.Smad7) which expresses Smad7 transgene at a mild level (~2 fold of the endogenous Smad7 in the skin). These mice did not have overt skin defects as shown from our previous Smad7 transgenic mice expressing much higher levels of the Smad7 transgene (EMBO J 2002, 21:2580-90). K5.Smad7 mice from the above low expressor line and non-transgenic littermates were subject to 6-mm full-thickness excisional wounding. K5.Smad7 mice exhibited early scab rejection, reduced inflammation, and accelerated re-epithelialization as compared with non-transgenic mice. In order to study stage-specific effects of Smad7 on wound healing, we generated a transgenic model in which Smad7 transgene expression can be induced in the epidermis and hair follicles (gene-switch-Smad7) by topical RU486 application. Smad7 induction after excisional wounding reduced inflammatory responses through suppression of a variety of inflammatory cytokines/chemokines in gene-switch-Smad7 mice when compared to control mice. Overexpression of Smad7 exhibited accelerated re-epithelialization, which likely correlated with increased expression of metalloproteinases and elevated Erk (extracellular signal-regulated kinases) signaling in the leading epidermal edges in gene-switch-Smad7 wounds compared to control wounds. Prolonged Smad7 induction after excisional wounding reduced dermal fibrotic response and angiogenesis in the dermis, resulting in accelerated tissue repair. We conclude that the effects of Smad7 on wound healing are likely due to blocking the inhibitory effects of TGF-beta on cutaneous wound healing.

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Smad2 gene deletion in the epidermis and hair follicles causes alopecia in aging mice

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Smad2 is a major intracellular mediator for the signal transduction of TGFβ, a cytokine that plays a pivotal role in the maintenance of skin homeostasis. The precise role of Smad2 in the skin remains largely unknown. Mice carrying germline deletion of the Smad2 gene caused early embryonic lethality, precluding their usage to further unravel its function in the skin. Taking advantage of a Cre-LoxP system (K5.CrePR1/Smad2$^{LoxP/LoxP}$), we have established a conditional knockout model that allows inducible deletion of the Smad2 gene in the epidermis and follicular epithelia (K5.Smad2$^{-/-}$) upon RU486 application. In the present study, RU486 was topically applied to the entire skin of neonatal K5.CrePR1/Smad2$^{LoxP/LoxP}$ mice to delete the Smad2 gene at birth. These mice did not exhibit overt phenotypes until they were 6-month-old. By that time most of the K5.Smad2$^{-/-}$ mice started to develop patchy baldness on the dorsal skin. The bald areas are well circumscribed and have a clear border with non-alopecia area. This phenotype grossly resembles alopecia areata in humans. None of control littermates, including wildtype and monogenic mice (K5.CrePR1 or Smad2$^{LoxP/LoxP}$) underwent similar hair loss. Histological analysis revealed numerous mononuclear cells infiltrated into and surrounding telogen hair follcles of the lesional skin. Interestingly, inflammatory cell infiltration also existed in the non-lesional area. Immunohistochemistry demonstrated that the infiltrated mononuclear cells are mainly CD4 lymphocytes and macrophages. In addition, significantly increased apoptotic cells were found in hair follicles specifically within the areas of hair loss in the K5.Smad2$^{-/-}$ mice. The epidermis of Smad2 knockout skin was slightly hyperplastic whereas epidermal differentiation remained normal. These phenotypes were similar to those observed in autoimmune-associated alopecial areata in humans. Taken together, our study suggests that normal Smad2 involved TGFβ signaling in the epidermis may play an essential role in the maintenance of immune homeostasis in the skin.

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Effect of the IGF-1R on UVB-induced signal transduction

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UVB irradiation of human keratinocytes causes damage to the cellular genome. How keratinocytes respond to UVB-irradiation is dependent of a variety of factors, including the UVB dose, cell-cell interactions, cell-ECM interactions, and the presence of specific growth factors. We have previously demonstrated at least three distinct responses of keratinocytes to UVB irradiation; 1.) complete repair of UVB-damaged DNA and continued cellular proliferation; 2.) no repair of UVB-damaged DNA and induction of apoptosis; or 3.) incomplete repair of UVB-damaged DNA and the induction of stress-induced senescence. The induction of apoptosis or stress-induced senescence is influenced by the activation state of the IGF-1R. Keratinocytes with activated IGF-1 receptors are more likely to be induced to undergo stress-induced senescence following UVB-irradiation, while keratinocytes irradiated without activated IGF-1R are more likely to undergo apoptosis. In this study, we have investigated differences in UVB-induced signal transduction pathways in keratinocytes with active and inactive IGF-1 receptors. When the IGF-1R is inactive in normal keratinocytes and the cells irradiated with UVB, specific changes in the phosphorylation status of the pro-apoptotic protein BAD were observed. Phosphorylation of serine 112 of the BAD protein is correlated with inhibition of apoptosis. In IGF-1R-active keratinocytes, no change in the phosphorylation status of serine 112 of the BAD protein was observed; however, phosphorylation of serine 112 on the BAD protein was significantly decreased in UVB-irradiated keratinocytes containing inactive IGF-1 receptors. These changes in BAD phosphorylation correlate with the increased sensitivity to UVB-induces apoptosis in IGF-1R-inactive keratinocytes.

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Mechanisms of HOXA7 and HOXA5 regulation of keratinocyte apoptosis

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The critical barrier function of the constantly renewing epidermis depends on the character and abundance of cells in each of the layers. The underlying architecture arises from the balance between the rate of keratinocyte mitosis and cell cycle withdrawal, and by the rate and specific program of differentiation. It is therefore important to understand the tight genetic regulation of proliferation, differentiation and apoptosis, processes that are perturbed following injury, through the course of inflammatory and neoplastic diseases, and through the action of topically applied medications. Homeobox transcription factors regulate differentiation and cell fate during development, and in tissues undergoing continuous renewal through proliferation and differentiation. Of the clustered, or class I homeobox genes reportedly expressed in keratinocytes, we detected predominately HOXA7 and HOXA5. We have documented the inhibition of keratinocyte differentiation by HOXA7, and the activation of apoptosis by overexpressed HOXA7 and HOXA5. Importantly, transduction with vectors harboring antisense, or truncated HOXA7 and HOXA5 cDNA attenuate both virally-induced and ultraviolet light (UV)-induced apoptosis, suggesting a role for the endogenous HOX genes in apoptotic activation or sensitization. We have now found that the protection against apoptosis by antisense HOXA7 and HOXA5 expression is associated with a concomitant increase in NF-kB expression. HOXA5 and HOXA7 overexpression trigger apoptosis in association with increased p53 activation and p21 expression, elements of the intrinsic apoptosis cascade. In addition, blocking jun-N-terminal kinase (JNK) activity, a kinase important in the UV induction and activation of p53, substantially blocks HOX activation of caspase-3, a measure of apoptosis. The HOX-induced apoptotic signal also responds to abrogation of caspase-8 activity, indicating simultaneous extrinsic, or TNF receptor family-mediated apoptosis activation, or cross-talk between the intrinsic and extrinsic pathways.

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A single cell suspension of hEGF-transfected autologous keratinocytes can improve reepithelialization of porcine full-thickness wounds

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In large wounds rapid wound closure is critical to reestablish barrier function of the skin. We investigated whether a single cell suspension of autologous keratinocytes (KC) applied to porcine full-thickness wounds can facilitate the formation of a normal epithelium, and serve as gene transfer vehicles. 1.5 x 1.5 cm full-thickness wounds were created in three Yorkshire pigs, 4-months old and filled with single cell solutions with either 1.25 x 10E5 or 3 x 10E5 autologous, or hEGF-transfected autologous KC. Control wounds received normal saline. Wounds were covered with vinyl chambers, ensuring a wet wound environment. Wound fluid was collected daily, wound contraction was measured every third day. H&E sections from biopsies taken on day 8 to determine re-epithelialization. EGF in wound fluids was measured by ELISA. The transplantation of 1.25 x 10E5 keratinocytes did not improve wound healing. However, on day 8, re-epithelialization in wounds transplanted with 3 x 10E5 KC was 46.3% in wounds receiving autologous KC, 58.2% with hEGF-transfected KC, and 39.15% in saline control wounds. Differences between KC treated groups and the saline control, and between untransfected and EGF-transfected KC wounds were statistically significant. Wound contraction did not differ between the groups. Wound fluid showed elevated hEGF levels in hEGF-transfected allogenic KC wounds, ranging from 4574 pg/ml on day 1, 7901 pg/ml on day 2, 2908 pg/ml on day 3 and then reduced on day 4 from 904 pg/ml to 74 pg/ml on day 9. No hEGF expression could be detected in the untransfected or saline control wounds. We were able to demonstrate that single cell solutions of KC can function as gene transfer vehicles for EGF cDNA and are able to further improve the rate of reepithelialization.

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Resveratrol-induced modulation of MEK-ERK signaling and cell cycle regulation in human epidermoid carcinoma A431 cells

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Resveratrol (trans-3, 4', 5-trihydroxystilbene) is a naturally occurring polyphenolic phytoalexin found in grapes, peanuts, seeds, and berries with promising cancer chemopreventive potential. To probe its mechanism of action we investigated the effects of resveratrol on cell cycle regulation and proliferation in the human epidermoid carcinoma A431 cell line. Resveratrol treatment (25 microM - 100 microM) of these cells resulted in a time- and dose-dependent accumulation of cells in G1/S. The resveratrol-induced G1/S arrest was associated with marked inhibition of cell cycle regulatory proteins, including cyclins A and D1 and cyclin-dependent kinase (cdk) 4 and 6. Cell cycle arrest occurred concomitantly with the accumulation of hypophosphorylated Rb and induction of p53 and the cdk inhibitor p21Waf1/Cip1. Resveratrol treatment specifically increased the phosphorylation of p53 at Ser15, but not at Ser20 or Ser392, residues known to regulate the stability, and thus the level, of the protein. p21Waf1/Cip1 was increased as detected by Western Blot and real-time RT-PCR. Resveratrol inhibited the proliferation of A431 cells as shown by a 2.5-fold decrease in [3H]-thymidine incorporation after 48 hours. To further investigate the upstream signaling components underlying the anti-proliferative effects of resveratrol we assessed the activation of extracellular signal-regulated kinases 1/2 (ERK1/2) that are linked to cell proliferation and survival in various cancers, including skin cancer. ERK activation is also shown to transcriptionally induce the cyclin D1 gene. Addition of resveratrol to A431 cells attenuated the phosphorylation-dependent activation of ERK1/2 through the MEK-ERK signaling pathway. These results suggest that resveratrol may act at multiple sites to inhibit cell proliferation and these effects may underlie its efficacy as a cancer chemopreventive agent.

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βadrenergic signaling pathways regulate dermal fibroblast migration and proliferation

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Understanding mechanisms that regulate cutaneous cell migration and proliferation is important in wound healing. Emerging studies point to a role of the adrenergic signaling pathway in this process. Although earlier work identified a role for catecholamines in vitiligo and atopic dermatitis, our laboratory has focused on their role in wound repair. We recently showed that ERK phosphorylation in keratinocytes is remarkably attenuated by β2 adrenergic receptor (β2 AR) activation, resulting in marked diminution of migration and ability to re-epithialize wounds in vitro (Pullar et al, JBC 278:22555, 2003). These findings imply that adrenergic signaling impairs wound healing. However, dermal fibroblasts are also required for wound repair, thus we extended our studies to these cells. In contrast to the effect in keratinocytes, we found that the migratory speed of isolated dermal fibroblasts was increased 31% over control in response to βAR activation. Likewise, fibroblast wound healing in vitro using the scratch assay was increased 23%. βAR activation also resulted in a 55% increase in fibroblast proliferation. The EGF receptor signaling pathway was transactivated by βAR activation, with a 2-fold increase in receptor phosphorylation within 15 min after βAR activation, There was a downstream activation of ERK, which likewise increased 2-3 fold by βAR activation. Cytoskeletal consequences of βAR activation included decreased actin and vinculin localization within focal adhesions, consistent with a more motile phenotype. The active cAMP analog, sp-cAMP, mimicked the effects of β-agonists on cell proliferation and cytoskeletal rearrangement, whereas the inactive analog, rp-cAMP, prevented the βagonist-mediated changes in the cytoskeleton and partly prevented the increase in proliferation. In conclusion, βAR activation in dermal fibroblasts is promotogenic and pro-mitogenic and partly cAMP-dependent, in contrast to its effects in keratinocytes, where it is anti-motogenic and cAMP-independent. Exploiting these divergent β-agonist responses in cutaneous cells may have novel therapeutic approaches for the control of wound healing.

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Differential roles of insulin and IGF1 in skin—an organotypic coculture model

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Diabetes is characterized by impaired insulin signaling, hyperglycemia and development of chronic complications. Among diabetes chronic complications, skin ulceration takes a staggering cost. Yet the problem of impaired wound healing is among the least known. In previous studies we have found that lack of IR expression results in impaired skin proliferation and differentiation, suggesting a role of impaired insulin signaling to the development of skin complications. To further investigate the role of insulin in skin we have used a unique model of 3D organotypic skin coculture. In this model murine skin keratinocytes are plated on top of a fibroblasts-containing collagen gel. Within a couple of weeks the cells reorganize into a skin equivalent structure, obtaining the histological characteristics of skin in vivo. Using this model we investigated the effects of insulin and IGF1 on skin organization. Adding insulin to the organizing organotypic coculture resulted in thicker epidermis. In addition, there seem to be an enhanced maturation of the skin, however the polarization and organization of the insulin treated coculture was maintained. In contrast, adding IGF1 to the coculture resulted in lack of polarization and abnormally delayed differentiation. The organotypic coculture shows incomplete polarization; no basal compartment was formed; only an initial organization of a spinous and granular compartments were identified; and keratin pearls, indicators for skin immaturity, were observed.

To further support the role of insulin in skin we examined the keratinocytes lacking the expression of insulin receptor (IR). The organotypic epidermis composted from IR null cells appeared thinner in comparison to WT coculture. Both insulin and IGF1 stimulation of the IR null organotypic coculture led to immaturity of the organotypic cocultured skin. These results demonstrate that insulin and IGF1 have distinct roles in skin, and impairment in their signaling or in the balance between the two hormonal signals might lead to abnormal wound healing in Diabetes.

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Opposite migratory responses of human keratinocytes and dermal fibroblasts to serum and plasma: implications in skin wound re-epithelialization

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Human keratinocytes (HKs) and human dermal fibroblasts (HDFs) represent the most abundant cells in skin. In intact skin, HKs and HDFs are bathed in a filtrate of plasma. In the wound bed, however, the cells come into contact with serum for the first time. We have recently shown that human serum promotes polarization and migration of HKs, whereas human plasma does not (LANCET, 361:574-576). These data suggest that serum factors may be responsible for the transition of HKs from non-motile to motile cells. In the current study, we found that HDFs and HKs respond to human serum and human plasma in opposite ways. While human serum promotes HK migration, it shows little motility-stimulating effect on HDFs. In contrast to its null effect on HKs, plasma significantly stimulates HDFs. TGF-beta inhibits HK division but does not inhibit HK migration (Sarret et al. J Invest Dermatol, 1991). We found that TGF-beta (3-10 ng/ml) completely blocks both plasma-stimulated and PDGF-BB-stimulated HDF migration. In contrast, TGF-beta (up to 100 ng/ml) has no inhibitory effect on human serum-stimulated or bovine pituitary extract (BPE)-stimulated HK migration. Human Cytokine Array analyses (RayBiotech, Inc.) showed that human serum contains higher amounts of TGF-beta than human plasma. Blocking TGF-beta in serum with a pan neutralizing anti-TGF-beta (1, 2 and 3) antibody transforms the serum into a pro-migratory agent for HDFs, just as serum is for HKs. This study reveals a novel mechanism, by which TGF-beta in human serum may determine the migratory behaviors of HKs and HDFs. This finding may be relevant to skin wound re-epithelialization.

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Metalloproteinase-mediated, context-dependent activation of EGF-like growth factors in human keratinocytes

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Human keratinocytes (KC) express multiple EGF-like growth factors (EGFs) including amphiregulin (AR), heparin-binding EGF (HB-EGF), TGF-α, betacellulin, and epiregulin in an autocrine fashion. All of these EGF receptor (EGFR) ligands are produced as membrane-bound precursors and require proteolytic cleavage to produce the soluble, active forms. We hypothesized that the presence of multiple ligands might reflect the existence of multiple mechanisms that become active in different cellular contexts. To test this hypothesis, we treated GF-deprived KC with the metalloproteinase (MP) inhibitor GM6001 (40 μM), the ErbB receptor tyrosine kinase inhibitor PD158780 (1 μM), or neutralizing antibodies (Abs) against various EGFs (5 μg/ml), followed by assays for cell migration, proliferation and ERK phosphorylation. KC migration in scratch wound assays was markedly inhibited by GM6001 and PD158780, and additively inhibited by neutralizing Abs against AR, HB-EGF and TGF-α (total blockade > 90%). Addition of EGF restored KC migration in the presence of a cocktail of blocking Abs, or GM6001, but not PD158780. Autocrine proliferation of KC was also blocked by GM6001 and by PD158780. However, in contrast to the involvement of multiple EGFs in KC migration, autocrine proliferation was selectively blocked by anti-AR Abs. GM6001, PD158780, and anti-AR mAb each reduced proliferation by more than 78 % (n=2-3). In contrast, neutralizing Abs against HB-EGF and TGF-α had only modest effects on proliferation (< 15% reduction; n=3). Autocrine ERK phosphorylation was also selectively and potently blocked by anti-AR Abs. In contrast, lysophosphatidic acid (LPA)-induced ERK phosphorylation was only prevented by anti-HB-EGF Abs. Autocrine and LPA-stimulated ERK phosphorylation could be blocked by GM6001 and restored by EGF. Taken together, these data indicate that autocrine KC signaling is strongly dependent upon MP-mediated cleavage of multiple EGFs, and that different ligands (and possibly different MPs) fulfill this role in different cellular contexts.

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Smad4 is required in the maintenance of hair follicle integrity

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Smad4 is a common signaling mediator of the transforming growth factor β (TGFβ) superfamily, which includes TGFβ, activins, and bone morphogenetic proteins (BMPs). Since the TGFβ superfamily plays a pivotal role in epidermal homeostasis, Smad4 is expected to mediate the functions of these molecules in the skin. We have generated keratinocyte specific (Keratin 5 promoter) knockout mice in which the Smad4 gene was deleted in the epidermis and hair follicles. Mice with homozygous Smad4 deletion in embryonic keratinocytes were born normally, but did not develop the first coat of hair. These mice grew retarded and remained hairless. The numbers of hair follicles in these mutant mice were similar to those in their wildtype controls, indicating that Smad4 deletion in keratinocytes does not preclude initiation of hair follicle morphogenesis. However, the follicles were disoriented and disorganized. Smad4 mutant follicles were not observed to form fully differentiated Inner Root Sheaths (IRS) and hair shafts at any age. The Smad4 mutant follicles were degenerate as early as 1 week old. In contrast, the sebaceous glands did not regress and accumulated in the skin. By 30 days, the epidermis of the mutant mice became hyperproliferative, but epidermal differentiation was not altered. At this stage, the hair follicle further degenerated into cysts and canals. Staining of hair follicle differentiation markers such as hair-specific acidic keratins (AE13), trycohyalin, and GATA-3 showed a patchy and disintegrated pattern. Our study suggests that normal Smad4 function in keratinocytes is essential for hair follicle differentiation and regeneration.

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The initiation, augmentation and directionality signals for human keratinocyte migration

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Extracellular matrices (ECMs) and growth factors (GFs) are known to act synergistically to stimulate cell motility. However, their functional relationships still remain elusive. We investigated the problem using HK migration driven by type I collagen and a mixture of soluble growth factors (containing bovine pituitary extract, insulin, EGF, transferrin and hydrocortisone) that exhibit optimal pro-motility activity in our in vitro assays. Our defined migration conditions were: 1) HKs plated without ECMs in the absence of GFs. 2) HKs plated with ECMs in the presence GFs; 3) HKs plated on collagen or other pro-motility ECMs without GFs; and 4) HKs plated on the same ECMs with GFs. Our results show that HKs migrate on a collagen matrix even in the absence of GFs. This indicates that a collagen matrix alone is able to initiate HK migration without any GFs! In contrast, however, GFs alone cannot initiate HK migration when the HKs are not plated on collagen or any other ECM. This shows that GFs alone cannot promote HK migration. Nevertheless, GFs do play a role in HK motility. First, GFs augment collagen-initiated motility by three fold. Further, GFs provide directionality to HKs migrating on ECM. To gain insights into the specificities of the actions of a collagen matrix and GFs on HK migration, we compared side-by-side the roles of the three major MAPK cascades, ERK1/2, p38 and JNK. We found that ERK1/2 mediates collagen and GF signaling. p38 is specific for only GF signaling, but not ECM signaling. JNK signaling does not play a role in HK motility. Double inhibition of p38 and ERK1/2 additively blocks HK migration. Simultaneous activation of p38 and ERK1/2 partially mimics the pro-motility effects of collagen and GFs. This study clarifies the functional relationships of GFs and ECMs in the control of HK motility and shows how they differentially regulate major MAPK signaling cascades required for optimal HK motility.

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A novel ERK-p38 MAPK complex controls keratinocyte cell death and survival—the role of individual p38 and ERK isoforms

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MAP kinase (MAPK) cascades are key regulators of cell growth, differentiation and survival. In keratinocytes, ERK kinases function to promote cell proliferation and survival. In contrast, p38δ MAPK drives keratinocyte differentiation and apoptosis. Thus, the interplay between p38δ and ERK1/2 MAPK isoforms is a critical determiner of keratinocyte fate. Multiprotein complex formation is an important mechanism for controlling the specificity of the signals transmitted by the MAPK cascades. An important and unique finding of the present study is the presence, in keratinocytes, of an ERK1/2-p38δ complex. This complex is constitutively present in both untreated and treated keratinocytes. Significant ERK1/2 activity, but little p38δ activity, is observed in untreated cells; however, treatment with various stimuli including TPA, okadaic acid or PKCδ overexpression, results in a reduction in ERK1/2 activity and an increase in p38δ activity within the complex. This finding alters some common assumptions regarding regulation by MAPKs, as MAPK cascades have been envisioned as linear regulatory pathways with minimal crosstalk among MAPKs (ERK, p38, JNK, etc). We now report that p38δ preferentially interacts with ERK1 while p38α interacts with ERK2. Remarkably, these p38 family members appear to differentially control the activities of the ERK isoforms - stimulation of p38δ activity results in ERK1 inactivation, whereas p38α activates ERK2. In addition, p38δ expression correlates with increased ERK1, but not ERK2, protein concentration, suggesting that p38δ may directly control the level of expression of its binding partner. This is an interesting observation, as MAPK level is not generally known to be regulated by upstream activators. Moreover, the p38δ-ERK1 complex translocates to the nucleus in response to p38δ activation where p38δ remains active and ERK1 is inactive. The biological outcome of this p38δ/ERK1 regulatory crosstalk is the cessation of keratinocyte proliferation and induction of apoptosis.

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Nerve growth factor expression as a requirement for normal skin regeneration

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Skin equivalent is a common model to study wound healing, skin function, etc. We construct dermal equivalent in triplicate with the use of five psoriatic from plaques and five healthy adult fibroblast lines. We placed foreskin epidermis on top of DE (dermal equivalent) after 24 hours. Media was changed every 24 hours and full skin equivalent was fixed cut and IHS was performed with NGF antibody on the 7th, 10th and 14th days. The result was assessed on the scale from 1 to 9. Statistical analysis was performed. We found skin equivalent expresses NGF at the 7th day in the area of beasel membrane and beasal keratinocytes with 4+ in normal and 7+ in psoriasis. On the 10th day psoriasis skin equivalent expresses stronger NGF in beasel membrane zone and beasel keratinocytes 8+ and 5+ in beasel area of normal skin equivalent. Mature 14th day psoriatic skin equivalent still express NGF in the same area 9+ and almost not expressed in mature normal skin equivalent. We conclude that NGF supports epidermal maturation through internal mechanism. After maturation of skin NGF is turned off in normal regeneration. In psoriasis automatic switch off of NGF does not happen supporting the overgrowth of epidermis as a symptom for psoriasis.

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Insulin signaling is essential for proper wound healing of skin

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Wound healing of skin involves complex interactions among many cell types, including skin epithelial keratinocytes, dermal fibroblasts and vascular endothelial cells. Any impairment in wound healing might lead to devastating results. One of the diseases associated with impaired wound healing is abnormal insulin signaling and diabetes, however the pathogenesis leading to diabetic skin pathology is not understood.

Recently, to investigate the role of insulin in skin we have studied insulin signaling in primary murine skin epidermal keratinocytes and dermal fibroblasts. We have found that insulin is essential for skin cell proliferation and for epidermal differentiation. Furthermore, lack of insulin receptor (IR) expression led to decreased proliferation and abnormal differentiation of skin. In order to investigate the clinical relevancy of these findings we studied the wound healing process in an epidermal skin-specific IR knockout mouse. Lack of IR expression in the epidermis led to a striking defect in the wound healing process. The strength of the healed wound was reduced by two folds compared to the normal littermates. Interestingly, the wound healing process deteriorated with age, worsening in mice aged 12 months and older. It should be emphasized that the expression of the insulin receptor in the dermal fibroblasts was normal, and the skin pathology resulted from lack of IR expression only in the epidermal keratinocytes. In conclusion, our results indicate that insulin signaling is essential for skin proliferation and differentiation and that impairment in insulin action in keratinocytes might lead to the development of skin pathologies. The epidermal skin specific IR knockout mouse can serve as an idle model for identifying the pathological processes leading to impaired diabetic wound healing, and as a model for testing new treatments for this devastating skin pathology.

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βadrenergic receptor agonists attenuate the contraction of dermal fibroblast-seeded collagen gels

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The ability to control wound contraction is important for preventing disfiguring scarring. Dermal fibroblasts are actively involved in wound healing by migrating to the wound site, synthesizing extracellular matrix, and generating mechanical forces within the wound to initiate wound contraction. Fibroblast-seeded collagen gels provide an in vitro model to study these processes. We are evaluating the role of the adrenergic signaling system in cutraneous wound repair, and recently found that keratinocyte migration, an essential step in wound re-epithelialization, is markedly decreased by β2-adrenergic receptor (β2AR) activation (JBC 278:22555 2003). As βARs are also expressed on dermal fibroblasts we initiated a study to determine the effects of β-agonists on dermal fibroblast-seeded type I collagen gel contraction. Control gels achieved maximum contraction by day 3-4 resulting in a 54% reduction in gel diameter. Isoproterenol (ISO), a β-agonist attenuated gel contraction at day 2 by 15-24%, (10nM - 10μM), respectively. When the cells within the gels were counted, there was a 2.5-fold increased in the ISO-treated gels, making the ISO-induced decrease in contraction (which is dependent on cell number) all the more remarkable. A non-specific β-antagonist, timolol (20μM), reversed the attenuation as did a β2-specific antagonist, ICI 118,551 (10nM), indicating that the attenuation of gel contraction was mediated by the β2-AR alone. The active cAMP analog, sp-cAMP, attenuated dermal fibroblast-mediated collagen gel contraction to the same degree as the β-agonist and when combined with ISO, the attenuation was enhanced suggestive of a cAMP-dependent mechanism. The inactive cAMP analog, rp-cAMP partially reversed the attenuation suggesting that the *beta*l-agonist-mediated attenuation of dermal fibroblast collagen gel contraction was mediated by the *beta*l2-AR and the mechanism was partly cAMP-dependent. In conclusion, β-agonists may provide novel therapeutic approaches for the control of wound healing by decreasing keratinocyte migration and attenuating dermal fibroblast-mediated wound contraction.

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Okadaic acid-dependent apoptosis in human keratinocytes - p38δ activation and ERK1/2 inactivation

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Okadaic acid is a protein phosphatase inhibitor that serves as a tumor promoter in mouse skin carcinogenesis. However, a new role for OA as an inducer of apoptosis in murine keratinocytes has been recently suggested. In the present study we show that okadaic acid-treated normal human keratinocytes display characteristic apoptotic features including phosphatidylserine externalization, cytochrome c release into the cytoplasm, and PARP cleavage. These changes are coupled with a marked change in keratinocyte morphology including cell rounding, loss of cell-cell contact, and increased cell detachment. We recently documented a central role for stress-responsive p38δ MAPK in regulating keratinocyte apoptosis. A remarkable feature of this regulation is the coupling of p38δ activation with suppression of pro-survival ERK1/2 activity. Our present studies show that OA-mediated keratinocyte death response is associated with concurrent activation of p38δ and inhibition of ERK1/2 activity. These results suggest that OA promotes keratinocyte death by shifting the balance between p38δ and ERK1/2 activity in favor of p38δ.

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p38 MAP kinase signaling mediates inflammatory responses in UVB-irradiated human skin

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Solar UVB radiation is a potent pro-inflammatory agent and the major causative agent for the development of human non-melanoma skin cancer. The p38 Mitogen-Activated Protein Kinase (p38 MAPK) signaling pathway responds to various cytotoxic insults and environmental stresses and is known to play an important role in photoaging and UVB-induced photocarcinogenesis. In human epidermal keratinocytes, UVB irradiation increases p38 MAPK activity, and inhibitors of p38 MAPK abrogate UVB-induced Cyclooxygenase 2 (COX-2) expression in vitro. In this study we assessed the effects of UVB on the activation of the epidermal p38 MAPK signaling pathway in six human volunteers with Fitzpatrick Type I and II skin. Relatively non-sun-exposed skin of the lower back of human subjects was irradiated with two doses of UVB (1 MED and 2 MED) and punch biopsies were obtained 2, 6, and 24 hours following exposure. The activity of MAP Kinase Activated Protein Kinase 2 (MAPKAPK-2), a specific substrate and downstream kinase of p38 MAPK, was determined. UVB irradiation dose-dependently induced MAPKAPK-2 kinase activity that led to the rapid phosphorylation of heat shock protein 27 (Hsp27), a known substrate for MAPKAPK-2. In the skin irradiated with 1 MED of UVB, phospho-Hsp27 began to appear at 2 hours and reached a maximum at 6 hours, whereas irradiation with 2 MEDs showed maximal phosphorylation at 2 hours, which was sustained for up to 6 hours. No Hsp27 phosphorylation was observed in non-irradiated control skin. COX-2 expression and the pro-inflammatory cytokine interleukin-8 were increased in human skin 24 hours after irradiation with 2 MEDs. Our data indicate that UVB irradiation of human skin activates epidermal p38 MAPK signaling and that COX-2 expression is downstream of p38 MAPK. Pharmacologic blockade of the p38 MAPK pathway may offer a novel approach to cancer chemoprevention by diminishing UVB-induced inflammatory responses that augment tumorigenesis.

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EGF receptor activation stimulates cutaneous wound reepithelialization via the transcription factor Slug

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Reepithelialization to cover exposed dermis is a crucial component of cutaneous wound repair. Many growth factors, including ligands for the epidermal growth factor (EGF) receptor, play a role in promoting the reepithelialization process. Expression of the EGF receptor and its ligands are transiently upregulated at the margins of healing wounds. We recently demonstrated that Slug, a protein belonging to the Snail family of transcription factors, is expressed at wound margins in vitro, ex vivo and in vivo. Furthermore, ectopic expression of Slug enhanced in vitro wound reepithelialization. Given the apparent importance of Slug and EGF receptor expression in promoting reepithelialization, we examined interactions of these two factors. We find that EGF receptor activation stimulates Slug, but not Snail, mRNA and protein expression in keratinocytes. Antisense to Slug inhibits in vitro wound closure. In addition, blockade of EGF receptor tyrosine kinase activity using the specific inhibitor tyrphostin AG1478 inhibits greater than 90% of basal and EGF-stimulated in vitro wound closure, indicating that EGF receptor activation is required for reepithelialization. Similarly, epithelial outgrowth from mouse skin explants ex vivo is reduced by AG1478. Ectopic expression of Slug reduces the concentration of EGF required for enhanced in vitro wound repair, suggesting that the two pathways cooperate in reepithelialization. Our findings indicate that Slug and EGF receptor both stimulate reepithelialization. EGF receptor dependent induction of Slug expression is a potential mechanism underlying EGF-stimulated reepithelialization.

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FasL/Fas-dependent activation of EGFR, ERK, and Akt in keratinocytes: potential implications for the self-restriction of spongiosis in eczemas

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Spongiosis in eczemas is preceded by keratinocyte death. Recent findings have implicated Fas/Fas ligand (FasL) in mediating the apoptotic death of keratinocytes in spongiotic lesions. Investigating the involvement of MAP kinases in contact allergy (ACD), we found that spongiosis is characterized by an elevated suprabasal activation of the ERK family of MAP kinases. We hypothesize that the elevated suprabasal ERK activity during ACD is a protective response of the skin to limit the destruction of the epidermis in the spongiotic areas (by means of ectopic proliferation and/or migration of unaffected keratinocytes to replace the dead cells in the areas of spongiosis). We postulated that apoptotic cells are the source of a signal that triggers activation of ERK in the surrounding cells. Indeed, treatment of keratinocyte cultures in vitro with FasL triggers, in addition to apoptosis, a profound phoshorylation of the Epidermal Growth Factor Receptor (EGFR) and of its downstream effectors ERK and protein kinase B (PKB/Akt). Using a variety of inhibitors and blocking antibodies, we demonstrated that: (i) apoptosis is required for the generation of the signal(s) leading to the activation of EGFR, ERK, and Akt; (ii) the activation of EGFR, ERK, and Akt by FasL is indeed mediated by its bona fide receptor Fas; (iii) the activation of EGFR is essential for the subsequent activation of ERK and Akt; (iv) apoptotic keratinocytes secrete soluble EGFR ligands that are processed (from membrane-bound proligand forms) by a protease identical or similar to TACE. Our findings demonstrate for the first time the existence of a program of self-restricting modulation of apoptosis in the skin. Thus, EGFR, MAP kinases, and/or Akt are potential targets of therapeutic intervention in eczemas.

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The expression of Aryl hydrocarbon Receptor Nuclear Translocator (Arnt) in human and mouse skin and its modulation by dioxin in hairless mice

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has a wide range of skin-specific adverse effects including chloracne, hair loss, hyperpigmentation and keratoderma. Among laboratory rodents, only hairless mice (hr/hr) are sensitive to TCDD skin toxicity. The major prerequisite of TCDD skin action is heterodimerization of ligand-activated dioxin receptor (AhR) with Arnt which is a helix-loop-helix transcription factor. Despite its obvious role in skin homeostasis control, the patterns of Arnt expression in skin were never assessed. Using immunohistochemistry and RT-PCR we studied Arnt expression in normal mouse and human skin, in TCDD-treated hairless mouse skin and in TCDD-treated wt and hr/hr keratinocytes in vitro at both, high and low Ca2++ conditions. In wt mouse epidermis, Arnt expression was seen in scattered suprabasal cells only, while in human epidermis basal layer was highly positive. In anagen hair follicle, Arnt expression was detected in ORS and hair matrix, while IRS, precortex, and follicular papilla cells were negative. In early catagen, Arnt expression was seen in regressing hair bulb, in ORS and in follicular papilla where it persisted during late catagen-telogen. In contrast to wt mice, in hairless mouse skin extensive Arnt expression was localized exclusively to the basal layer of epidermis and proliferating part of utricular epithelium with weak expression in dermal cysts. TCDD treatment of hairless mouse skin resulted in complete loss of Arnt expression in the basal layer and utriculi along with its upregulation in dermal cysts. Cell culture studies showed that basal level of Arnt expression is much higher in hr/hr keratinocytes than in wt cells. TCDD treatment slightly induced Arnt expression in both proliferating and differentiating wt cultures while hr/hr keratinocytes responded to TCDD differently: differentiated cells appeared to be much more sensitive to TCDD action than proliferating cells. Collectively, our results provide insights into the role of Arnt protein in skin and hair follicle biology and toxicology.

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Langerhans cell differentiation is dependent on integrin-mediated activation of TGF-β1

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Transforming growth factor-beta 1(TGF-β1) has a critical role in the generation of Langerhans cells (LCs), as evidenced by the striking absence of LCs in TGF-β1 -/- mice. TGF-β is secreted in a latent form and must be activated (i.e. released from its inhibitory propeptide) in order to bind its receptors and elicit biological functions. The integrin αvβ6, which is expressed in the epidermis, was recently shown to activate TGF-β1. Therefore, we tested whether αvβ6 also regulates LC differentiation. Epidermal sheets were isolated from the ears and backs of mice with the integrin β6 knockout mutation (β6-/-) and were stained with an antibody against MHC-II, which is expressed at high levels by LCs. β6-/- mice have no LCs in the ear epidermis, whereas back epidermis from these mice has ~60% reduction in the number of LCs. We also stained β6-/- ear epidermis with an antibody against the γδ-T cell receptor to identify dendritic epidermal T cells, immune cells that are not dependent on TGF-β1 for localization in epidermis, and found normal numbers. Thus, β6-/- mice do not have a generalized defect in migration of leukocytes into the epidermis. Our results suggest that integrin-mediated TGF-β1 activation generates the active TGF-β1 required for LC differentiation. In ear epidermis, αvβ6 may be the only integrin involved in LC generation, whereas in back epidermis other RGD-binding integrins may play a role. Recently, integrin αvβ8 was shown to also bind and activate latent TGF-β1. Whether this integrin is also involved in LC differentiation will be assessed in the future.

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A new method for purifying amelanotic melanocytes from human hair follicles

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We report a new method to establish long term pure culture of amelanotic melanocytes derived from human hair follicles. Normal corpse human scalp, which was taken just after death 1 h, was transected 1 mm below the epidermis, and hair follicles in the remaining dermis were isolated by two step enzyme treatment⟨first incubated in 0.50% Dispase and second in 0.50% Collagenase V ⟩.Hair follicle cell suspensions were prepared by 0.50% Trypsin treatment for about 30 min, and cultured in a optimized culture medium. Cells attached to the substratum were mostly amelanotic melanocyte in early stage, only a few of keratinocytes and none of fibroblasts. The keratinocytes were removed by differential trypsinization. After the fourth passage, the cells were all amelanotic melanocytes confirmed by immunostaining with monoclonal antibodies to HMB45 and NKI/beteb melanosomal antigens and observation with transmission electron microscope. Thus, this new technique is potentially more suitable for cultivated amelanotic melanocytes. The availability of the pure culture of the hair-follicle amelanotic melanocytes will facilitate investigations of the role of those cells in migration and differentiation during treatment of vitiligo.

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Expression of pIRES2-EGFP-hVEGF165 in HaCaT cells and pig hair pappilae cells

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Human keratinocytes and dermal papillae cells (DPCs) have been documented to secrete Vascular Endothelial Growth Factor (VEGF), which plays a significant role in hair growth and if uncontrolled relates to hail loss or other hair diseases. In this study, we investigate the feasibility of transfecting VEGF165 gene into HaCaT cells and pig DPCs in vitro. pIRES2-EGFP-hVEGF165, a recently established fluorescent reporter gene vector containing hVEGF165, was transfected into HaCaT cells and pig DPCs by lipofectamine 2000. Cells transfected with pIRES2-EGFP-VEGF165 emit green fluorescence that can be observed clearly under fluorescence microscope. The flow cytometer was utilized to analyze the efficiency of transient transfection. The expression of VEGF in cultured cells and supernatants was measured by Streptavidin Biotin Complex kit and ELISA kit respectively. Flow cytometry was utilized to examine the effect of transfecting pIRES2-EGFP-hVEGF165 on cell apoptosis. We found that HaCaT cells and pig DPCs were successfully transfected with IRES2-EGFP-VEGF165 with lipofectamine 2000. The efficiency of transient transfection in HaCaT cells and pig DPCs HPC was 9.32% and 3.25% respectively. Compared with negative control, the mean level of VEGF in cultured supernatants was statistically higher especially in HaCaT cells. Immunohistochemical analysis showed that positive staining was both in cytoplasm and nucleus, with stronger intensity in the latter. Transfecting cells with pIRES2-EGFP-VEGF165 inhibited cell apoptosis. Taken together, our data demonstrate that pIRES2-EGFP-VEGF165, successfully transfected into HaCaT cells and pig DPCs in vitro, promotes the expression of VEGF and prevents the cells from apoptosis. Since VEGF is an important mediator in hair growth and hair cycle and patients with androgenic alopecia or alopecia areata usually have low level expression of VEGF in hair follicle, our study may have potential value for clinical management of hair loss.

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Spontaneous alopecia and ulcerative dermatitis in C57BL/6J laboratory mice

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C57BL/6J inbred mice and closely related substrains, collectively known as B6 mice, develop transient alopecia and small, punctate ulcers that wax and wane, sometimes progressing to severe ulcerative dermatitis. Frequency of disease varies between substrains and is modulated by husbandry practices. Histologic and scanning electron microscopic evaluation of groups of 20 mice at weekly intervals for 10 weeks after weaning (at 4 weeks) revealed that the first lesion noted was scattered follicular dystrophy affecting only late anagen and early catagen hair follicles. Degradation of the inner root sheath resulted in defects in the cuticle and hair fiber. Fibers that twisted within follicles sometimes punctured the root sheaths and caused hyperplasia of the root sheaths and foreign body granulomas around free fibers in the dermis and hypodermis. Trauma (scratching) induced small ulcers that either healed by pseudoepitheliomatous hyperplasia and dermal scarring or expanded into large, coalescing ulcers with underlying deep beds of granulation tissue. These inner root sheath changes and foreign body reactions resemble central centrifugal cicatrical alopecia variants (follicular degeneration syndrome, pseudopelade, or folliculitis decalvans). To evaluate whether these changes were primarily found in B6 mice, wax stripping was done to simulate excessive grooming behavior. After wax stripping, C3H/HeJ mice had minor epidermal changes followed by rapid and consistent onset of anagen within 7 days. By contrast, B6 skin developed follicular dystrophy and ulcers with marked epidermal hyperplasia. These studies suggest that B6 mice may not be suitable models for certain skin and hair diseases as they may develop a scarring alopecia similar to human central centrifugal cicatrical alopecia (follicular degeneration syndrome).

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Differential expression of a gene homologous to a teashirt gene in neonatal mouse skin during development of hair follicles

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Neonatal mouse skin is useful for studying changes in gene expression during development of hair follicles, as the mitotic activity of skin cells changes shortly after birth. Using RNA differential display, a 261-nt message has been identified in the skin, specifically on days 3-5 but not on day 2 after birth. Confirmation of its expression by ribonuclease protection assay showed that stronger expression is seen on days 3-5 compared with days 1-2. Using RNA ligase-mediated rapid amplification of 5' cDNA ends, we have successfully isolated a 3046-bp gene, which has 93% sequence homology to a mouse teashirt1 gene. Amino acid analysis showed that it has 73% identity to the mouse teashirt1 protein and possesses zinc finger motifs 1, 2 and 3. In situ hybridization data revealed that it is mainly expressed in the follicle bulb including dermal papilla and matrix cells. As the proliferation of bulb cells is important to follicle development during this period, the finding of its strong expression on days 3-5, suggests that the identified gene is a potential candidate for follicle growth. Our current data revealed that a gene homologous to a mouse teashirt gene is differentially expressed in neonatal skin during development of hair follicles.

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Adenosine stimulates fibroblast growth factor 7 expression in human dermal papillae cells

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Adenosine (Ado) plays various physiological functions such as cAMP regulation through adenosine receptors known as four subtypes A1, A2a, A2b and A3 (AdoRs). It has been previously reported that minoxidil up-regulates vascular endothelial growth factor in dermal papillae (DP) cells through Ado and its receptors pathway, resulting in the activation of hair growth[1]. Here we studied other biological functions of Ado in DP cells and the possible involvement of AdoRs in hair growth. Total RNA isolated from DP cells which exposed to 10µM Ado for 4 hours was served for Human 1 cDNA Microarray (Agilent technologies). A quantitative RT-PCR of mRNA expression was performed utilizing LightCycler and FastStart DNA Master SYBR Green I (Roche diagnostics). Immunohistochemistry for AdoR 2b was performed on 5µm formalin fixed sections of human scalp specimen, with rabbit polyclonal anti-human AdoR 2b antibody (Chemicon International). DNA microarray analysis revealed a pronounced up-regulation of fibroblast growth factor-7 (FGF-7) mRNA expression in the DP cells treated with Ado, as compared with control treated DP cells. A quantitative analysis using real time RT-PCR showed that EC_{50} for the up-regulation of FGF-7 was approximately 5×10^{-5} M. AdoR 2b specific antagonist alloxazine, but not other antagonists specific for AdoR A1, A2a, or A3 inhibited the up-regulation of FGF-7 mRNA expression. More, immunohistochemical stains detected an AdoR 2b in DP cells of human scalp specimen. These data suggest that Ado stimulated FGF-7 mRNA expression via AdoR 2b in DP cells. Previous reports suggest that an FGF-7 expressed in DP cells play a critical role in the control of hair growth[2]. Taken all together, these data suggest that Ado might activate hair growth by the stimulation of FGF-7 expression in DP cells.

1) Li *et al., J. Invest. Dermatol.* 117, 1594-, 2001
2) Kulessa *et al., EMBO J.* 19, 6664-, 2000

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Peripheral blood mononuclear cells from patients with advancing alopecia areata exhibit an activated state and resistance to apoptosis

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Alopecia areata (AA) is a putative hair follicle autoimmune disease. We recently noted in a mouse model of AA that CD4+CD25+ regulatory T cells (Treg) levels are depressed and that expression of CD44v7 is transiently upregulated. Here, we explored whether similar patterns are seen in AA patients' peripheral blood mononuclear cells (PBMC). PBMC of 43 AA patients, 26 treated and 17 untreated, and of 31 healthy volunteers were tested. AA patients' PBMC differed from healthy donors' PBMC by an increase in CD16 and TNFα expressing cells. These features were independent of the disease state and treatment. Additional changes in the activation state of PBMC, upregulation of the costimulatory molecules CD40 and CD80, of the accessory molecule CD154 and of IFNγ expression, were observed only in patients with an advancing disease state while PBMC from stable and successfully treated patients did not exhibit activation. PBMC from patients with progressive AA contained an increased percentage of CD4+CD25+ cells. However, the majority of these cells exhibited a freshly activated phenotype with expression of CD154 and very weakly inhibited T cell proliferation in in vitro cell stimulation assays. PBMC from patients with progressive AA also displayed increased resistance towards apoptosis accompanied by a decrease in CD95L+ cells and an increase in CD44v7+ cells. Notably, the expanded population of CD4+CD25+CD154+ T cells in progressive AA patients' PBMC was apoptosis resistant and expressed CD44v7. Thus, a progressive state of AA is accompanied by a systemic activation of potentially pathogenic CD4+CD25+CD154+ T cells. Survival of activated T cells in progressive AA patients' PBMC might be sustained by downregulation of CD95L and upregulation of CD44v7 which is known to be associated with anti-apoptotic gene expression. Furthermore, susceptibility to AA may be associated with an increase in CD16+ (candidate NK cell phenotype) and TNFα expressing PBMC.

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PTHrP influences angiogenesis of the hair follicle

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In developing organs PTH/PTHrP receptor (PPR) signaling inhibits proliferation and differentiation of mesenchyme-derived cell types resulting control of morphogenic events. Throughout most of the hair cycle low levels of PPR transcripts are diffusely expressed in the dermis, but during early anagen cells along the dermal sheath express high levels this mRNA. Treatments of murine skin with PPR agonists and antagonists as well as transgenic overexpression of the PPR ligand, parathyroid hormone-related protein (PTHrP), have suggested that this ligand receptor combination might contribute to the regulation of the anagen to catagen transition in the hair cycle. To further the understanding of the precise role of PTHrP and the PPR in the hair cycle we have evaluated cellular and cell fate markers in depilated K14-PTHrP mice and a novel human versican promoter driven PPR1 over expressing line. In the K14-PTHrP mice that exhibit premature catagen, there was a 50% reduction in proliferating cells in the hair follicle matrix 13 to 15 days after depilation. CD31 staining revealed a decrease in perifollicular vasculaturization in anagen VI of the K14-PTHrP mice as compared to controls. Immunoblotting and immunohistochemistry indicated that during anagen VI the outer root sheath of K14-PTHrP hair follicles contained elevated levels of the antiangiogenic factor thrombospondin-1 but levels of the proangiogenic factor VEGF were not decreased. Surprisingly, transgenic overexpression of the PPR in the dermal papilla and dermal sheath did not influence hair shaft length. Taken together, these findings suggest that the influence of PTHrP on the hair cycle is not mediated by fibroblasts associated with hair follicle but instead the recruitment of sufficient vasculature to the structure. Whether the effect of PTHrP on angiogenesis is mediated by direct signaling of the PPR on endothelial cells or by a PPR-independent modulation of antiangiogenic factor production by the ORS remains to be determined.

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Foxn1 promotes epithelial morphogenesis by modulating protein kinase C

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Foxn1, the product of the nude locus, is a transcription factor essential for the proper development of several epithelial tissues or organs, including the epidermis, hair follicles, mammary gland, and thymus. In mammalian skin, epithelial cells induce Foxn1 during the early stages of terminal differentiation. Here, we present evidence that Foxn1 acts as a negative regulator of protein kinase C (PKC). In the absence of Foxn1, mouse primary keratinocytes upregulated the levels and activity of several PKC isoforms, resulting in the increased phosphorylation of PKC substrates. This elevated PKC signaling then led to the overproduction of late markers of keratinocyte differentiation. Consistent with these findings, *Foxn1* overexpression rendered keratinocytes resistant to 12-O-tetradecanoylphorbol-13-acetate (a PKC activator) and suppressed late differentiation markers. Thus, our results suggest that Foxn1 downmodulates PKC signaling, enabling Foxn1 to influence the extent or timing of events in the differentiation program. As PKC is thought to play critical roles in epidermal development and hair growth, the modulation of PKC may explain, at least in part, how Foxn1 promotes the morphogenesis of the epidermis and hair.

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The role of BMP signalling in the control of ID3 expression in the hair follicle

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Both the production of the hair shaft in anagen and the initiation of a new hair cycle at telogen are the result of reciprocal interactions between the dermal papilla and the overlying epithelial cells. Secreted factors, such as those of the bone morphogenetic protein (BMP) family, play a crucial role in moderating these interactions. Analysis of hair follicles in different stages of the hair cycle showed BMP signalling was only active during anagen and again during telogen. During catagen, no BMP signalling via SMAD1 occurred in the dermal papilla. ID3, a gene expressed in the dermal papilla of both vibrissa and pelage follicles, is a BMP target and as such we found that ID3 was expressed from the earliest stages of morphogenesis. During the hair cycle, ID3 was only expressed in the dermal papilla at middle anagen and telogen. To test the significance of ID3 expression in the dermal papilla, we cultured dermal papilla cells and found that ID3 expression fell significantly after a single passage. ID3 expression was returned to in vivo levels in low and high passage cells by culturing to high confluence or by the addition of BMP4. These studies reinforce the requirement for active BMP signalling and cell-cell contacts in the dermal papilla during specific stages in the hair cycle.

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Cotton honeydew oligo-saccharides yield very interesting results in hair care cosmetics

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Hair is particularly important for the psychological equilibrium of humans, and the concept of normal, beautiful hair, takes today a central place in cosmetic products. Cotton honeydew extract is an original composition of unusual oligo-saccharides, such as fructose, glucose, inositol, melezitose, saccharose, trehalose and trehalulose. Previous studies have demonstrated very interesting protective, nutritive and stimulating properties of these oligo-saccharides. Such an original association of these oligosaccharides is endowed with effect on stimulating keratin synthesis, protecting against nutrient deprivation and osmotic stress. Therefore, we were interested in studying the effect of these oligo-saccharides on normal and damaged human hair, by scanning electron microscopy, in order to study the scale margins which are essential for the shine and feel of the hair. Standardized human hair samples were used in the first study to determine the effect of a rinse-off mask with 3% of cotton honeydew extract on the hair ultrastructure aspect. In addition, hair samples were submitted to different aggressions, following different experimental protocols, including exposure to detergent and to air blowing dryer, in the presence or absence of the cotton honeydew extract mask. Hair samples were then prepared for scanning ultrastructural studies to evaluate any damage to hair shaft. Scanning electron micrograph showed that, without extra aggression, in cotton honeydew extract-treated samples, the cuticle scales appeared lying more smoothly in the hair than in the untreated samples, they exhibited less lifting from the surface and remained flatter against the hair surface and so are less prone to chipping. In contrast to the control-untreated hair where the hair kept a dry and damaged aspect. The cuticle scales appeared significantly lifting from the surface of the hair, making them prone to chipping and progressive break off. These results demonstrate a great application for cotton honeydew extract in hair cosmetics and care products.

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Desmoglein 4 is expressed in diverse tissue types and is localized to the desmosome

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Recently, we reported cloning of a novel desmosomal cadherin gene, Desmoglein 4 in the human, mouse and rat genomes. DSG4 is highly expressed in suprabasal layers of the epidermis and throughout the hair follicle with the exception of the outer root sheath (ORS). Mutations in DSG4 underlie the human disorder, localized autosomal recessive hypothrichosis (LAH) (OMIM 607903), in which affected members display hypothrichosis restricted to the scalp, chest, arms, and legs. Furthermore, mutations in mouse and rat Dsg4 result in lanceolate hair (lah) phenotype. We sought to better define the role of Dsg4 in the normal epidermis and the hair follicle, and examine its expression pattern in humans and rodents. Using a new reagent, the Imgenex Tissue Array, we analyzed the expression of human and rat DSG4 in multiple tissues. In both species, we detected cell border expression of DSG4 in all the epithelial tissues examined including cornea, oral epithelium, transitional bladder epithelium, esophagus, tongue, and cervix. Interestingly, between weeks 14 and 18 of early human fetal skin development, DSG4 is expressed highly throughout all the layers of the epidermis. During the next 12 weeks, the expression of DSG4 becomes diminishingly detectable in the basal layer, and by week 31, the protein is only present in the suprabasal layers of the epidermis. DSG4 is also present throughout the epithelial compartment of the developing human fetal hair follicle. We confirmed the localization of DSG4 protein to cell borders in cultured keratinocytes, and found that it co-localizes with desmoplakin. These findings demonstrate that DSG4 is expressed in a diverse range of mammalian tissues, and raises the possibility of functional roles beyond those defined for DSG4 in epidermis and hair follicle.

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Oligonucleotide treatment inhibits hair growth and stimulates apoptosis-driven hair follicle involution

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Increased evidence suggests that telomere homolog oligonucleotides (T-oligos) are capable of inducing a variety of cellular responses in skin including cell cycle arrest and apoptosis. To assess the effects of T-oligos on hair growth, thymidine dinucleotide (pTT), 1/3rd of the TTAGGG telomere sequence, were administered intracutaneously at distinct time points of the depilation-induced hair cycle in mice. Penetration of T-oligos into the hair follicle (HF) was monitored by using FITC-labeled pTT and confocal microscopy. pTT treatment on days 1-5 after hair growth induction (i.e. at the early anagen) significantly ($p<0.01$) retarded anagen development and resulted in formation of the HFs with the reduced size of proximal hair bulbs, compared to the vehicle-treated controls. pTT treatment on days 12-16 after hair growth induction (i.e. at late anagen-beginning of catagen) significantly ($p<0.05$) accelerated catagen development. Hair growth inhibition and acceleration of HF involution induced by pTT were accompanied by increased keratinocyte apoptosis in the HFs, as detected by TUNEL. By Western blot analysis, pTT-treated skin showed marked increase of p53 protein and several other pro-apoptotic markers (CD95/Fas, Bax, caspase-3), compared to the control skin. By multi-color immunofluorescence, HFs treated by pTT showed marked increase of p53, Fas, Bax and caspase-3 expression and co-localization with TUNEL-positive cells. Taken together, these data suggest that pTT activates a p53-dependent apoptotic response in actively growing HFs, as well as in regressing HFs. This raises a possibility for using synthetic T-oligos for hair growth inhibition and for the correction of excessive hair growth (hirsutism) in clinical practice.

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Expression of estrogen receptor α, β and androgen receptor in human follicular dermal papilla *in vivo*

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Hair growth and cycles are under the influence of sex hormones such as androgen and estrogen. Dermal papilla cells (DPCs) are known to be the target of hair growth regulation by such hormones affecting the transcription of modulatory signals from these cells and therefore, play an inductive role in hair follicle morphogenesis and cycling, The aim of this study is to investigate whether there is a difference in the expression pattern of androgen receptor (AR), estrogen receptor α (ERα) and β (ERβ) in dermal papillae from vertex and occipital scalp skin. We compared the immunohistochemical staining characteristics of the receptors in dermal papilla from each portion. We also compared the degree of expressions of 3 receptors in DPCs microdissected from hair follicles by Western blot and semi-quantitative RT-PCR. Dermal papilla from vertex scalp showed higher AR positivity and stronger AR expression than that from occipital scalp. ERα was expressed more in DPCs from occipital scalp, although rarely detectable by immunohistochemistry. ERβ exhibited a similar pattern of expression to ERα evaluated by Western blotting and RT-PCR.

The differential expression of AR, ERα and ERβ in dermal papillae from androgen-dependent (vertex scalp) and androgen-independent tissue (occipital scalp) suggests delicate interactions of androgen and estrogen. The expression pattern of estrogen receptors implicate they may have a regulatory effect on the expression of AR.

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A novel hair growth promoter, t-flavanone, suppresses membrane bound urokinase on keratinocytes

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We have previously reported that trans-3, 4'-dimethyl-3-hydroxyflavanone (t-flavanone) is a novel hair growth promoter that improves male pattern baldness. TGF-beta has been considered as one of the critical factors in controlling hair growth. TGF-beta is secreted by follicular papilla upon androgenic stimuli and is highly active in inducing the catagen phase of the hair cycle. t-Flavanone reduced the amount of activated TGF-beta in the mixed cultures of follicular dermal papilla cells and keratinocytes. We investigated the effects of t-flavanone on TGF-beta activation by keratinocyte urokinase. Urokinase inhibition by t-flavanone; 0.1-100 mg/ml t-flavanone was added to mixtures containing commercially available urokinase and a synthetic substrate. Urokinase activities were then measured using a fluorometer. Effects of t-flavanone on membrane bound urokinase; after keratinocytes were treated with/without 0.1μM t-flavanone for 16 hrs, membrane bound urokinase was collected by washing with HCl/glycine buffer. Activities and amounts of urokinase were measured by zymography and by western blotting, respectively. t-Flavanone did not inhibit urokinase directly in the cell free system. However, t-flavanone decreased the activity and amount of membrane bound urokinase protein. These findings suggest that t-flavanone promotes hair growth through TGF-beta, by activating a cascade controlling urokinase activity.

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Molecular mechanisms of neurotrophin-induced apoptosis in autoreactive CD8 cells in alopecia areata

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Neurotrophins are a family of structurally and functionally related polypeptides that include nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF). They bind a 75 kD transmembrane receptor (p75), and also each neurotrophin binds a distinct receptor of the Trk family. When p75 and Trk are coordinately bound by neurotrophins, survival signal is initiated through Trk. However, in Trk absence, p75 is activated alone to induce apoptosis. Alopecia areata (AA) is an autoimmune dermatosis that involves the development of an inflammatory infiltrate, composed primarily of CD8+ and CD4+ lymphocytes, around and within hair follicles. Because many autoimmune conditions involve neurotrophins and their receptors, we asked if neurotrophins may play a role in AA pathogenesis. CD8 lymphocytes were isolated from involved skin and peripheral blood of AA-affected C3H/HeJ mice as well as from non-affected control mice. In contrast with peripheral blood CD8 cells that displayed low p75 levels, cells from AA involved skin expressed significantly higher p75 level ($p<0.001$, FACScan analysis). Multi-color immunofluorescence microscopy showed that p75 positive CD8 cells were assembled specifically around the hair follicles as well as inside the follicular epithelium. However, by RT-PCR, these cells were negative for Trk (TrkA, TrkB, TrkC) receptor expression, suggesting that neurotrophin administration could induce apoptosis of these inflammatory cells. Consistent with this hypothesis, NGF and BDNF induced apoptosis in CD8 cells isolated from skin of mice affected by AA and cultured in vitro, as compared to diluent alone. Furthermore, in vivo administration of agarose beads soaked with NGF or BDNF resulted in significant reduction of CD8 cells ($p<0.01$) in AA-affected skin. These data suggest that neurotrophins by stimulating p75 mediated signaling in CD8 cells could be used as a novel therapeutic intervention to arrest the development of AA.

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Edar signaling is involved in the control of hair shaft formation and catagen development

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Ectodysplasin (Eda) and its receptor (Edar) are required for normal development of several ectodermal derivatives including hair follicles in humans and mice. In order to elucidate the expression and function of the components of the Eda/Edar signaling pathway during postnatal hair cycle, we employed the murine depilation model. By RT-PCR, the expression levels of the transcripts for EdaA1, Edar and its signal transucing components EDARADD and TRAF6 are minimal in the telogen and maximal in late anagen-catagen skin (days 8-16 post depilation). By in situ hybridization, EdaA1 mRNA was strongly expressed in the hair matrix, outer and inner root sheaths of late anagen hair follicles. By multi-color immunofluorescence, Edar expression in late anagen follicles was seen in the uni-lateral cluster of hair follicle matrix cells that also expressed Shh protein. Intracutaneous administration of a fusion protein that selectively binds EdaA1 and block Edar signaling resulted in severe defects of hair shaft formation in late anagen hair follicles and also inhibited catagen development, compared to the skin treated by vehicle control. Hair shaft abnormalities seen after pharmacological blockade of the Edar signaling were accompanied by alterations in the expression of the selected hair keratins and other regulatory molecules that are involved in their synthesis (Shh, Lef-1, Smad1/5). Thus, our data demonstrate that Edar signaling is involved in the control of hair shaft formation and catagen development in mice. These data also suggest a cross-talk between Edar signaling and other regulatory pathways (Shh, Wnt, BMP) that are involved in the control of postnatal hair cycle.

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An *ex vivo* human model for chemotherapy-induced hair loss: a role for p53 signaling pathway in doxorubicin-induced apoptosis in the hair follicle

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Chemotherapeutic agents induce apoptosis in proliferating malignant cells via activation of the p53 signaling pathway. Many side effects of chemotherapy are also mediated by p53, and it was shown that p53 is essential for chemotherapy-induced hair loss in mice. However, the roles of p53 and its target genes in the control of apoptosis induced by chemotherapy in human hair follicle appear to be unknown. To study the effects of chemotherapy on human hair follicles, the ex vivo human model for chemotherapy-induced hair loss was established. Hair follicles isolated from human scalp were cultured ex vivo in presence of different concentrations of doxorubicin or vehicle control. Doxorubicin induced rapid and dose-dependent anagen-catagen transition in human hair follicles, associated with appearance of TUNEL-positive cells in the hair follicle matrix and outer and inner root sheaths. By multi-color immunofluorescence, doxorubicin-treated hair follicles showed the increase of p53 protein, compared to control hair follicles. Analyses of the expression of the proteins encoded by p53 target genes showed the increase of Fas and Bax expression and decrease of Bcl-2 protein in doxorubicin-treated hair follicles, compared to controls. These data suggest that isolated human hair follicles cultured ex vivo may be used as a model for studying the mechanisms of chemotherapy-induced hair loss and that topical application of p53 inhibitors to the scalp may serve as a tool for preventing apoptosis in human hair follicles induced by cancer chemotherapy.

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Transgenic expression of noggin targeted to the hair follicle epithelium or mesenchyme leads to distinct skin and hair follicle phenotypes

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Bone morphogenetic proteins (BMPs) and the BMP antagonist noggin are short-range morphogens that play important roles in the control of hair follicle (HF) development and cycling. To define the effects of general inhibition of BMP signaling in distinct HF compartments, transgenic mice overexpressing noggin in the outer root sheath (promoter: keratin 5) or dermal papilla (promoter: versican) were generated. K5-Noggin mice showed both epidermal and hair follicle phenotypes, compared to wild-type (WT) mice. Epidermal phenotypes observed in K5-Noggin mice included the decrease of keratinocyte apoptosis, retardation of the epidermal differentiation and eyelid opening accompanied by the reduced expression of apoptotic receptors (Fas, p55TNFR), Id3 protein, involucrin and loricrin in the epidermis of neonatal mice. Hair follicles of K5-Noggin mice showed significantly accelerated morphogenesis and increased size, failure to generate the zig-zag hairs and retarded catagen. In contrast to K5-Noggin mice, Ver-Noggin mice did not show changes in epidermal apoptosis and differentiation. HFs in Ver-Noggin mice were capable of generating all hair types including zig-zag hairs. However, HFs in Ver-Noggin mice showed significantly increased hair bulbs and retarded catagen, compared to WT mice. Expression analyses of the markers involved in the control of HF development and growth (Lef-1, Foxn1, Shh, Edar) revealed distinct patterns of alterations specific for the K5-Noggin and Ver-Noggin mice. Thus, inhibiting BMP signaling in specific HF compartments (epithelium versus mesenchyme) leads to different HF phenotypes, suggesting specific and local roles for BMP signaling in distinct HF compartments during its development and cycling.

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Recapitulation of the hair cycle in dermal papilla cells *in vitro*

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Transgenic mice expressing GFP under the control of versican regulatory sequences have been employed to purify a cell population from skin that is enriched for dermal papilla cells. When mixed with primary keratinocytes and used to reconstitute skin in a grafting assay, these cells promote the formation of hair follicles in the regenerated skin. Both GFP expression and the ability to promote hair growth are lost when the sorted GFP-positive population is maintained in culture. We have shown previously that exposure to Wnt3a maintains both GFP expression and the ability to promote hair growth in these cells, but it is not sufficient to reactivate GFP expression or hair growth inducing activity in the population that has lost these properties in vitro. We therefore sought to determine whether the loss of GFP expression and hair inductive activity from the cultured cells corresponds to a transition from an "anagen DP " like population to DP cells with characteristics associated with the catagen or telogen phase of the hair cycle, or whether the cells were losing DP character altogether. In this work, we report that as the sorted GFP positive population loses GFP expression in vitro, it also loses the expression of other genes expressed in the anagen DP. However, these cells activate markers found in DP cells during the catagen or telogen phase, suggesting that they acquire characteristics associated with the catagen or telogen phase. We further show that in the context of appropriate inductive signals, these cells reactivate both GFP and other anagen DP gene expression, suggesting that they retain DP cell character. To assess the inductive properties of these "reactivated" cells, we have developed an in vitro follicle induction assay and shown that the inductive capacities of these cells are partially restored. These results demonstrate that this cell preparation may be useful for identifying signals that activate the DP during the hair cycle and may also have application to the generation of cultures of active DP cells for autograft therapy.

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Mammary mesenchyme reprograms keratinocytes from hairy regions to form nipple epidermis

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The nipple is a specialized epidermal appendage that forms after the development of mammary epithelium. Emerging evidence suggests that the primary mammary mesenchyme-derived fibroblasts have a central role in the process, but it is unclear what role the epithelial components play in forming the nipple. We used grafted cellular recombinations of the nipple-like skin from the ventral surface of the K14-PTHrP mouse and wild type littermates in conjunction with label retaining cell analysis to address this issue. Combinations of dermal fibroblasts from the dorsal skin of wild type mice and dorsal or nipple keratinocytes produced a thick tuft of hair. In contrast, grafts that contained mammary mesenchyme derived fibroblasts and nipple or dorsal keratinocytes lacked hair. Evaluation of the grafts indicated that isolated fibroblasts from the ventral skin of the K14-PTHrP mouse were sufficient to produce many of the epidermal and connective tissue features of the nipple regardless of the source of keratinocytes. However, recombinant grafts derived from fibroblasts from the ventral skin of the K14-PTHrP mouse and dorsal keratinocytes from wild type mice contained epithelial invaginations that lacked dermal papilla-like structures. Using the labeling windows of nipple morphogenesis, the early neonatal period and late pregnancy, BrdU retaining cells were never found within the nipple or the ventral epidermis of the K14-PTHrP mice, but were present in the surrounding bulge of hair follicles. On the basis of these findings we conclude:1) the mammary mesenchyme-derived fibroblasts have the capacity to direct interfollicular keratinocytes to nipple-like epidermal differentiation pattern, 2) mammary mesenchyme-derived fibroblasts cells cannot be reprogrammed to form dermal papilla cells, 3) nipple keratinocytes can be reprogrammed to produce hair follicles by dermal papilla cells. 4) hair follicles may be the ultimate source of epidermal stem cells for the murine nipple.

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Epidemiologic and disease associations in participants of the National Alopecia Areata Registry registrants

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To better understand the pathogenesis of the organ specific autoimmune disease, Alopecia Areata (AA), a National Alopecia Areata Registry (NAAR) was created to collect epidemiologic data, DNA, and sera samples from well-characterized subjects. Phase I of the registration process through a website [http://www.AlopeciaAreatarRegistry.org] solicits flare factors and associated diseases from all registrants who have completed initial questionnaires (n=2900). This is a preliminary analysis of self-reported data from the first 1,151 AA patients who have registered, of whom 60% are female. Registrants are divided among clinical phenotypes: severe - universalis or totalis (AU=376, AT = 194), intermediate -(persistent patchy AA= 318), or mild (transient = 263). Seventy-four percent of all AA patients reported the co-existence of at least one of the following 63 possible autoimmune diseases. Forty percent reported some form of allergy, 21% had atopic eczema, and 15% had asthma with similar distribution in each severity group. Arthritis was reported by 7%. Surprisingly, thyroid disease was reported only by 13% of all AA but by 19% of AU, and 9-10% of pts. with either AAT or AAP. As expected, the frequency of reported diabetes was low (2%) in all patients. Thirty-three percent of patients reported the existence of a possible trigger factor preceding the onset of their hair loss as follows: stress - 54%, infection - 28%, surgery - 7%, pregnancy - 7%, or vaccination - 4%. Trigger factors were more commonly reported in 40% of AU patients compared to 22% of AAP (p=0.03) or 19% of AAT (p = .000, Mann-Whitney). The preliminary self-reported registry data suggest that patients with severe alopecia universalis are more likely to have a history of thyroid disease or atopy and to report putative flare factors more commonly than patients who have less severe phenotypes of the disease.

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Reprogramming of adult mouse bladder epithelial cells to hair follicle and sebaceous glands under inductive dermal influences

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Adult epidermal stem cells renew the epithelial compartment of the skin throughout life and are the most accessible of all adult stem cells. Most importantly, epidermal stem cells can be efficiently cultivated and transplanted, a significant advantage for cell and gene therapy. Gene therapy in the skin has been hampered by the inability to target (or identify) a stem cell, and the lack of sustained gene expression. Several lines of recently emerging evidence about the plasticity of stem cells in general has prompted our laboratory to consider a novel approach to the treatment of inherited skin disorders. We asked whether we could reprogram other epithelia into skin under the appropriate inductive (dermal) influences in vivo. We reasoned that if the donor cells were taken from an immunologically-compatible individual or did not elicit an immune response, such cells could overcome the two major obstacles in gene therapy approaches: Extending the previous work of Ferarris et al.(2000), we previously reported cornea and amnion epithelial cells can reprogram into new skin including hair follicle and sebaceous glands. In this study, we build upon our earlier work and have used adult bladder as the source of epithelium. We performed tissue recombination experiments using embryonic mouse dermis from day 14.5 embryos combined with adult mouse bladder epithelial cells. Recombinant grafts were implanted beneath the kidney capsule of normal mice and retrieved after two weeks. Fully formed pilosebaceous units with mature hair shafts and sebaceous glands had been induced. We have demonstrated that even differentiated adult cells (bladder) have the capacity to regenerate epidermis under the appropriate dermal influences. We are currently expanding this technology to include other sources of donor epithelium, as well as developing methodology to recapitulate the molecular events of epithelial reprogramming in vitro.

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Interaction of hairless and thyroid hormone receptor is not involved in the pathogenesis of atrichia with papular lesions

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Atrichia with papular lesions (APL, MIM 209500), is a rare autosomal recessive disease characterized by early-onset of atrichia, followed by a papular eruption within the first years of life. Recent studies demonstrating linkage to chromosome 8p21 and further mutation detection in the hairless gene (HR) have established the molecular basis of APL. HR has been shown to interact with thyroid hormone receptor (TR) via two specific interaction domains in HR. A putative role for hr-TR in the regulation of hair follicle cycling has been speculated upon, however, several lines of evidence argue against this as a crucial factor in APL observed in human and mouse HR mutants. In this study, we describe a 16 year-old female with APL due to a missense mutation, D1012N, in the hr-thyroid hormone receptor interacting domain 2 (TRID2) of the hairless gene. Using functional and biochemical analysis, we determined that this mutation does not significantly affect hr-thyroid hormone receptor interaction. Our data suggests that the TRID2 domain is dispensable in the hr-TR interaction, and does not appear to be involved in the pathogenesis of APL.

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Hairless suppresses vitamin D receptor transactivation in human keratinocytes

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The phenotypes of vitamin D receptor (VDR) null and Hairless (Hr) null mice (and humans) are very similar: alopecia beginning with the first postnatal hair follicle cycle. The ligand for VDR, 1,25-dihydroxyvitamin D, stimulates epidermal differentiation, but does not appear to be required for hair follicle cycling. Hr has no known ligand, and its role in the epidermis and hair follicles is poorly understood. To explore the role of Hr in the epidermis and hair follicles and its potential impact on VDR regulated transcription, we investigated the interaction between Hr and VDR in both human and mouse keratinocytes. The Hr antibody was generated by immunizing rabbits using a peptide synthesized based on both human and mouse Hr sequences. The specificity of the antibody was determined by western analysis and immunohistochemistry with preabsorption of the antibody by the peptide blocking the reaction. Immunohistochemistry localized Hr and VDR in the nuclei of cells in the basal layer of the epidermis, the outer root sheath and dermal papilla of hair follicles. Gel shift analysis showed that Hr antibody enhances the binding of keratinocyte nuclear extracts to the VDR response elements of 24-hydroxylase, phospholipase C-γ1 and involucrin, suggesting that Hr blocks the binding of VDR to VDRE. Transfection with antisense constructs of human hairless cDNA in keratinocytes enhances the induction of vitamin D responsive genes including involucrin, transglutaminase and PLC-γ1 by 1,25-dihydroxyvitamin D_3. Overexpression of Hr in human keratinocytes suppresses the induction of these vitamin D responsive genes by 1,25-dihydroxyvitamin D. These data suggest that Hr suppresses the transcriptional activation of VDR. How the loss of this regulation in the absence of either VDR or Hr results in cessation of hair follicle cycling remains to be established.

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WNT/beta-catenin signaling is required at early stages of tooth development

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The formation of epidermal appendages, such as hair, teeth and mammary glands, depends on epithelial-mesenchymal interactions. We have previously shown that WNT paracrine intercellular signaling is required for the initiation of hair follicle and mammary placode development. We hypothesize that WNT signaling is also necessary for tooth initiation, which would suggest a broad requirement for WNT signals in the adoption of appendageal fate by embryonic epidermal stem cells. To test this hypothesis we asked whether WNT pathway genes are expressed, and WNT signaling is active, during tooth initiation. We detected expression of beta-catenin and Wnt10b mRNAs in the dental lamina beginning at embryonic day (E) 10.5 and E11.5 of mouse development, respectively. WNT/beta-catenin signaling activity, revealed by in vivo expression of a WNT reporter transgene, TOPGAL, was present in the dental lamina and underlying mesenchyme at E11.5, and in tooth buds at E12.5. To determine whether forced activation of WNT signaling promotes tooth development, mandibles from E11.5 TOPGAL-transgenic embryos were cultured in the presence or absence of 50 mM LiCl, which blocks the function of the intracellular WNT antagonist glycogen synthase kinase-3. LiCl-treated mandibles showed increased levels of TOPGAL expression, indicating elevated WNT signaling. In addition, the TOPGAL expression domains were broader than in controls, suggesting expansion of incisor and molar tooth rudiments. Conversely, we asked whether inhibition of WNT signaling blocks tooth formation. We found that in vivo transgenic expression of Dickkopf1 (Dkk1), a potent secreted WNT inhibitor, under the control of a Keratin 5 promoter causes reduced or absent TOPGAL activity in the oral cavity at E12.5 and arrest of tooth morphogenesis prior to the bud stage. These results demonstrate that WNT signaling plays an essential role at early stages of tooth development, and suggest that it may act to promote tooth fate in oral epithelium.

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Hair cycle specific immunolocalization of retinoic acid synthesis markers

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This study was performed to examine the sites of endogenous retinoic acid (RA) synthesis in cycling hair follicles. C3H/HeJ mice were wax stripped to initiate a synchronized anagen hair cycle and samples taken at various time points after depilation. Immunohistochemistry was performed with antibodies against one retinol dehydrogenase (eRoldh), two retinal dehydrogenases (Aldh1a2 and Aldh1a3, formally called Raldh2 and Raldh3), cellular retinol binding protein (Crbp) and cellular RA binding protein type II (Crabp2) involved in RA synthesis. In early anagen, Crbp, eRoldh, Aldh1a3, and Crabp2 are expressed in the sebaceous gland or its duct. This expression remains throughout the hair cycle, except the expression of eRoldh decreases by late anagen, through catagen and increases again in some telogen hair follicles. In the downward growing hair follicle, eRoldh and Aldh1a2 are expressed in early anagen, while Crabp2 expression occurs later during anagen at this site. In addition, expression in the bulb region (precortex and dermal papilla) of Crbp, eRoldh, Aldh1a3, and Crabp2 begins in mid anagen and extends throughout anagen. Crabp2 is also expressed in the dermal papilla during telogen. The inner layer of the outer root sheath expresses Crbp, Aldh1a3, and Crabp2 once it forms and throughout anagen. Some hair fibers express Aldh1a3 and Crabp2 during anagen. During catagen the regressing part of the follicle expresses Crbp, Aldh1a2, and eRoldh. Throughout the hair cycle Aldh1a2 is expressed in the outer root sheath and stratum granulosum, while Crabp2 is expressed in the stratum corneum. This expression pattern indicates that endogenous RA may be important for: sebaceous gland function, hair follicle growth and regression, hair fiber growth and release from its sheath, and epidermal barrier function.

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Structural and molecular hair abnormalities in trichothiodystrophy

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Trichothiodystrophy (TTD) is a rare inherited disorder with brittle hair having low cystine content and alternating light and dark (tiger tail) bands with polarizing microscopy. To better understand the clinical and molecular basis of TTD, we examined 13 TTD patients. They exhibited a wide range of phenotypes, ranging from the hair defect only to PIBIDS [photosensitivity, ichthyosis, brittle hair, intellectual impairment, decreased fertility, and short stature] with recurrent infections, developmental delay and neurological findings. Light microscopic examination showed tiger tail bands in all hairs from all patients under polarized light. Shaft abnormalities (trichoschisis, trichorrhexis nodosa, or ribbon/twist) varied in severity among patients. All hairs had reduced sulfur content. We observed a significant inverse correlation (Rval=0.9) between hair sulfur content and the percent of hairs with shaft abnormalities, demonstrating the role of sulfur in hair toughness. There was no correlation between the presence of photosensitivity, neurological or immune findings and the percent of abnormal hairs. Scanning electron microscopy revealed numerous surface irregularities. Confocal microscopy captured structural features of breaks and hair autofluorescence. Raman spectra of hair samples of 3 TTD patients and volunteers were recorded. Deconvolution of the disulfide stretching region yielded the contributions of three individual conformers. For the TTD hairs, a larger contribution of the energetically less favored conformers, (g-g-t) and (t-g-t), was found. Thus our data indicate that alterations in sulfur content and disulfide conformations contribute to the fragility of TTD hair; however, there does not appear to be a correlation between severity of hair disease and clinical phenotype.

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Role of TSC-22 in homeostasis of murine skin

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TSC-22 (TGFbeta-stimulated clone 22) is a member of a poorly understood family of leucine zipper transcription factors, the TSC-22/DIP/BUN family. TSC-22 has been implicated in TGFbeta-regulation of proliferation in osteoblasts and colon epithelia, as well as TGFbeta-induced apoptosis in human salivary gland tumor cell lines. Another member of the family, GILZ, has been implicated in differentiation of adipose cells and regulation of activated T cell survival. There are four family members in mammals, but only one family member, bunched, in the model organism Drosophila. In Drosophila, the single TSC-22/DIP/BUN gene is essential for embryonic viability. We are interested in understanding the function of TSC-22 in tissue homeostasis and tumor suppression, so we have initiated an investigation into its function in mouse skin. Our published data on TSC-22 mRNA accumulation and immunoreactivity during the murine hair cycle may be consistent with regulation by TGFbeta2 from the outer root sheath during anagen, but is not consistent with a direct role in apoptosis during remodeling of the hair follicle at the anagen/catagen transition. We have noted that TSC-22 immunoreactivity is present in the basal layer of mouse epidermis, but that the level of nuclear TSC-22 is variable in this population. Given the role of this family in regulation of proliferation and differentiation in other tissues, we wish to determine whether TSC-22 may regulate proliferation in the basal layer or the onset of differentiation. We have generated a TSC-22 knock-out mouse strain, which is viable with normal appearance. We are presently investigating the role of TSC-22 in more challenging circumstances, such as cutaneous wound healing.

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MICA alleles and extended haplotype suggest phenotypic variation in alopecia areata multiplex families

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Alopecia areata (AA) is an autoimmune, T-cell mediated disease directed at the hair follicles and major histocompatibility complex (MHC) DR and DQ loci associations have been reported. MCH class I chain related gene-A (MICA) is a stress antigen located 46.5 bp centromeric to the HLA-B region. Allelic polymorphisms due to variation in number of GCT repeats (A4, 5, 5.1, 6, 9) are described, and allele 5.1 and 6 are associated with psoriasis and Behcet s syndrome respectively. To determine whether MICA is associated with AA, DNA from 431 individuals in 100 AA multiplex families were genotyped using MICA specific PCR amplification, gel electrophoresis, and/or DNA sequencing. Transmission disequilibrium test (TDT), family based association test (FBAT) and haplotype analysis were applied. Phenotypic severity was defined as mild or patchy alopecia areata (AA) (85 informative trios) or as severe alopecia totalis (AT)/ alopecia universalis (AU) (83 informative trios) based on degree and duration of hairloss. Five MICA alleles were detected by genotyping in AA affected individuals. Family based association test (FBAT) suggests the MICA allele 5.1 is associated with mild, patchy AA (p= 0.02) while allele 6 is bordline associated with AT/AU (p= 0.087). Two extended haplotypes, HLA-DQ*6, DR6, MICA*5.1 (p=0.004) and DQ1, DR3, MICA*5.1 (p=0.009), were significantly associated with AA (patchy and AT/AU). Differences in MICA alleles, in addition to HLA-D loci alleles, may underlie phenotypic severity of alopecia areata in multiplex families.

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Phenotype characterization of the rough coat mice

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Rough coat arose spontaneously as a recessive mutation in the C57BL/6J strain. Homozygous mice were born indistinguishable from their normal littermates, but showed unkempt looking coat by weaning age, and developed cyclic and progressive hair loss. The rough coat locus has been mapped to chromosome 9, but the gene mutation has not been identified. In this study, we carried out phenotype characterization of these mice. Loss of pelage hair and vibrissae was observed during catagen/telogen, and hair re-growth was observed during the subsequent anagen. Scanning electron microscopy revealed that hair loss in the rough coat mice was not due to hair shaft breakage. However, the hair shafts from the rough coat mice appeared much smoother than those from normal C57BL/6J mice. Histological analysis showed severe sebaceous gland hypertrophy. Additionally, rough coat mice over 10 months old also developed persistent ulcerated lesions in the skin at a high incidence, suggesting a defect in epidermal maintenance. Because the multipotent keratinocyte stem cells are the source for the cyclic growth of the hair follicle root sheath and the proliferating basal cells of the sebaceous glands as well as epidermal maintenance upon wounding, our observations suggest a possible shift of the stem cells from hair follicle to sebaceous fate in the rough coat mice.

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Immunohistochemical analysis of bone morphogenetic protein-5 in human skin

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Bone morphogenetic proteins (BMPs) are novel subfamily members of the transforming growth factor beta (TGF-β) supergene family. Originally found to stimulate cartilage and bone formation in vivo, BMPs are now known to show specific expression patterns in the epidermis, regulate cell proliferation and differentiation, and have been implicated in hair follicle morphogenesis and cycling. The patterns of expression of BMP-5 another member of the BMP family has been understudied in human skin, therefore the objective of this study was to look at the immunohistochemical expression patterns in normal human skin and hair follicles. Archived paraffin blocks of human skin were immunostained with anti-human BMP-5 ligand antibody followed by the peroxidase method. Previous immunohistochemical studies have shown BMP-2/4 to be localized to the cytoplasm of suprabasal and spinous cells in normal and benign samples of oral mucosa and hard palate, where as BMP-5 was localized to the cytoplasm of basal cells and some suprabasal cells in samples from the same locations. Our study results show that BMP-5 is diffusely immunolocalized in the cytoplasm of cells in all layers of the epidermis, the external root sheath of the hair follicle, nerve bundles, endothelial cells, and the secretory and ductal elements of eccrine glands. In our opinion BMP-5 plays a crucial role in hair follicle morphogenesis and cycling. These results enable functional studies of BMP-5 and its receptor in normal and abnormal skin and its appendages.

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Developmental analysis of nail development

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Skin appendages (nails, hairs, eccrine glands) are micro-organs that convey mechanical and regulatory properties to the epidermis and associated dermis. Development of these skin appendages involves three distinct, yet overlapping, stages: specification, morphogenesis, and differentiation. Our lab focuses on the genetic regulators that play a role in nail and eccrine gland development during these 3 stages. A developmental foundation crucial to understanding the phenotypes we observe, however, is unavailable in the literature. The current focus of our work has been the creation of a developmental timetable for the nail appendage. We have conducted histological and expression analyses of candidate regulatory and differentiation markers in the mouse nail unit. The presumptive nail is first discernible by a thickening of the epidermis at the dorsal digit tip at E14.5. This region is delineated by *Dlx2* gene expression as indicated by a *Dlx2-LacZ* allele. *Dlx2* continues to be expressed in the developing nail bed and hyponichium (the ventral component of the nail) until at least P6, thus serving as a useful marker of the developing nail unit. In addition, the onset of nail development coincides with a sharp increase in alkaline phosphatase (AP) activity in the mesenchyme underlying the nail field at E14.5 and a loss of AP activity in the remnant apical ectodermal ridge (AER). AP activity remains robust in the nail mesenchyme postnatally, differentiating it from more proximal non-appendageal dermal mesenchyme. By E16.5, the progeny of nail bed cells differentiate into a pre-nail structure (indicated by expression of hard keratins). At the same time, the cells of the proximal nail epithelium, which include the presumptive nail matrix cells, begin to invaginate. By birth, the proximal matrix cells are enveloped by mesenchyme and their progeny differentiate into the mature nail plate, indicated by histological staining and hard keratin expression. We also show that the transcription factor *Engrailed-1* and trichohyalin, a keratin binding protein, are selectively expressed in the nail hyponichium beginning at E15.5.

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Semaphorin A is expressed in the bulge region of the hair follicle

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Semaphorins comprise a large family of cell surface and secreted molecules that play important roles for neuronal axon guidance. The class III semaphorins are secreted and are also involved in the control of angiogenesis. Other neuronal factors such as substance P and PDGF and angiogenesis-related factors such as VEGF and thrombospondin-1 have been shown to be expressed in the hair follicle in a hair cycle-dependent manner. However, the localization and function of semaphorins in hair biology have remained unclear. Using a tyramide-based sensitive in situ hybridization (ISH) technique, we detected mRNA expression of one of the class III semaphorins, semaphorin A (SemaA), in the bulge region beneath the sebaceous gland during the telogen phase of the postnatal murine hair cycle. During the depilation-induced hair cycle in adult mice, SemaA mRNA was selectively expressed by cells in the bulge region. The ISH results were confirmed by immunostains for class III semaphorins. Double immunofluorescence stains for class III semaphorins and for BrdU performed in mice that were exposed to BrdU twice daily from day 3 to day 5 after birth revealed that label-retaining cells (LRCs) co-localized with class III semaphorin-positive cells. In human skin, immunoreactivity for keratin15 partially overlapped with class III semaphorin-positive cells in the bulge region of anagen hair follicles. These data reveal that expression of class III semaphorins is restricted to the bulge region of hair follicles and they suggest that class III semaphorins might be involved in the control of the migration of follicular stem cells.

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High commitment of embryonic keratinocytes to terminal differentiation through a Notch1-caspase 3 regulatory mechanism

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Embryonic cells are expected to possess high growth/differentiation potential, required for organ morphogenesis and expansion during development. However, little is known about the intrinsic properties of embryonic epithelial cells due to difficulties in their isolation and cultivation. We report here that pure keratinocyte populations from E15.5 mouse embryos commit irreversibly to differentiation much earlier than newborn cells. Notch signaling, which promotes keratinocyte differentiation, is up-regulated in embryonic keratinocyte and epidermis, and elevated caspase 3 expression, which we identify as a transcriptional Notch1 target, accounts in part for the high commitment of embryonic keratinocytes to terminal differentiation. *In vivo*, lack of caspase 3 results in increased proliferation and decreased differentiation of interfollicular embryonic keratinocytes, together with decreased activation of PKC-δ, a caspase 3 substrate which functions as a positive regulator of keratinocyte differentiation. Thus, a novel Notch1-caspase 3 regulatory mechanism underlies the intrinsically high commitment of embryonic keratinocytes to terminal differentiation.

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Shh and Gli 1 are involved in the control of human hair follicle cycle and growth *ex vivo*

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Sonic Hedgehog signaling (shh) is implicated in murine hair follicle morphogenesis and growth through the regulation of the Gli transcription factors. The purpose of this study is firstly to examine the expression of the mRNAs and proteins of shh and its downstream effector Gli1 in the human hair follicle at different stages of the hair cycle using immunolabelling and in situ hybridization with biotin-labelled shh or Gli1 specific probes.

We demonstrated by these two approaches a same localization of shh and Gli1 in the outer root sheath keratinocytes near the bulge region at the anagen stage. With transition to catagen and telogen stages, expression was detected in the lower part of hair follicle. The shh gene presence was investigated on isolated hair follicle ex vivo. Total RNA was extracted from isolated human anagen hair follicles and genes (shh, Gli1) expressions were analyzed by semi-quantitative RT-PCR. We found a significant amount of shh and Gli 1. We then attempted to introduce the shh gene into isolated hair follicle for induction or inhibition of hair shaft growth. Hair follicles were subjected to transfection with lipofectin. Transfected hair follicles with shh antisense probes show a significant inhibition of hair shaft elongation ex vivo. Targeting isolated hair follicle with shh or Gli1 encoding mRNAs may find an application for intervention in hair follicle diseases and/or in alopecia.

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Vellus versus terminal hair follicle: cytokeratin expression *in vitro* and *in vivo*

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The human hair follicle can undergo transformation from strong terminal hair follicles (THF) to fine vellus hair follicles (VHF) as in androgenetic alopecia, and conversely, as it occurs in hirsutism. Although structurally similar to THF, there is evidence for a characteristic histomorphology of VHF. To better understand the structure and the regulation of the human VHF, fresh skin samples from different body sites including scalp, face and trunk were stained with a large panel of anti-cytokeratin (CK) antibodies ranging from CK5 to CK20 and differentiation markers such as trichohyalin. In both THF and VHF, anti-CK19 and anti-CK17 strongly stained the outer root sheath (ORS), while anti-CK13 and trichohyalin were confirmed as markers of the inner root sheath (IRS). However, staining was strong and homogenous in VHF, while a spotted pattern was found in THF. To further investigate this observation, primary keratinocyte cultures were established from THF and VHF. Consistent with our immunohistochemical data, the percentage of cells positively stained for cytokeratin 17 and 19, with 80% and 35%, respectively, was higher in VHF-derived keratinocytes than in THF-derived keratinocytes, yet both cultures only partly expressed outer root sheath markers. Furthermore, up to 10% of VHF keratinocytes were trichohyalin positive, indicating IRS-like differentiation, while anti-CK13 stained less than 1% of the cells in all cultures. In contrast to cultured primary keratinocytes, whole organ cultures from THF consistently maintained the physiological expression pattern of cytokeratins over several days in vitro, indicating that models of the whole hair follicle are essential tools in experimental hair research. Our findings underline the need of a suitable in vitro model of the whole human vellus hair follicle to further investigate vellus hair biology and the transformation to terminal hair follicles.

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***In vitro* reactivation of inactive dermal papilla cells isolated from mouse whisker follicles**

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Dermal papilla cells (DPCs) in anagen phase are a source of inductive signals involved in the formation of hair follicles in vivo. When DPCs from a transgenic mouse line that expresses green fluorescent protein (GFP) during anagen are separated from the epithelium and cultured in vitro as monolayers for several passages, they lose GFP expression and their inductive ability (1). An exogenous source of Wnts can maintain GFP-expression and hair follicle inducing activity of such cultured cells. Wnts, however, cannot re-activate GFP-expression and hair follicle inducing activity of cultured cells (2). In the present study with manually dissected whisker dermal papillae, GFP expression could be maintained for as long as one month when the cells were maintained as dermal aggregates. However, GFP expression was lost in cells that migrated out of the dermal papillae as a monolayer. We have been successful in re-expressing GFP in DPCs that had been cultured as a monolayer for several passages by inducing the formation of cellular aggregates in culture. In these monolayer derived aggregates expression of GFP, HGF and IGF-1 was up-regulated and expression of BMP-4 was down regulated. When DPCs grew out from the GFP-re-expressing aggregates on cell culture dishes, GFP expression was again lost in the monolayers. When the GFP-re-expressing DPC aggregates were combined with normal mouse keratinocytes and grafted to nude mice, one mouse out of three revealed formation of hair follicles. These results suggest a possibible new technology for in vitro amplification and reactivation of DPCs for therapeutic purposes in cases such as alopecia areata. [1, Kishimoto et al. (1999) PNAS 96:7336; 2, Kishimoto et al. (2000) Genes Dev 14:1181]

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The morphogenesis of scales in the zebrafish: a model for hair/teeth formation

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The morphogenesis of scales in the zebrafish is analogous to the formation of hair and teeth in mouse and human. Mesenchymal-epithelial interactions are known to be essential for the development of hair and teeth. Our recent studies in the zebrafish indicate that the formation of scales likewise involves molecular signaling between the epithelial and mesenchymal components of scale placodes. We analyze the in vivo expression of many genes thought to be important in the morphogenesis of hair and teeth in scale placode development in the zebrafish. These expression studies reveal many similarities between the development of scales, and of hair and teeth. We detect the expression of various components of the Wnt, Bone Morphogenetic Protein (BMP), Sonic Hedgehog (SHH), and Notch/Delta signaling pathways in scale placodes many days prior to the appearance of scales. We utilize a transgenic zebrafish line TOPdGFP (Dorsky RI, et al.), which consists of a destabilized GFP mutant downstream of a promoter responsive to Wnt signaling, to show that Wnt signaling occurs during the morphogenesis of scale placodes. We also detect the stabilization of B-catenin protein in cells of the scale placode. These lines of evidence strongly support the hypothesis that Wnt signaling is involved in the scale formation of scales in the zebrafish, similar to hairs/teeth in mouse and human. We are analyzing the phenotype of a zebrafish mutant that lacks scales. We confirm that this mutant does not form scales by alizarin red staining. We have investigated the expression of various components of the Wnt, BMP, SHH, and Notch/Delta pathways in this mutant during stages of scale morphogenesis. We report a severe reduction or absence of expression of each and all of these genes in the scale placodes of this mutant. We conclude that the gene mutated is required at a very early stage for scale placode development in the zebrafish.

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Defining the Hedgehog-PTHrP signaling axis in the skin

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Sustained and orderly growth of long bones depends on a balance between Indian hedgehog-driven cellular proliferation and differentiation and inhibition of these processes by parathyroid hormone-related protein (PTHrP), a factor that appears to be indirectly activated downstream of the hedgehog-Gli cascade. Using a series of transgenic models that lack or over express components of the Sonic hedgehog (Shh) signaling pathway or PTHrP, we investigated interactions between these two pathways in the skin. Using Q-RT-PCR, we found that PTHrP mRNA levels in skin extracts were modestly decreased in E18.5 Shh null mice but transcripts were increased 10-fold in adult K5-driven N-terminal deleted Gli2 (K5-Gli2DN2) mice, in which the hedgehog-Gli pathway is modestly upregulated. To determine if PTHrP signaling could influence the consequences of dysregulated hedgehog signaling in skin, we crossed K14-PTHrP mice with K5-Gli2DN2 mice. PTHrP over expression reduced the length of longer guard hairs that characterize the first hair cycle of K5-Gli2DN2 mice by 25%. The K5-Gli2DN2 mice do not undergo a normal second hair cycle and progressively lose hair shafts beginning at 6 weeks. Alopecia in the K14-PTHrP;K5-Gli2DN2 mice is markedly delayed and greater numbers of the remaining hair follicles attained a normal telogen-like morphology. At 6 months, the skin of the K5-Gli2DN2 mice had a thickened epidermis that expressed markers of hyperproliferation, contained many keratinized cysts in the hypodermis and a dense very cellular dermis. In contrast, the K14-PTHrP;K5-Gli2DN2 mice had few cysts and the epidermis and dermis were not as severely affected. Thus, in the skin, endogenous PTHrP gene expression appears to be activated as a consequence of increased Gli2 mediated transcription; however, overexpression of this calcitropic peptide inhibits Gli2-driven phenotypic alterations, suggesting a functional interaction between these signaling pathways in skin.

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Human hair follicle organ culture as a screening tool for “hair drug” discovery: applications, limitations and optimization

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Organ culture of human hair follicles, pioneered by Philpott *et al.* (J Cell Sci 97: 463, 1990), still is the only reliable *in vitro*-method for predicting how a test agent might affect human hair growth *in vivo*. Here, we reconsider this assay and propose modifications for its optimisation. Microdissected human scalp hair follicles in anagen VI are cultured up to 10 days during which time hair shaft elongation proceeds at an *in vivo*-like rate. In its traditional form, this assay is best-suited for testing candidate hair growth-inhibitors, since anagen VI hair bulbs show already maximal growth and are highly susceptible to inhibitory compounds. Also, this assay imitates only systemic drug administration and is limited in its physiological relevance due to the absence of large portions of the pilosebaceous apparatus. To overcome these limitations, we have modified and optimised the assay, e.g. by enlarging and standardizing the read-out parameters for evaluating the modulatory properties of a candidate “hair drug”. Besides hair shaft elongation, proliferation and apoptosis of hair matrix cells, hair cycle stage, hair pigmentation (melanin quantity/distribution and melanocyte detection) and indications of compound toxicity (signs of follicle dystrophy) are assessed. This allows highly instructive test compound screening for its effects on spontaneous catagen development, “physiological” catagen development (induced by TGF-beta2), premature “pathological” (i.e. inflammation-associated) catagen development (induced by IFN-gamma). In parallel, we have applied strict parameters for quality control of organ-cultured hair follicles and included automatized measurements by customized digital analysis software. These modifications greatly enhance the instructiveness, sensitivity and predictive value of this assay as a screening tool for candidate hair growth and/or hair pigmentation-modulatory molecules, while simultaneously offering indications of a test compound’s potential toxicity in the human system.

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Gene targeting on embryonic skin explants: lessons and outcomes

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Continuous renewal of the epidermis is provided by so-called epidermal stem cells that give rise to the progeny that forms suprabasal layers of the epidermis. Several studies revealed that these lineage-specific stem cells might reside in the basal layer of epidermis and in the bulge area of the hair follicles. The absence of any molecular or genetic markers for these stem cells confines further development of the applicable gene therapy approaches for treatment of the dermatological disorders. We sought an approach where subsets of stem cells can be marked directly in developing embryonic mouse skin by using oligodeoxynucleotide (ODN) mediated gene targeting. We generated a transgenic mouse model, where a mutant b-galactosidase with inactivating mutation (G1651A; E523K) is expressed in the epidermis. When cells containing this mutant reporter gene are corrected by gene targeting, the enzymatic activity of the b-galactosidase is restored and corrected cells can be visualized by histochemical staining. We hypothesized that ODNs can correct the point mutation in epidermal stem cells and that clonal expansion of corrected cells can be visualized by staining. We have established appropriate conditions for the in vitro culture of the embryonic mouse skin, where epidermis and its appendages can be fully differentiated and developed. Embryonic skin was isolated from the E13 mouse embryos and treated topically with ODNs. Three weeks after the initial treatment, skin explants were harvested and cryosections were analyzed for the active b-galactosidase. Several positively stained cells were detected in the stratified layer of epidermis. The majority of the b-galactosidase positive cells were detected in conjunction with hair follicles, not with basal layer of epidermis, suggesting that during embryonic development epidermal stem cells may primarily migrate to the hair follicles. Further optimization of gene targeting on skin explants and live embryos, as well as histomorphometric analysis may shed important information of the lineage of epithelial cells.

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Regeneration of human hair follicles by cellular grafting

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Our studies have focused on the possibility of human hair follicle regeneration from the view of epithelial-mesenchymal interactions (EMI). As a first step, we examined the potential of human epidermal keratinocytes for follicular differentiation in combination with mouse dermal papilla (DP) cells as an active mesenchymal source. We have previously shown that versican-expressing DP cells possess hair inductivity utilizing a transgenic model. We further applied this finding to establish large-scale preparation of DP-enriched fraction from non-transgenic mouse skin using a freeze-thaw procedure. After confirming these DP cells have hair inductivity with both mouse and rat new born epidermis, fresh epidermal cells or cultured keratinocytes derived from human new born foreskins were co-grafted with mouse DP-enriched cells. Detection of human originated cells was confirmed by Hochest 33258 nuclear staining and human specific AluI DNA in situ hybridization. Results demonstrated follicle-like formation with a keratin fiber-producing hair shaft structure in the grafting area with fresh human epidermal cells. Mouse DP cells recruited to the human follicular epithelial cells were also evident. These characteristics were observed in graft with cultured human keratinocytes as well and were maintained up to the third passage. Furthermore, adult epidermal keratinocytes both from young human foreskins and from rat dorsal skins were also capable of differentiating into hair follicles with mouse DP cells. These results demonstrate that: 1) mouse DP possess hair follicle inductive properties across the species, 2) epidermal keratinocyes from non-hair forming skin are capable of differentiating into the follicular epithelium, and 3) epidermal cells from a mature stage are also capable of differentiating in response to inductive signals from DP cells, indicating the importance of EMI in follicular generation and the higher differentiation potential of epidermal keratinocytes than previously thought.

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Gene expression profile of epithelial stem cells induced by dermal papilla using Genechip microarrays

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The epithelial-mesenchymal interactions between keratinocyte stem cells and dermal papilla cells are crucial for normal development of the hair follicle as well as during hair cycling. In order to characterize the events occurring during the process of epithelial stem cell fate determination, we utilized a co-culture system by incubating human hair follicle keratinocytes stem cells with dermal papilla cells. Using Genechip microarrays, we analyzed changes in gene expression profiles within the stem cells upon induction by dermal papilla over a 1, 2 and 5 day time-course. After normalization and filtration, 272 genes were up- or down-regulated by >3-fold. Among up-regulated genes, the hair-specific keratin 6 hair follicle (K6hf) gene increased 7.9 fold, with a resulting 2-3 fold increase in the protein levels. The up-regulation of K6hf was unique to dermal papilla-induced differentiation since expression of K6hf was not induced by increased calcium. We also found that the more committed transit-amplifying cells of the hair matrix, are more readily differentiated by the dermal papilla than epithelial stem cells. For matrix cells the expression of K6hf protein peaked at day 2, while for stem cells it took 3-4 days. Since the beta-catenin signaling pathway has been implicated in the initiation of hair follicle development, we also examined the status of beta-catenin in the co-culture system. Although no changes are seen in beta-catenin gene expression, there was a significant increase in nuclear localization indicating that beta-catenin-regulated gene expression is occurring.

Our results show that the co-culture system recapitulates the epithelial-mesenchymal interactions which induce hair differentiation of the multi-potent keratinocyte stem cells and identify markers of differentiation as well as pathways involved in fate determination. Our finding also show that K6hf can be used as a marker for the initiation of dermal papilla-induced hair follicle differentiation of keratinocyte stem cells.

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Loss of epithelial *Bmpr1a* results in continuous proliferation of hair follicle epithelium and the development of matricomas

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Signaling by Bone Morphogenic proteins (BMPs) is thought to play important roles in the regulation of hair follicle differentiation and cycling. We have previously reported that Keratin 14-Cre mediated deletion of the BMP receptor IA (*Bmpr1a*) gene from the surface epithelium and epidermis of mice carrying a floxed allele of *Bmpr1a* results in reduced or absent expression of several hair follicle transcription factors and failure of differentiation of the hair shaft and inner root sheath. These results provided definitive genetic evidence that BMP signaling is required for differentiation of hair follicle matrix cells. Here we show that *Bmpr1a* mutant hair follicles also fail to cycle normally, being maintained in an abnormal, proliferative, anagen-like state. Analysis of older mutant mice reveals that, while some mutant follicles eventually degenerate to form follicular cysts, a subset of follicles continues to proliferate, forming large benign tumors. The tumor epithelium does not express keratins, except for very low levels of K17. The lack of follicular differentiation within the epithelial proliferations, together with absence of epithelial nuclear beta-catenin, indicates that these tumors are comprised of undifferentiated matrix cells and are similar to human matricomas. These results demonstrate a novel role for BMPRIA in regulating the proliferation of hair follicle epithelial cells, and suggest that loss of epithelial BMP signaling may be involved in the etiology of human matricoma.

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Expression of an olfactomedin-related gene in cultured rat hair follicular papillary cells

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The proliferating matrix keratinocytes located at the bottom of the hair follicle enclose almost completely a group of specialized mesenchymal cells called follicular papilla (FP) cells. Earlier studies showed that FP cells, but not their closely related dermal fibroblasts (DF), can maintain hair growth suggesting cell type-specific molecular signals. To define the molecular differences between these two cell types, we generated a subtraction cDNA library highly enriched in follicular papilla-specific cDNAs. Differential screening identified about 30 EST sequences and known genes that are preferentially expressed in cultured follicular papilla cells vs. fibroblasts. One of these cDNAs, which we named FP-1, represents the most abundant sequence in the subtraction library. The full-length FP-1 cDNA is 2,283 bp, that encodes a protein of 502 amino acids containing a signal peptide, a collagen-related domain and a olfactomedin-like domain. FD-1 message is highly abundant in cultured rat vibrissa follicular papillary cells, can be detected at low levels in stomach and ovary, but is absent in cultured dermal fibroblasts and in lung, heart, spleen and other 10 rat tissues. Monospecific rabbit antibodies to FP-1 recognizes in cultured FP cells a single 72kd glycoprotein band. FP-1 was not detectable in normal follicular papilla in vivo but become upregulated when FP cells were placed in culture. FP-1 may play a role as an extracelluler matrix protein involved in the reorganization of dermal papilla during certain phases of normal or pathological hair growth.

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Cleavage of caspase-14 coincides with stratum corneum formation during development of fetal mouse skin

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In adult human skin caspase-14, a protease implicated in terminal differentiation of keratinocytes, is present as complex of large and small subunits indicating catalytic caspase activity. In order to determine whether cleavage of the proenzyme is a secondary event relative to formation of mature epidermis or if it represents an integral step within this process, we investigated caspase-14 processing during fetal skin development in the mouse. Whole skin was sampled at different days of embryonic development and analyzed by immunohistochemistry and Western blot. Immunohistochemistry revealed that keratinocytes in suprabasal layers expressed caspase-14 as early as day E14.5 whereas the periderm and all other skin compartments were negative. By Western blot analysis, caspase-14 proenzyme was detected on E14.5 and at all later time points whereas cleavage products of the same size as those in adult mouse skin appeared on day E16.5. This coincides with the formation of orthokeratotic stratum corneum, but precedes the establishment of the permeability barrier and of the epidermal calcium and pH gradient. Our study demonstrates that caspase-14 processing parallels the development of stratum corneum in utero. These data provide a basis for screening of potential substrates and activators for caspase-14 that are present in this stage of development.

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Feather regeneration—the whole story

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Appreciation of feathers as model systems for understanding molecular controls of pattern formation and cytodifferentiation in epidermal appendages is inhibited by the lack of a comprehensive review of data concerning feather replacement. All recent studies are rooted in a 1972 account that was conceptually flawed and lacked reference to the presence of both αand βkeratins. For obvious reasons, it did not include allusion to the advances that have been made in our knowledge of keratinization processes and their control that have appeared over the last 30 years: unfortunately, relevant data for sauropsid (avian and reptilian) epidermis - a system now arguably better known for a variety of species than for the more familiar mammalian models - have been largely ignored. Feather anatomy and development can only be understood in the context of "vertical alternation of keratogenesis" (Baden and Maderson, J Exp Zool, 1972). This concept, that emphasizes how daughter cells arising from an apparently homogeneous germinal population may undergo quite distinct protein synthetic pathways, has been refined and explains how somatic growth accommodates the role of αkeratogenic tissues in providing a barrier to water loss. A review of studies on sauropsid epidermis since 1876, combining classical histology with "modern" ultrastructural, biophysical, physiological, biochemical and molecular data has allowed the preparation of a completely new, comprehensive series of graphic representations of stages in feather regeneration that (a) permits comparison with the cyclic activity of a hair follicle and (b) spotlights a number of significant aspects of keratinocyte behavior during feather morphogenesis that have no counterparts in mammalian epidermal appendages. This developmental model emphasizes the single unique feature of feathers - postmitotic patterning mechanisms establish spatially organized regions wherein loss of cell-cell adhesion permits separation of populations of mature keratinocytes resulting in a "branched morphology." This model accommodates all known natural feather morphs and explains phenotypes of genetic mutants and results from experimental perturbations.

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Isolation and characterization of adult hair follicle stem cells

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The characterization of presumptive hair follicle stem cells in the bulge has been hampered by the inability to target these cells genetically. Here, we use a keratin (K15) promoter to target bulge cells in transgenic mice with an inducible Cre recombinase construct or Enhanced Green Fluorescent Protein (EGFP), which allow for lineage analysis and for isolation of the cells. Lineage analysis demonstrated that bulge cells in adult mice normally generate all epithelial cell types within the intact follicle during follicle cycling. After isolation, adult bulge cells retained their multipotent nature and generated all components of the cutaneous epithelium in skin reconstitution assays. Bulge cells also have an increased in vitro proliferative potential relative to K15-EGFP-negative cells. Genetic profiling of the isolated hair follicle stem cells reveaed differentially expressed genes previously associated with inhibition of proliferation and differentiation, as well as novel receptors potentially important for maintaining the stem cell phenotype. For example, the inhibitor of differentiation genes, ID-2 and ID-4, Basonuclin, Growth arrest specific 1, Tenascin C, PBSF/SDF-1, Embigin, and the S100 calcium binding proteins have all been associated with quiescent or undifferentiated cells, and were up-regulated in hair follicle stem cells. Expression of key signaling genes known to be important for hair growth was downregulated in the stem cell population, suggesting that other downregulated genes novel to the follicle may represent new targets for hair growth manipulation. For example, Wnt3a and Wnt10a were depleted while inhibitors of the WNT pathway, including Sfrp-1, disabled 2 and Dkk-3, were prominently expressed. Overall, our findings point to the preferential expression of a remarkably diverse set of genes with apparently redundant functions related to maintaining the stem cell phenotype.

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Human hair follicles display a functional equivalent of the hypothalamic–pituitary–adrenal axis and synthesize cortisol

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It has been proposed that mammalian skin contains a peripheral equivalent of the hypothalamic–pituitary–adrenal (HPA) axis, which controls adrenal cortisol production and serves as a major stress–response regulator and endocrine integrator system in mammalian organisms. Hypothalamic corticotropin releasing hormone (CRH) is the most proximal element of the HPA axis, and the major regulator for the pituitary expression of the proopiomelanocortin (POMC) gene and for the processing of POMC–derived peptides, such as α–melanocyte stimulating hormone (MSH), β–endorphin, and adrenocorticotropic hormone (ACTH), which induces adrenal cortisol production. Human skin indeed expresses transcripts and/or immunoreactivity (IR) for most of the key components of the HP axis, including CRH and CRH–receptor, POMC, α–MSH, ACTH and melanocortin receptors and even expresses key enzymes for cortisol synthesis. However, convincing evidence that a functional equivalent of the HPA axis operates in normal skin *in situ* and that normal human skin actually synthesizes cortisol, is still missing. Here, we show that human scalp hair follicles display a fully functional equivalent of the HPA axis, synthesize cortisol, and recruit classical HPA feedback loops.

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Hair growth control by BMP: more complex than suspected

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Bone morphogenetic proteins (BMPs) are key regulators of hair follicle morphogenesis and cycling. Here, we have further investigated this role in transgenic mice that overexpress a potent BMP inhibitor, noggin, under the control of the neuron−specific enolase (NSE) promoter. Transgene expression was seen in hair matrix keratinocytes, hair follicle melanocytes, and skin nerve fibers, and appeared only after the induction of both primary (tylotrich) and secondary (non−tylotrich) pelage hair follicles. Noggin overexpression resulted in a dramatic loss of hair shafts postnatally, while the density of developed hair follicles was not significantly different from WT controls. After an apparently normal but shortened postnatal hair follicle morphogenesis, only secondary hair follicles of NSE−noggin tg mice showed premature onset of catagen (with massive and abnormal intrafollicular apoptosis), and sebocyte−like differentiation was frequently seen in the proximal ORS. Noggin tg mice also showed a much accelerated onset of the first anagen, following an abnormally short first telogen phase with defective club hair formation. These hairs were subsequently lost by shedding (i.e. were not retained, as in WT mice), explaining the increasing postnatal alopecia. This supports the concept that BMP signaling is specifically required for proper hair follicle cycling and ORS keratinocyte differentiation/apoptosis in non−tylotrich hair follicles and that inhibition of BMP signaling stimulates the telogen−anagen switch. Unexpectedly, this also indicates that BMP inhibition can also trigger premature catagen onset anagen hair follicles.

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Role of laminin-10 in hair development

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Previously, we showed that laminin-10 is the primary laminin expressed by elongating hair germs, and that laminin-10 is required for hair follicle development. In the current study we sought to identify hair-promoting laminin domains, and to determine the role that laminin-10 plays in hair follicle formation. Lama5 -/- transgenic mice, which lack laminin-10, do not survive beyond E16.5, however development of lama5 -/- skin can be studied after grafting E16.5 day skin to nude mice. While lama5 -/- skin showed a complete lack of hair development after grafting, incubation of lama5 -/- skin in a laminin-10 solution prior to grafting, restored the development of fully formed hair as shown 16 days after grafting. Two other cutaneous laminins showed profoundly divergent abilities to rescue hair formation in lama5 -/- skin. While exogenous laminin-5 restored hair follicle development to similar levels as that of laminin-10, as shown by quantitative morphometric analysis, exogenous laminin-1 failed to show any effect. These results suggest that laminin-5 and laminin-10 share common structural hair promoting domains which laminin-1 lacks. Previously we showed that sonic hedgehog (SHH) was reduced in lama5 -/- skin, suggesting a role for laminin-10 in the maintenance of SHH expression. To further examine this, we treated lama5 -/- skin with SHH- adenovirus prior to grafting. This resulted in the formation of large hair germs, which failed to elongate. These results suggest that laminin-10 may play a role in hair germ elongation downstream of SHH. Finally, we studied whether lack of laminin-10 could cause a feedback inhibition of other early markers of hair follicle formation. By RT-PCR, we found that E16.5 lama5 -/- skin showed downregulation of noggin, Msx1 and Lef-1 at E16.5, however Wnt 10a, Wnt 10b, BMP2, and Msx2 showed normal expression, compared to wild type controls. These results implicate laminin-10 in the maintenance of expression of several important early hair markers.

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Detailed characterization of eccrine gland development

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Human disorders in which the eccrine glands are lost or are non-functional can be life threatening during infancy and childhood due to impaired thermoregulation. In addition, eccrine sweat contains critical components of the innate immune system and may serve an important role in defense against skin pathogens. In spite of the clinical significance of eccrine glands, their development has not been well studied. To provide a foundation for our analyses of mouse mutants displaying eccrine gland defects similar to those observed in human disorders, we have undertaken a detailed characterization of the morphological and molecular changes that occur during normal gland development. Our data show that eccrine glands develop in two waves, one that begins at E14.0 and one that begins perinatally (E18.5). The first visible sign of insipient gland development is the formation of elevated dermal pads. Mesenchymal cells of the early pad express high levels of alkaline phosphatase, differenting them from adjacent non-appendageal dermal cells. A variety of developmental regulators are expressed in volar skin at this time, including the homeobox transcription factors En1 and Dlx2. The former is throughout volar ectoderm, whereas the latter is restricted to ectoderm overlying the pads and nail field. A day or two later, focal clusters of basal epidermal cells overlying the pads elongate and become wedge-shaped, forming ectodermal placodes of the presumptive glands. By birth, the first wave of eccrine glands have developed to the peg stage, characterized by an elongated ectodermal downgrowth with a slightly widened distal tip. By P6, the coiled secretory portion is visible and by P8 the spiraled duct through the epidermis can be delineated by selective K6 expression in the suprabasal cells of the epidermis. En1 continues to be expressed by the gland and overlying epidermis throughout the animal's lifetime whereas Dlx2 is restricted to the distal tip of the elongating gland anlaga and is downregulated upon gland maturity. These studies provide the first detailed delineation of distinct stages of eccrine gland morphogenesis.

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Glial cell line-derived neurotrophic factor (GDNF) and neurturin (NRTN) as a new player in human hair follicle biology

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GDNF and another related member of the GDNF family, neurturin (NRTN), as well as their cognate receptors (GFRα−1, GFRα−2) are essential for nervous system development, and are also involved in murine hair cycle control (Am J Pathol 156:1041, 2000). However, it is still unknown whether GDNF and NRTN play any role in human hair biology. As a first step towards to exlpore this, the immunoreactivity (IR) for GDNF, NRTN, GFRa−1, GFRa−2 and their common signal transduction element, c−Ret, was studied in human scalp hair follicles (HF) *in situ* by immunohistology. GDNF IR was strongest in the ORS, in hair matrix cells, in the dermal papilla (DP) of anagen VI hair follicles, while it was only weak in catagen and telogen HFs. NRTN IR was most prominent in the DP, CTS, and IRS of human anagen VI scalp hair follicles. Strong GFRα−1 IR was found in the DP, CTS, ORS, while GFRα−2 IR was also detected the IRS, and the IR for both receptors was weak or absent during catagen and telogen. c−Ret IR was strongly expressed thoughout the hair follicle. GDNF mRNA transcripts were detected by RT−PCR in both in isolated hair follicles and human scalp skin. *In vitro*, GDNF partially protect human HF from TGFβ2−induced premature catagen development. These observations provide the first indication that GFRα−mediated signaling plays an important role not only in murine, but also in human HF biology.

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The hedgehog target gene BEG4 associates with a Gli signaling complex

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Hedgehog (hh) signaling has diverse, context-dependent functions while controlling the proliferation, differentiation and movement of epithelial cells during development. Unregulated hh signaling has been shown to be an important contributor to epithelial carcinogenesis. While the transcriptional output of the hh pathway is largely governed by the Gli family of transcription factors, how Gli target genes are selected in different contexts remains largely unknown. We have previously shown that the novel hh target gene, BEG4, is expressed in both human and mouse transgenic basal cell carcinomas. When expressed ectopically in keratinocytes with Gli transcription factors, BEG4 alters Gli transcriptional activity and induces epithelial growths in regenerated human skin. An important question is whether BEG4 acts directly or indirectly with the Gli signaling complex. Using GST affinity columns, we find that BEG4 interacts with Gli-containing complexes. BEG4 can also co-immunoprecipitate Gli in cell extracts. Further, analysis of interactions with other members of the Gli signaling complex demonstrates BEG4 can also associate with Suppressor of Fused. Together these data suggest that the BEG4 regulatory effects on Gli transcriptional activity involve (at least in part) interactions with known regulators of hh signaling. Our data are consistent with the idea that BEG4 may control organ growth and tumorigenesis by directly regulating the Gli signaling complex.

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The murine hair follicle is a melatonin target

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The aim of this study was to prove that murine and/or human hair follicles in situ are indeed peripheral melatonin targets and express melatonin membrane receptors (MT1, MT2) and/or the mediator of nuclear melatonin signaling, the orphan nuclear receptor RORα. Immunohistochemical examination of MT1 and RORα on c57BL/6 mouse hair follicle, and semi-quantitive RT-PCR for MT1 and MT2, and quantitive real time PCR for MT1, MT2 and RORα on mouse skin cDNA has been studied. Short term-mouse skin organ culture stimulated with melatonin was studied to examine the effect of melatonin on keratinocyte apoptosis. Murine hair follicle keratinocytes show both MT1-like immunoreactivity (IR) and RORα-like IR, both of which changed substantially in a hair cycle-dependent manner. Both semi-quantitive RT-PCR for MT1 and MT2, and quantitive real time PCR for MT1, MT2 and RORα revealed that all three genes are transcribed in normal mouse skin in hair cycle-dependent manner. Functionally, melatonin (0.01 to 1 nM) significantly inhibited the constitutional level of epidermal and hair follicle keratinocyte apoptosis in short term-mouse skin organ culture. The prototypic pineal hormone, melatonin reputedly exerts many functional effects on mammalian skin and/or its isolated cell populations in culture (e.g., melanogenesis inhibition, melanocyte growth inhibition, regulation of seasonal changes in the pelage), and is recognized as a potent free radical scavenger. We here provide evidence that normal murine skin is a prominent target for melatonin bioregulation, that it expresses MT1, MT2 and RORα and that at least some of these regulators are functionally active in situ.

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Genetic and phenotypic analysis of the near naked (Hrn) phenotype in mice

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The near naked (Hrn) is a semi-dominant mutation in mice, which is characterized by complete hairlessness since birth in homozygous animals, and sparse hairs in heterozygotes. The molecular basis and pathomorphological features of this mutation are unknown. Based on crossbreeding studies, linkage of this mutation to hairless (hr) locus on chromosome 15 was proposed over 20 years ago [Stelzner et al., 1983]. In order to assess the possible allelism of Hrn and hr, direct sequencing of the coding region of the hairless gene in Hrn mutants was performed and no mutation was identified. The histological studies of skin and hair follicles of mutant mice (Hrn/Hrn and +/ Hrn) at different stages of postnatal development and their comparison to hairless (HRS/J hr/hr) mice revealed that Hrn phenotype is quite different from hairless. Specifically, the defect in Hrn is characterized by dramatic abnormalities in the process of differentiation in precortex region and in hair shaft formation, while in contrast the dominant feature of the hairless phenotype is hair follicle disintegration. We devised a breeding scheme to directly assess the allelic nature of the Hrn and hr. Backcross breeding of double heterozygotes (+/Hrn:+/hr) to homozygous hairless rhino mice (hrrh/hrrh) resulted in the absence of wild type offspring in the 176 classified progeny. These data suggest that Hrn and hr are closely located, but different genes. The Hrn mutation may serve as a model of autosomal dominant Marie Unne Hypotrichosis in humans, which is closely linked to, but not allelic with the human hairless gene.

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The lowermost portion of the bulge forms the secondary hair germ during late catagen

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The lowermost portion of the telogen follicle consists of the bulge and secondary hair germ. At anagen onset, cells within both the bulge and secondary hair germ proliferate and form the new lower follicle, however, the relationship between the bulge and secondary hair germ remains unclear. Are secondary hair germ cells derived directly from the bulge or do they arise from cells in the anagen bulb that survive catagen? To address this question, we used bromodeoxyuridine (BrdU) labeling strategies to follow bulge cells during hair follicle cycling. We administered BrdU twice per day for three days during anagen onset after hair plucking. This labeled the bulge cells and secondary hair germ. We then chased for different time periods and collected tissue for detection of BrdU. We found that in the catagen follicle, 18 days after plucking, labeled cells were present exclusively in a cylindrical configuration in the basal outer root sheath of the bulge region. In contrast, after examining serial sections we could not detect any labeled cells within the catagen bulb at 18 days. In the ensuing telogen stage, the majority of cells in the secondary hair germ were label retaining cells (LRCs), which could only have originated from the bulge. Careful histological examination of hair follicles in catagen showed that the secondary hair germ forms at late catagen when the lowermost bulge cells collapse around the ascending club hair.

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Differential expression of p63 isoforms during epidermal development

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p63 is a transcription factor required for the initiation of an epithelial stratification program during epidermal development. p63 knockout mice have truncated limbs and lack all stratified epithelia and their derivatives. Alternative promoter usage and alternative splicing give rise to six different p63 isoforms, three of which contain a transactivation domain (TA isoforms) and three of which lack this domain (ΔN isoforms). In mature epidermis, ΔN isoforms are the predominantly expressed p63 isoforms. However, the expression patterns and roles of the different p63 isoforms during epidermal development are not known. To determine the expression profile of p63 isoforms during embryonic development, we performed RT-PCR using primers specific for the TA and ΔN isoforms. TA isoforms were first detected at E7.5, before the commitment of the single-layered surface ectoderm to a stratification program. In contrast, ΔN isoforms were not expressed until E9.5, after commitment to stratification but before the onset of terminal differentiation. These findings support our model that TAp63α is required for the initiation of stratification and that ΔNp63α counterbalances the effect of TAp63α such that terminal differentiation can occur. Although the role of p63 in epidermal development has now been established, the target genes that mediate the effects of p63 are not known. To identify target genes of p63, we have performed gene expression profiling on RNA isolated from the skin of wild type and p63 knockout mice using Affymetrix microarrays. Putative target genes of p63 are being validated by Real Time RT-PCR, RNase protection assays and immunohistochemistry. The identification of p63 target genes will provide further insight into the molecular function of p63.

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A cell surface marker for the purification of dermal papilla cells

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The formation of follicles during embryogenesis and regeneration of follicles in the context of the hair cycle depend on inductive signaling between the epithelial and dermal components of the hair follicle. In particular, inductive signals from the dermal papilla (DP) are sufficient to drive follicle formation in competent epidermis and are thought to drive follicular regeneration during the hair cycle. Therefore, an efficient method for purification of DP-cells may provide an important tool towards the development of techniques to encourage follicle rejuvenation or formation in the adult to combat alopecia. We have used a transgenic line expressing GFP under the control of the versican promoter to enrich for dermal papilla cells, and have performed differential expression screens that identified a number of candidate genes to mediate inductive signaling from the DP. One of these genes, designated DPS-1 (for Dermal Papilla Specific 1), exhibits the properties required for DP purification. In the skin, it is expressed specifically and exclusively in the DP. Expression begins during embryogenesis at the dermal condensate stage and is maintained throughout the anagen phase of the hair cycle in both juveniles and adults. However, DPS-1 is not observed in the DP during the telogen phase, rendering its expression in correlation with hair inductive activity. More important, DPS-1 encodes for type II transmembrane protein and therefore can be used as a cell-surface marker to purify DP cells. Antibodies raised against the extra-cellular domain of DPS-1 specifically stain the DP and can be used to purify this population by FACS.

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c-Myc alters stem cell fate by directly regulating genes involved in WNT and Hedgehog signaling

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The oncogene c-Myc has recently been shown to be involved in stem cell fate determination. Our constitutive K14.myc2 mouse model, in which c-Myc expression is targeted to the basal layer of the epidermis and to multipotent stem cells residing in the bulge region of hair follicles, exhibits an accelerated depletion of stem cells and an increase in size and number of sebaceous glands. These results suggest that deregulated expression of c-Myc may alter the fate of multipotent stem cells. However, the mechanism by which c-Myc alters the fate of stem cells remains to be resolved. A recent report suggests that c-Myc alters the expression of cell adhesion molecules, which prevents stem cell migration and therefore causes the cell to be influenced by fate-determining signals from the surrounding microenvironment. To further study the fate-determining role of c-Myc, we developed an inducible mouse model in which c-Myc expression is targeted to epidermal stem cells and is regulated by topical application of RU486. Induction of c-Myc at E14.5 results in an acceleration of sebaceous gland development, while induction of c-Myc at E10.5 results in a defect in hair follicle development and epidermal hyperplasia. The inducible mouse model is an ideal system to identify downstream targets of c-Myc in an in vivo environment. Therefore, we subjected RU486-treated skin samples to microarray analysis and Real Time RT-PCR. One of the potential downstream targets of c-Myc, DKK1 (dickkopf1), has been shown to prevent hair follicle formation, while other targets of c-Myc such as IHH (Indian Hedgehog) and Scd1 are involved in sebaceous gland development. Interestingly, the promoters of both DKK1 and IHH contain potential binding sites for c-Myc and chromatin immunoprecipitation assays confirmed that c-Myc was bound to these promoters in vivo. These data suggest that c-Myc alters stem cell fate by activating direct targets involved in both the WNT and Hedgehog signaling pathways.

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Microarray analysis of gene expression in the bulge of human anagen hair follicle following laser capture microdissection

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To characterize bulge area of human anagen hair follicles (HF), repository of stem cells, we analyzed and compared its gene expression profile to other portions of the HF using precisely navigated laser capture microdissection (LCM) and microarray analysis. To isolate the bulge outer root sheath cells (ORS) by LCM, serial transverse sections of scalp biopsies were made (approximately 40 HF/section). The bulge area was defined by the presence of label-retaining cells in human scalp xenograft studies and could be identified by high keratin 15 expression in ORS and arrector pili muscle desmin staining, when compared to ORS below the bulge. The bulge ORS and ORS below the bulge were separately microdissected by LCM from unstained transverse sections. Total RNA was extracted (11.5-24.7ng), amplified and hybridized with Affymetrix oligonucleotide microarrays (HG-U133A genechip, gene probe sets n=22283). The quality and comparability of separate and matched pairs of bulge ORS and below bulge ORS genechips was demonstrated by the 40% gene expression "present calls" on each chip and by the 89% overlap of gene expression "absent and present calls" on all chips. Comparing bulge ORS to below bulge ORS from 3 individual biopsies, 45 genes were upregulated in all 3 chip pairs and 117 genes were upregulated in 2 out of 3. When categorized by cellular component gene ontology, 40 membrane, 47 intracellular and 15 extracellular genes were upregulated. Highly expressed bulge ORS genes include those involved in developmental processes such as epidermal differentiation and neurogenesis. The increased fold changes observed in inhibitors of the Wnt signaling pathway, negative regulators of cell proliferation, and anti-apoptosis proteins are consistent with quiescent bulge stem cells in anagen HF. Other upregulated biological processes include protease inhibition, hormone metabolism, and response to external stress, that may provide protection and maintenance of this special niche.

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Increasing cellular energy promotes hair growth in hair follicle explants

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Energy in the form of ATP is required for many processes in the cell. Some of these processes include DNA synthesis, cell proliferation and active transport. We hypothesized that increasing cellular energy will lead to an increase in hair follicle cell proliferation, resulting in increased hair growth. Pretreatments with AMP or creatine were found to increase human follicle dermal papilla cell DNA synthesis. Furthermore, creatine was found to increase hair growth in human hair follicle explants by 70% compared to the untreated follicles. In addition, this increase in hair follicle growth was up to 250% when creatine was used in combination with N-acetyl carnitine, and nicotinamide adenine dinucleotide (NADH). The creatine/creatine kinase system is known to have an important role in mitochondrial function, and is one of the cell's energy(ATP) storage system. Moreover, carnitine is vital to mitochondrial function, while NADH is vital to the electron transport chain. For those reasons, we postulate that supplementing hair follicles with creatine, carnitine, and NADH, leads to increased cellular efficiency in ATP formation, ATP storage, as well as ATP utilization, resulting in increased cellular proliferation and hair follicle growth.

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Neuroimmunophilin ligand analogs induce anagen hair growth in C57BL/6J mice

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Application of topical FK506 has been shown to induce anagen hair growth in C57BL/6J telogen mice, the C3H/HeJ mouse, and the Dundee Experimental Bald Rat. Hair growth induced by this naturally-occurring immunophilin ligand has been attributed to its immunosuppressive effects. However, FK506 which binds to FKBP-12 (a cytosolic protein found in the central and peripheral nervous system and the immune system) is also neurotrophic. Our goal is to understand the neuronal component of hair growth and the hair cycle. We examined the effects of topically-applied small molecule neurotrophic nonimmunosuppressive FKBP-12 immunophilin ligands GPI1046 and GPI1511 (Guilford Pharmaceuticals, Baltimore, MD), as well as FK506. The backsides of 50 day old C57BL/6J mice were carefully shaved to remove hair, and these mice were treated with nothing, ethanol vehicle, 1 mol FK506, 1 or 6 mol GPI1046, or 1 or 6 mol GPI1511 three times per week over 6 weeks. We found that FK506 initiated onset of anagen within 11 days after application whereas GPI1046 and GPI1511 also initiated onset of anagen but not as quickly as FK506. At 42 days of treatment, anagen hair growth was greatest and statistically significant with topically applied 1 µmol GPI1511, 1 µmol GPI1046, and 6 µmol GPI1046 vs. 6 µmol GPI1511, 1 µmol FK506, vehicle and shaving only. Percent hair coverage of the treated area at Day 42 was also higher in the GPI1046-, and GPI1511-treated mice vs. the mice in the FK506, EtOH, or shaved-only groups. This study suggests a neuronal component may indeed be an important component of hair growth and the hair cycle in the C57BL/6J mouse model. Clinical application for specific human alopecias can only be speculated on at this point. We have initiated the use of xenografts (full thickness human skin grafts onto immunodeficient mice) as a transition model to evaluate the function of these compounds on specific hair diseases.

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Morpho-regulation of ectodermal organs: pathological changes or phenotypic variations?

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Ectodermal organs are composed of keratinocytes organized in different ways during induction, morphogenesis, differentiation, and regenerative stages. We hypothesize that an imbalance of fundamental signaling pathways should affect multiple ectodermal organs in a spatio-temporal dependent manner. We produced a K14-Noggin transgenic mouse to modulate BMP activity and test the extent of this hypothesis. We observed thickened skin epidermis, increased hair density, altered hair types, faster anagen re-entry, and formation of compound vibrissa follicles. The eyelid opening was smaller and ectopic cilia formed at the expense of Meibomian glands. In the distal limb, there were agenesis and hyper-pigmentation of claws, inter-digital webbing, reduced footpads, and trans-differentiation of sweat glands into hairs. The size of external genitalia increased in both sexes, but they remained fertile. We conclude that modulation of BMP activity can affect the number of ectodermal organs by acting during induction stages, influence the size and shape by acting during morphogenesis stages, change phenotypes by acting during differentiation stages, and facilitate new growth by acting during regeneration stages. Therefore during organogenesis, BMP antagonists can produce a spectrum of phenotypes in a stage-dependent manner by adjusting the level of BMP activity. The distinction between phenotypic variations and pathological changes are discussed.

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Role of B cells in leprosy

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Leprosy, is an infectious disease of the skin, where clinical manifestations correlate with the type of immune response mounted to the pathogen, Mycobacterium leprae. We used DNA microarrays to characterize gene expression patterns in skin biopsies from individuals with a diagnosis of tuberculoid leprosy and compared those to the patterns of gene expression seen in biopsies from lepromatous leprosy. We observed that genes characteristically required for B cell activation and function were significantly up-regulated in lesions of lepromatous patients, those suffering from the disseminated form of the infection. Lepromatous skin lesions exhibited marked up-regulation of genes related to humoral immunity, including immunoglobulin (Ig) heavy and light chains and molecules involved in B cell activation as CD19 and CD22. To further investigate the identity and localization of B lymphocytes in leprosy tissue, immunohistochemistry was performed. We found large clusters of CD20+ B cells in patients with the diseminated lepromatous as compared with the localized tuberculoid form of the disease. We also found an increase in the expression of different markers for B cells (CD20, CD19, CD22), and plasma cells (CD138) in lepromatous lesions compared with tuberculoid lesions.

Our data indicate that B cells are present at the site of disease in leprosy and suggests a role for a B cell dependant mechanism that contributes to the disease progression and tolerance in leprosy.

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Pimecrolimus does not affect the migration capacity of cutaneous dendritic cells

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Migration of antigen-laden Langerhans cells (LC) into draining lymph nodes is a pivotal step in the development of the primary immune response in contact hypersensitivity (CH) We have examined the effects of the calcineurin inhibitor pimecrolimus on the migration and maturation capacity of LC in a skin organ culture assay and *in vivo*. Ears of BALB/c mice were treated topically with 10 µl 1% pimecrolimus or with ethanol (vehicle), twice at an interval of 8 h on day 1. Forty hours later, the ears were dissected and the dorsal, mechanically separated, sides floated on culture medium for 3 days prior to analysis. LC in epidermal/dermal sheets and the migrated LC and dermal dendritic cells (DDC) in the culture medium of pimecrolimus- and vehicle-treated samples were similar, both in numbers and degree of maturation. Antigen-induced migration of LC into the draining lymph nodes was studied in mice treated orally with 90 mg/kg pimecrolimus or placebo (vehicle) 2 h before and 4 h after FITC application. After 24 h, the numbers of antigen-laden $FITC^{+}MHC\ II^{+}$ cells in lymph nodes were not statistically different in pimecrolimus- and vehicle-treated mice (1.40 vs. 1.54%). In mice treated intraperitoneally with 200 µg anti-LFA-1 antibody (M 17.4), used as a positive control, only 0.25% $FITC^{+}MHC\ II^{+}$ cells were detected ($p > 0.05$). Taken together, our data show that neither topically nor systemically administered pimecrolimus interferes with the migratory and maturation capacity of LC/DDC. This is in line with previous data, which showed that oral treatment of mice with doses up to 120 mg/kg pimecrolimus did not impair the induction phase of CH in contrast to cyclosporine A and tacrolimus, whereas the elicitation phase was potently inhibited with lower doses (J Invest Dermatol, 121:77-80,2003). In conclusion, the data indicate that pimecrolimus leaves the indigenous immune system of the skin unaltered.

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Prevention of UV-induced immunosuppression by IL-12 is dependent on DNA repair

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Ultraviolet radiation (UV) suppresses the induction of contact hypersensitivity (CHS) and induces hapten-specific tolerance which is mediated via regulatory T cells (Tr). Interleukin (IL)-12 is able to prevent the suppression of CHS by UV and even to break UV-induced tolerance by yet unknown mechanisms. IL-12 recently was shown to exhibit the capacity to reduce UV-induced DNA damage via the induction of nucleotide excision repair (NER). Since UV-induced DNA damage is an important molecular trigger for UV-mediated immunosuppression we studied whether the restoring effect of IL-12 is linked to its capacity to reduce DNA damage. Injection of IL-12 into wild type mice (wt) which were sensitized through UV-exposed skin restored the CHS response completely. In contrast, Xpa knock out mice (Xpa-/-) which are deficient in NER were not able to mount an immune response after UV exposure despite the injection of IL-12. This indicates that the prevention of UV-induced suppression of CHS by IL-12 is dependent on functioning DNA repair. In contrast, IL-12 was able to break already established UV-induced tolerance both in wt and Xpa-/-, indicating this effect to be independent of NER. Accordingly, adoptive transfer of suppression via injection of Tr into naive recipients was inhibited by IL-12 both in wt and Xpa-/-. Inhibition of sensitization by UV is due to the depletion of Langerhans cells (LC) which is caused by UV-induced DNA damage. Accordingly, LC depletion by UV was prevented upon injection of IL-12 into wt but not in Xpa-/-.Taken together, these data indicate that the prevention of UV-induced inhibition of CHS by IL-12 is linked to its capacity to induce NER. In contrast, breaking of UV-induced tolerance and the activity of Tr by IL-12 is independent of NER and mediated via another yet to be determined mechanism. These data demonstrate for the first time a link between NER and the prevention of UV-induced immunosuppression by IL-12.

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T cells, B cells, and antigen presenting cells are activated, depart from secondary lymphoid organs, and infiltrate the skin in an animal model of atopic dermatitis

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We previously generated an interleukin-4 transgenic (Tg) mouse line that develops a chronic inflammatory skin disease, closely resembling human atopic dermatitis. To characterize the activation status and the dynamic movement of inflammatory cells involved, we examined the skin, skin-draining lymph nodes (LNs), and spleen of non-Tg mice and Tg mice before disease onset, at acute and chronic skin disease stages by flow cytometry (FACS) and immunofluorescence microscopy (IF). As the disease progresses, T cells, B cells, and antigen-presenting cells (APCs) decrease in the spleen, while they increase in the LNs, particularly in the chronic disease. The minor cell types, monocytes/macrophages and natural killer cells, slightly increase in both LNs and spleen. Despite their decrease, T cells and B cells in the spleen are marked by the increase of activating molecules CD44 and CD69, as occurred in LNs, coincided with the disease progression. In LNs, the T cells and APCs are activated, particularly in chronic disease, marked by increased inducible co-stimulatory molecules ICOS and PD-1 on T cells and their ligands CD80, CD86, B7h, B7-DC on APCs. APCs were also found in large number in the skin of Tg mice with disease. T cells in LNs of the Tg mice with disease proliferate spontaneously and proliferate in a significantly enhanced manner in responding to anti-CD3, mitogen (PHA), and superantigens (Staphylococcus enterotoxin A & B). By IF, CD3+, CD4+ and CD8+ T cells were detected in the lesional epidermis of Tg mice. In the dermis of Tg mice with disease, CD3+, CD4+, CD8+ T cells, B cells, and macrophages were detected in a significantly increased manner, with CD4+ cells predominate the T cell population. Together, our data suggest that, as the disease progresses, the helper and cytotoxic T cells, B cells, and APCs are activated and expanded, departing from secondary lymphoid organs and infiltrating the skin where they cause inflammatory disease.

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Th1, Th2, and non-Th cytokines are involved in pathogenesis in an animal model of atopic dermatitis

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We determine the cytokine milieu in an epidermally expressed IL-4 transgenic (Tg) mouse line which develops a skin inflammatory disease resembling human atopic dermatitis (AD) and fulfills the clinical diagnostic criteria of human AD. cDNA microarray, reverse transcription (RT)-real time PCR and flow cytometry (FACS) were utilized to examine the cytokine mRNA and protein expressions in the skin and skin-draining lymph nodes (LNs). cDNA microarray screening indicated that the mRNAs encoding IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-13, TNF-α, IFN-γ were up regulated in the skin of chronic lesion (CL) (n=7) and to a lesser degree in non-lesion (NL) (n=7) when compared to that of non-Tg mice (n=2), with fold increases range from 1.9 to 23.1. Verifying the microarray data was carried out by quantitative RT-real time PCR with total RNA (n=10-15) from the skin of non-Tg mice and skin from Tg mice before onset (BO), NL, acute lesion (AL), and CL. When compared to Non-Tg mice, the cDNA copy numbers of IL-1β, IL-4, IL-6, IL-10, TNF-α and IFN-γ from skin of CL, AL and NL significantly increased. IL-2 and IL-12p40 cDNA copy numbers were increased in AL, but not in CL. IL-5 and IL-13 cDNAs were increased from 1.3 to 4.4 fold, significantly in the skin of BO, AL, CL and NL. Interestingly, IL-1β, IL-4, IL-5, IL-6, TNF-α, and IFN-γ cDNAs were increased in skin of Tg mice BO with some (IL-1β, IL-4, IL-6, IFN-γ, TNF-α) reached statistical significance. FACS analyses showed that the frequency of keratinocytes producing IL-4 increased as the disease progressed. The frequencies of Th1 cytokine (IL-2 and IFN-γ) and Th2 cytokine (IL-4 and IL-10) producing T cells and IL-12-producing antigen-presenting cells in both LNs and lesional skin also increased, especially in mice with CL. These results suggest that not only Th2, but also Th1 and non-Th cytokines, play a role in initiating and maintaining the inflammatory disease in this animal model.

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UV-induced regulatory T cells inhibit the effector phase of contact hypersensitivity only upon injection into the area of challenge

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Epicutaneous application of haptens onto UV-exposed skin induces hapten-specific regulatory T cells (Tr). Tr inhibit the induction of contact hypersensitivity (CHS) when injected i.v. into naive recipients, but not the elicitation when injected i.v. into sensitized recipients. In contrast, when UV-induced Tr were injected intracutaneously (i.c.) into the ears of sensitized mice, elicitation of CHS was suppressed. Depletion of either CD4+ or CD25+ fractions resulted in the loss of transfer of suppression upon i.v. or i.c. injection, indicating that UV-induced Tr belong to the CD4+CD25+subtype. I.c. injection of dinitrofluorobencene (DNFB)-specific Tr into the ears of oxazolone (OXA)-sensitized mice did not affect the challenge with OXA, indicating hapten-specificity. However, when ears of OXA-sensitized mice were injected with DNFB-specific Tr and painted with DNFB before OXA challenge, CHS response was suppressed. This indicates that the activation of Tr is hapten-specific, however, once they are activated their suppressive activity is non-specific (bystander suppression). The inhibitory effect correlated with the local expression of IL-10. Accordingly, suppression was blocked by i.c. injection of an anti-IL-10-Ab. Depletion studies also revealed that UV-induced Tr express the lymph node homing receptor L-selectin (CD62L) which was functionally relevant since incubation with anti-CD62L-Ab prevented transfer of suppression when injected i.v. into naive mice. In contrast, FACS analysis demonstrated that Tr do not express the ligands for the skin homing receptors E- and P-selectin. Thus, we conclude that UV-induced Tr though being able to inhibit T effector cells do not suppress the elicitation of CHS upon i.v. injection since they do not migrate into the skin due to the expression of lymph node homing but not of skin homing receptors.

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Interleukin-4 strongly enhanced eotaxin-3/CCL26, but not eotaxin-2/CCL24, production in a human keratinocyte cell line, HaCaT cells

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Eotaxin-2/CCL24 and eotaxin-3/CCL26 are CC chemokines and their receptor, CC chemokine receptor 3 (CCR3) is preferentially expressed on eosinophils. Previous reports showed the production of eotaxin-3/CCL26 in vascular endothelial cells and dermal fibroblasts. However, the regulation of eotaxin-2/CCL24 and eotaxin-3/CCL26 production in keratinocytes (KCs) has not been well documented. We investigated the expression and production of eotaxin-2/CCL24 and eotaxin-3/CCL26 in a human KC cell line, HaCaT cells. HaCaT cells were cultured with various stimuli for 24 hours. RT-PCR was performed using these cells and ELISA was carried out using supernatant of HaCaT cells. The expression and production of eotaxin-2/CCL24 in HaCaT cells was not enhanced by TNF-α and IFN-γ, but slightly enhanced by IL-4. The expression and production of eotaxin-3/CCL26 in these cells was not enhanced by TNF-α or IFN-γ, but strongly enhanced by IL-4 in a dose dependent manner. Dexamethasone and IFN-γ inhibited IL-4 enhanced eotaxin-3/CCL26 production in these cells. These results suggest that KCs may be involved in the migration of CCR3 positive cells such as eosinophils under the Th2-dominant situation like atopic dermatitis.

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Generation of regulatory T cells from naive T cells by retroviral gene transfer of Foxp3

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Immunological unresponsiveness to self is mediated in part by regulatory CD4+CD25+ T cells (Treg), which can actively suppress self-reactive T cells that have escaped thymic selection. The isolation of large numbers of Treg is difficult, since CD4+CD25+ cells constitute only a small subset of CD4+ T cells. Recently, the transcription factor Foxp3 has been identified to be a key regulatory gene for the development of Treg. The Foxp3 cDNA was cloned into a MCSV-based retroviral vector encoding eGFP under control of an IRES site and used to transfect the GPE86 packaging cell line. For controls a retrovirus containing only eGFP was employed. Retrovirus-containing culture supernatants were utilized for Foxp3 gene transfer into murine naive CD4+CD25- T cells to investigate if these cells would develop a regulatory phenotype. Control virus-transfected T cells proliferated, whereas Foxp3 transfected T cells were anergic. Flow cytometry analysis of Foxp3 transfected T cells revealed expression of CD25, CTLA-4, and neuropilin-1, all molecular markers associated with Treg. Subsequently, GFP+ T cells were added to CD4+CD25- T cells to study their suppressor function. Naive CD4+CD25- cells that were control virus-treated failed to inhibit T cell proliferation. In contrast, naive T cells that had been transfected with the Foxp3 containing retrovirus showed strong suppressor activity. A mechanism by which Treg suppress other T cells is the production of IL-10. Only Foxp3 gene-transfected T cells but no control virus-treated T cells expressed IL-10. We are currently using Foxp3 transfected T cells for the treatment of systemic autoimmunity in K14-CD40L transgenic mice in order to ameliorate disease. Together, gene transfer of Foxp3 converts naive T cells towards a regulatory phenotype similar to that of natural Treg. Furthermore, Foxp3 gene transfer may be useful to generate large numbers of Treg for immunotherapy of autoimmune disorders.

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Characterization of necdin expression and interaction with IL-1a in epidermal skin cells

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The MAGE-related protein necdin was originally identified as a neuronal-specific protein and is known to be deleted in Prader-Willi syndrome. It was isolated from retinoic acid-treated embryonal carcinoma cells and is reportedly expressed in postmitotic cells. Expression of necdin induces growth arrest and it has been shown to interact with the transcription factor E2F, p53 and the p75 neurotropin receptor. We studied the expression of necdin in skin. Immunofluorescent staining of human face skin demonstrated that necdin immunoreactivity occurs throughout the epidermis with positive staining concentrated in the cytoplasm of basal layer keratinocytes. Western blot analysis of human, mouse, and rat epidermal keratinocytes confirmed the expression of necdin in skin epidermal cells. Necdin immunoreactivity was also detected in cultured human keratinocytes from human foreskin, face, and a squamous carcinoma cell line (SCC13). Recently, yeast two hybrid studies have identified IL-1a as a potential binding partner for necdin. Since we have previously demonstrated high levels of IL-1a in skin, we also examined the cellular localization of 31kDa IL-1a in the cultured keratinocytes to attempt to colocalize necdin and IL-1. Ectopic expression of a 31kDa IL-1a-GFP fusion protein resulted in nuclear localization in the majority of cells and co-staining for necdin suggested overlap of the two proteins in vitro. Immunostaining of the human keratinocytes and SCC13 cells for endogenous IL-1a confirmed the nuclear localization in cultured keratinocytes. While most of the cultured cells displayed strong nuclear staining, a small fraction exhibited positive staining in the cytoplasm as well. In larger cells and in calcium-treated cultures, IL1a immunoreactivity shifted to the cytoplasm, similar to the results observed for necdin. This raises the possibility that the interaction of these two proteins may be related to the differentiation state in normal human keratinocytes.

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TLR5 and TLR9 are expressed in different layers of human skin and are induced in primary keratinocyte organotypic cultures after TGF-α stimulation

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In addition to acting as a barrier against pathogenic microorganisms, epidermal keratinocytes have been shown to express certain Toll-like receptors (TLR) which can be activated by microbial-derived components and subsequently elicit immune responses. Here, we investigated TLR5 and TLR9, which are respectively activated by bacterial flagellin and unmethylated bacterial CpG motifs. Both TLR5 and TLR9 were detected by immunohistochemistry in epidermal keratinocytes of normal human skin, verruca vulgaris, condyloma acuminatum, and in psoriasis. However, TLR5 labeling was greatest in the basal keratinocytes, and in contrast, TLR9 expression was found predominantly in the upper layers of the epidermis. TLR5 and TLR9 had a similar expression pattern in organotypic air-lifted primary keratinocyte cultures, which form layers simulating normal epidermis. In addition, TLR5 and TLR9 mRNA levels (qPCR) were increased (2-fold and 3-fold, respectively) after stimulation with the autocrine keratinocyte growth and differentiation factor TGF-α. The TLRs in keratinocyte organotypic cultures were functional as indicated by increased levels of IL-8 (2-3 fold) in response to the TLR5 or TLR9 ligands. In summary, the regulated and differential expression of TLR5 and TLR9 by human epidermal keratinocytes can contribute to host immune mechanisms against microbial pathogens.

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Production of TARC and MDC by CCR4+ adult T-cell Leukemia cells: an implication for malignant T cell aggregation by chemokine/chemokine receptor paracrine loop

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Adult T-cell leukemia/lymphoma (ATL) is a peripheral CD4+CD25+ T cell malignancy caused by infection with human T-cell leukemia virus type I. The tumor cells frequently infiltrate in the skin, lymph nodes, and other organs, especially forming prominent cutaneous masses. However, the cellular and molecular mechanisms underlying the malignant T cell aggregation remains unelucidated. Recently, ATL cells have been shown to express Th2 chemokine receptor CCR4. Here, we found that CCR4+ ATL cells produce CCR4 ligands TARC and MDC by analyzing peripheral blood CD4+ T cells and skin tumor-infiltrating lymphocytes. By flow cytometry, CD4+CD25+ ATL cells highly expressed Th2 chemokine receptor CCR4, but not Th1 receptor CXCR3, although they did not necessarily exhibit the Th2 cytokine profile. When these neoplastic cells from peripheral blood and skin tumors were cultured for 96 h in the presence or absence of anti-CD3 and -CD28 mAbs, they produced or expressed markedly higher levels of CCR4 ligands TARC and MDC than did normal subjects′ CD4+ cells, as assessed by ELISA and RT-PCR. Th1 chemokine CXCR3 or MIG was not produced by peripheral blood tumor cells except for one case. Immunohistochemically, both CCR4 and TARC were expressed in ATL cells infiltrating into the skin. A blocking study revealed that aggregation of ATL cells enhanced by anti-CD3/CD28 mAbs was significantly inhibited by the addition of anti-CCR4 mAb to the culture. These findings suggest that the tumor cells aggregate by means of paracrine loop composed of chemokines and their ligands in ATL.

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Withdrawn

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Inhibition of the class II pathway limits induction of a T regulatory phenotype in cutaneous T cell lymphoma

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We have shown that tumor cells cultured from patients with cutaneous T cell lymphoma (CTCL) adopt a T-regulatory (T-reg) phenotype and function when stimulated with autologous dendritic cells (DC) that have been pulsed with apoptotic cells. T-reg CTCL cells up-regulate CD25, CTLA-4, secrete IL10 and TGF-β and suppress normal T cell alloproliferative responses. We propose that CTCL is a malignancy of T-reg cells whose proliferation is driven by DC presentation of peptides in class II molecules. To determine if CTCL cell interaction with DC class II molecules was required for T-reg CTCL cell induction, we tested inhibitors of the class II pathway. CTCL cells were rendered apoptotic by CD3-antibody (3.5μg/ml) binding and fed to autologous DC overnight in the presence of an anti-DR antibody that binds to class II MHC on DC but does not trigger apoptosis (25μg/m), and two inhibitors of the class II pathway: Brefeldin A (10μg/ml), and Cystatin C (1μg/ml). CTCL cells were added to the loaded DC overnight in the presence of the inhibitors and the phenotype compared to non-inhibited cultures.The results demonstrated that class II antibody blocked detection of 84% of the membrane DR molecules on the DC and inhibited approximately half of the CTCL cells from expressing a Treg phenotype (48% reduction in CTLA-4 and 53% decrease in CD25 expression). Cystatin also inhibited 48% of CTCL cell CTLA-4 expression, while Brefeldin A treatment caused a 71% inhibition of CTCL cell CTLA-4 expression and a 57% reduction in CD25 up-regulation. These results demonstrate that inhibitors of DC class II interaction with CTCL cells decrease CTCL cell expression of a T-reg phenotype, thereby, supporting our hypothesis that DC class II molecules potentially displaying peptides derived from apoptotic CTCL cells are required for CTCL cell adoption of T-reg characteristics. Furthermore, these studies indicate that T-reg cell induction may be limited through interference with the DC class II pathway.

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Conversion of peripheral blood monocytes to dendritic cells by physical perturbation

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Monocytes activated by leukapheresis harvest and passage through a plastic plate transition into the dendritic cell (DC) pathway. Overnight co-incubation with apoptotic cells results in DC loading and maturation into semi-mature DC. To determine the factors mediating this rapid monocyte to DC conversion, methods of physical activation were tested. Normal peripheral blood (PB) was rotated for 2hr. Additionally, leukocytes were isolated, rotated, and passed through a micro-column matrix, and incubated overnight in the absence of cytokines with or without apoptotic cells. Markers of DC maturation included: semi-mature DC co-expressing membrane class II/CD83; and immature DC co-expressing membrane CD36 or DR and cytoplasmic CD83. In addition, cutaneous T cell lymphoma (CTCL) cells were incubated with CD3-beads and passed through a column. D3-separated fractions were incubated overnight and apoptosis, DC maturation and loading measured by phenotyping. The results demonstrate that when unseparated whole blood cells were rotated for 2hr and incubated overnight, 11% of the monocytes became semi-mature DC, while 28% were immature DC. Addition of apoptotic cells, increased immature DC to 37%. Isolated leukocytes were rotated for 1hr and incubated overnight driving 14% of the monocytes to co-express markers of semi-mature DC, which increased to 24% after column passage, with 32% of the monocytes becoming immature DC. CD3-treated CTCL leukocytes passed over a micro-column became apoptotic (23% annexin+)and 21% were ingested by activated DC (32% immature DC). These studies confirm overnight induction of transitioning DC from PB monocytes can be achieved by simple rotation and physical interaction with a plastic surface. Column passage further promotes acquisition of a DC phenotype. CTCL cells can be rendered apoptotic and used to directly load activated immature DC in a one-step procedure. These methods can be used to develop clinically practical means for DC preparation and loading.

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Development of a dendritic cell-based biosensor system for discovery of novel adjuvants

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Dendritic cells (DC) in peripheral tissues require activation signals for their maturation into fully potent immuno-stimulators, and the nature of such signals governs the resulting immune responses. We observed that Toll-like receptor ligands (e.g., LPS and CpG oligonucleotides) primarily stimulated NFkB- and AP1-dependent gene transactivation pathways in DC, whereas other DC-stimulatory agents (e.g., ATP, ADP, γ-interferon, and necrotic cells) each activated different transcription regulatory pathways. These agents also differed from each other in their impacts on gene expression profiles, surface phenotypes, and functions of DC, suggesting that one can control the magnitude, direction, and class of immune responses by administering rationally selected DC-stimulating agents as vaccine adjuvants. All the above agents were found to share a common feature of inducing robust IL-1β mRNA expression in DC. Based on this key finding, we have developed a DC-based biosensor system by engineering our murine skin-derived XS106 DC line to express the yellow fluorescence protein (YFP) gene under the control of 4.5 kB IL-1β promoter. All tested DC-stimulatory agents induced significant YFP expression by the resulting DC clone (XS106-IL1/YFP) in dose-and time-dependent fashions, with LPS at 10 ng/ml causing >100-fold YFP induction over the baseline level within 6 hr. By screening 2,000 small chemicals in the NCI library with the XS106-IL1/YFP biosensor clone, we have identified 6 “hits”. One of them may represent a prime lead for adjuvants because this small compound (MW = 389) and its three derivatives each triggered, at sub-micromolar concentrations, significant (P<0.01) upregulation of I-A and CD40 expression by bone marrow-derived DC without causing robust production of a wide array of cytokines and chemokines (except for IL-1β). The DC biosensor system represents a major breakthrough for the development of “designer’s adjuvants” that are formulated to selectively activate given arms of DC function for initiating desired types of protective immunity for individual infectious pathogens and cancer cells.

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Topical immunization by *E. coli* vector

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Our aim is to develop a simple vaccine delivery system by topical application of recombinant E. coli encoding foreign antigens. Our hypothesis is that the high expression of antigen by E. coli, the multiple immune adjuvants of E. coli, and the potent immune induction capability of skin would lead to strong humoral immune responses. We constructed several E. coli K12 strains expressing tetanus toxin C-fragment (E. coli-tetC), anthrax protective antigen (E. coli-PA) and lethal factor (E. coli-LF) respectively. A single topical application of 1010 colony forming unit of E. coli-tetC on mouse skin elicit robust antibody response and conferred a full protection against lethal challenge. Anti-PA and anti-LF antibody were also elicited by topical application of E. coli-PA and E. coli-LF. Topical application of Gamma-irradiated replication-defective E. coli is as effective as its live counterparts, which enhances the safety margin of the E. coli vector. Topical application of sonicated E. coli cell-free filtrates can enhance the immune responses against the co-administered prototype antigen hen egg lysozyme. The IL-4 and INF-? ELISPOT assay indicate that E. coli-vectored topical immunization produce a Th2 biased immune response. While its adenovirus counterpart produce a combined Th1 and Th2 responses. We also found those ??-T cells are rapidly mobilized in skin after topical application of E. coli. After topical application of E. coli expressing fluorescent EGFP, no bacteria were found in the skin. However, E. coli DNA can be amplified by PCR in internal organs following topical application. Using radiolabeled bacteria and autoradiography technique, we found that radiolabeled compound are trapped and concentrated in hair follicles 4 hours after topical application of 35S-labeled E. coli. The radioactivity diminished 48 hours after inoculation, and radioactive spots presents outside hair follicles in dermis. As the topical application of E. coli-delivered vaccines is simple, painless, effective, and potentially safe, it may boost the vaccine coverage.

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Role of IL-6 in mediating the ability of UV-induced skin iC3b to differentiate precursor monocytes

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UV-irradiated skin produces significantly altered immune responses such as tolerance induction following antigen sensitization. Additionally, the UV-skin micromilieu accumulates bioactive complement product iC3b, which can bind the monocyte (Mo) CD11b receptor and in vitro promote precursor Mo maturation toward the macrophage (Mac) phenotype, while preventing differentiation into dendritic cells. In this study, RNA collected from freshly isolated human peripheral blood Mo treated +/- iC3b was hybridized to oligonucleotide microarrays. At 20 hrs post-iC3b, Mo demonstrated a 76-fold increase in the suppressor of cytokine signaling 3 gene (SOCS3), confirmed by RT-PCR to be selectively increased in iC3b-exposed Mo. Because SOCS3 can selectively block IL-6 signaling via binding to STAT3, we examined iC3b/Mo culture supernatants by ELISA, which showed that IL-6 was produced at 4-8 hrs, and peaked at 12 hrs, with a 61-fold increase compared to control (726pg/ml, N=4, p=0.017); IL-6 decreased by 24 hrs, at which time SOCS3 has become markedly increased. Similarly, soluble IL-6 receptor (sIL-6R) had a 2-fold increase at 12 hrs as compared to control. In chronic inflammation with Mo infiltration, the IL-6/sIL-6R complex induces upregulation of Mac chemotactic protein-1 (MCP-1). ELISA of iC3b/Mo culture supernatants for MCP-1 showed a 33-fold increase at 24 hrs (n=2, p=0.007). Blocking antibodies to either IL-6R or its signaling receptor gp130 drastically diminished MCP-1 (>60%). In summary, cutaneous formation of iC3b after UV induces IL-6 and sIL-6R in newly infiltrating monocytes, which is critical for subsequent MCP-1 production and amplification of Mo recruitment into UV-damaged skin. The robust SOCS3 induction after iC3b likely represents a compensatory response to IL-6/STAT3 signaling, as SOCS3 inhibits STAT3. IL-6 is known to upregulate MCSF-R on Mo, activating Mo to drive their own differentiation. In conclusion, IL-6 signaling may be a critical mediator of iC3b-modified differentiation of precursor monocytes entering sunburn skin.

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Establishment of cutaneous eosinophilic inflammation and its regulation by CpG oligodeoxynucleotide

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The number of patients with severe atopic dermatitis (AD), who show an eosinophil-related late phase skin reaction and increased levels of IgE, is increasing, although there is accumulating evidence revealing its pathomechanism. Ag-nonspecific immunosuppressants have been used for the treatment of AD. These therapeutic agents sometime cause the deterioration of the host immunologic defense. There is a real need for developing an antigen-specific treatment to selectively suppress Th2 cell-mediated responses. Oligodeoxynucleotides (ODNs) containing CpG motifs have recently been highlighted as an immunomodulator that reduces Th2-mediated responses by biasing the T-cell response toward a Th1-dominant phenotype. To substantiate the effect of CpG ODN in the Th2-mediated skin inflammation, we introduced a unique cutaneous model of a protein-Ag-induced eosinophilic inflammation with increased levels of serum IgE in BALB/c by three-time i.p. priming with ovalbumin (OVA)/alum and following a week-OVA skin patching. Intradermal administration of CpG ODN diminished the number of infiltrated eosinophils and IgE systemic response. Interestingly, intradermal CpG ODN administration with OVA significantly augmented the inhibitory effects on both the eosinophil infiltration and IgE levels. Our data suggest that a cutaneous administration of CpG ODN with Ag can work as a novel Ag-specific immunomodulatory treatment for patients with severe AD.

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Effect of Epigallocatechin-3-gallate on expression of iNOS mRNA and production of NO in HaCaT cell

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Nitric oxide (NO) plays an important role in inflammation and multiple stages of carcinogenesis. Green tea (Camellia sinensis) polyphenols are potent antiinflammatory agents and have been shown to inhibit NO production in tumor cell lines. In the present study, we examined the effect of epigallocatechin-3-gallate (EGCG), a green tea polyphenol, on the expression of inducible NO synthase (iNOS) mRNA and generation of NO in HaCaT cells. HaCaT cells were treated with 10μM EGCG and 100μM NAC for 1 hour. After 1 hour later, they were irradiated with 50mJ/cm2 UVB and treated with 200 μg/ml LPS for 12 hours, respectively. The iNOS mRNA was determined by reverse transcription-polymerase chain reaction (RT-PCR) and NO production was assessed by spectrophotometric method based on Griess reaction. Nuclear factor κB (NF-κB) binding activity were determined by electrophoretic mobility shift assay (EMSA). EGCG inhibited UVB and LPS induced expression of inducible nitric oxide synthase. HaCaT cells cotreated with EGCG produced significantly less iNOS mRNA and NO compared with HaCaT cells stimulated with UVB irradiation or LPS. The inhibition of iNOS mRNA and NO production correlated with the suppression of expression of NF-κB dependent gene iNOS. EGCG inhibited the activation and translocation of NF-κB to the nucleus. Our datas suggest that EGCG inhibits the UVB and LPS-induced production of NO in HaCaT cells by interfering with the activation of NF-κB through a novel mechanism. Our results further suggest that EGCG may be a therapeutically effective in UVB and cytokine induced cutaneous inflammation.

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Cutaneous and nasal immunity is L-selectin-dependent, whereas cooperation between L-selectin and β7 integrin is critical for intestinal immunity

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Lymphocyte migration into lymphoreticular tissues is dependent on tissue-specific adhesion molecules such as L-selectin and α4β7 integrin. For migration into skin-draining lymph nodes, L-selectin is important. Among mucosal sites, migration into nasal mucosal sites is L-selectin-dependent, whereas for migration into intestinal mucosal sites, α4β7 integrin is more critical than L-selectin. To address the importance of α4β7 integrin and L-selectin for humoral responses, tetanus toxoid was immunized to wild type, L-selectin-/-, β7 integrin-/-, and L-selectin/β7 integrin-/- mice. When immunized gastrically, only antigen (Ag)-specific IgA titers in serum and mucosal samples were decreased in β7 integrin-/- mice. Surprisingly, the deficiency of both L-selectin and β7 integrins lead to severe impairment of all isotypes of Ag-specific Ig titers in serum and mucosal samples, which correlated with the number of Ag-specific antibody forming cells in spleen and mesenteric lymph nodes (MLN). In contrast, Ag-specific Ig responses after nasal immunization required L-selectin and additional deficiency of β7 integrins further reduced the responses. These responses resembled those after subcutaneous immunization where L-selectin/β7 integrin-/- mice showed even more delayed humoral responses than L-selectin-/- mice although systemic humoral responses were intact in L-selectin/β7 integrin-/- mice. The deficiency of L-selectin and β7 integrins did not significantly change the number of CD11c cells in spleen and MLNs, and the number of intraepithelial lymphocytes and lamina propria lymphocytes. These data suggest that the defective humoral responses after gastric immunization in L-selectin/β7 integrin-/- mice is due to the decreased number of lymphocytes and defective lymphocyte migration into MLNs and Peyer's patches. Thus, β7 integrin together with L-selectin regulates humoral responses in intestinal mucosal tissues.

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The dendritic cell repertoir of atopic dermatitis skin: evidence for two non-indigenous cell populations

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Because of their unique ability to initiate primary immune responses in T- lymphocytes, dendritic cells are thought to play a key role in the pathogenesis of atopic dermatitis. To learn more about the process initiating and propagating the cutaneous allergic reaction, we phenotypically analyzed various dendritic cell subsets in both skin biopsies (n=6; n=5) and peripheral blood (n=10; n=8) of atopic dermatitis patients and healthy controls. Our findings confirm the presence of the resident types of dendritic cells in normal human skin, namely epidermal $CD1a^+$/$Langerin^+$ Langerhans cells and $CD1c^+$ dermal dendritic cells. In atopic dermatitis lesions two non-indigenous dendritic cell subsets occurred besides increased numbers of Langerhans cells and dermal dendritic cells: (i) $CD1a^+$/$Fc\epsilon RI^+$/$CD1c^+$ DC in the epidermis (inflammatory dendritic epidermal cells) and dermis and (ii) $CD123^+$/$BDCA\text{-}2^+$/$CD45RA^+$/$CD68^+$ cells in the dermis. These latter cells exhibit the phenotypic features of plasmacytoid dendritic cells and, interestingly enough, display FcεRI on their surface. A detailed characterization of dendritic cell subsets in the skin and in the peripheral blood including their activation status and their ability to bind IgE should form the basis for the investigation of dendritic cell-mediated events operative in the pathogenesis of the eczematous response.

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The *Propionibacterium acnes* mitogen is not a bacterial heat shock protein

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Propionibacterium acnes has been shown to stimulate lymphocytes via mitogenic mechanisms and this activity is associated with the bacterial cell wall. It is possible that heat shock proteins (HSP) may be associated with the cell wall and account for this mitogenicity. Two HSP of P. acnes (HSP60 and HSP70) have been purified. P. acnes HSP were used in a lymphocyte transformation assay time course experiment from day 3 to 7 with peripheral mononuclear cells (PBMNC) from patients with inflammatory acne and from cord blood (CBMNC) to investigate their T-cell stimulatory properties. PBMNC and CBMNC samples were also investigated with and without pre-incubation with TU39, a monoclonal mouse anti-human MHC class II antibody, to block antigenic stimulation. 9/12 and 10/12 CBMNC were stimulated by HSP 60 and 70, respectively with 5/9 showing an early response. 3/9 samples showed two peaks of stimulation, suggesting two different mechanisms of lymphocyte activation. In contrast, only 3/11 of the PBMNC samples were stimulated by HSP 60 and 70 from day 5 onwards. Lymphocyte stimulation could be blocked completely by TU39. It can be concluded that P. acnes HSP 60 and 70 evoke an antigenic reaction and that other P. acnes products are responsible for the mitogenic activity. This is consistent with reports on immune reactions induced by mycobacterial HSP, which show a high degree of amino acid sequence homology with P. acnes HSP.

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Regulation of interleukin 4 expression by small molecule tyrphostins

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CD4+ T cells can differentiate in a polarized manner into at least two effector cell types characterized by distinct cytokine production profiles. T helper type 1 (Th1) cytokines regulate cell-mediated immune responses and are involved in certain autoimmune diseases including psoriasis and experimental allergic encephalomyelitis. T helper type 2 (Th2) cytokines regulate humoral immune responses and are involved in allergic disorders such as atopic dermatitis and asthma. Tyrphostins are a class of small molecule kinase inhibitors. Previous studies have shown that the tyrphostin AG490 diminishes the symptoms of Th1-mediated disease in mice. Interleukin 4 (IL-4) is a potent immunomodulatory cytokine secreted by Th2 cells and it promotes the differentiation of naive T cells into Th2 cells. In this study, we utilized a strain of mice carrying a modified IL-4 gene containing green fluorescent protein (GFP) that enables sensitive detection of IL-4 expression to examine the immunological effects of tyrphostins. Exposure of naive or concanavalin A (ConA)-stimulated CD4+ T cells to tyrphostins in vitro stimulated expression of GFP in a dose-dependent manner. The correlation of GFP expression with IL-4 expression was confirmed by real-time PCR in cells from both wild-type and IL4-GFP mice. Tyrphostin 5H4, a derivative of AG490 with increased kinase inhibitory activity, is also more effective at stimulating IL-4 gene transcription. The increased level of GFP is sustained for at least 2 days after the removal of tyrphostin molecules. Increased GFP expression was also detected in CD8+ T cells, although primarily in the absence of stimulation. Tyrphostin treatment also increased the GFP expression in non-lymphoid cells, in both non-stimulated and stimulated conditions. The GFP+ cells do not express the activation marker CD69, indicating that the effect of tyrphostins on the IL-4 expression is independent of T-cell-receptor (TCR) signaling. These data suggest that tyrphostin molecules have the potential to modulate Th1/Th2 balance and may be valuable for intervention in Th1 or Th2-mediated immune diseases.

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Antibody-based targeting of tumor antigens to dendritic cells *in vivo* induces protective tumor immunity in a B16 melanoma model

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The antigen receptor DEC-205 is exclusively expressed by dendritic cells and enhances antigen presentation up to 500-fold as compared to pinocytosis. We therefore aimed to use antibodies against DEC-205 to load DCs in situ with antigens. A fusion protein of the tumor antigen TRP-2 and the green fluorescent protein (EGFP) was coupled to anti-DEC-205 antibodies (αDEC) and injected together with CpGs into mice. Lymph node cells (LNC) were prepared from draining LN 2h to 24h later. FACS analysis revealed that 6h after injection of these conjugates up to 20% of the CD11c$^+$ DCs displayed EGFP fluorescence. B220$^+$as well as CD11b$^+$ cells were negative for EGFP fluorescence, indicating that EGFP-TRP2-αDEC conjugates had selectively been taken up by LN DCs. To test whether this targeting resulted in induction of TRP2 specific CD8$^+$ T cells, spleen cells from conjugate plus CpG injected mice were restimulated with TRP2-peptide and in ELISPOTs we were able to detect IFN-γ producing T cells. No IFNγ producers were detected in control samples. Moreover, we demonstrated induction of melanoma specific antibodies in TRP2-αDEC-conjugate injected mice, whereas mice that received uncoupled TRP2 protein developed only weak antibody responses. Finally we tested whether immunization with TRP2-αDEC-conjugates plus CpG leads to protective tumor immunity in a melanoma model. Therefore mice were immunized with TRP2-αDEC-conjugates and respective controls and were challenged for tumor growth by injection of $4x10^5$ B16 melanoma cells. 14 days later, mice were sacrificed and lung metastases were counted. These experiments revealed that injection of TRP2-αDEC-conjugates prevented tumor growth in more then 75% of the mice, whereas no tumor free mice could be detected in untreated - or TRP2 injected groups. Notably, when CpG was omitted from the immunization, tumor growth was not prevented, indicating that activated DCs are mandatory for successful immunization. Thus these data show that in vivo targeting of tumor antigens to activated DCs induces protective tumor immunity.

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Phenotype and TCR diversity analysis of T cells isolated from normal human skin

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Studies of T cell homing to cutaneous sites have largely focused on T cells isolated from inflamed skin. The study of normal immunosurveillance mediated by T cell trafficking into non-inflamed skin has been hampered by difficulties in obtaining sufficient numbers of cells to analyze. We report here isolation of significant numbers of CLA$^+$ memory T cells from normal human skin by culture of skin explants on three-dimensional matrices. We performed comprehensive comparisons of these cells to CLA$^+$ cells derived from blood. We found >90% co-expression of CLA and CCR4 and >80% co-expression of CLA and CCR6 on skin derived T cells. L-selectin was expressed at lower levels on skin T cells and CD69 was expressed on skin T cells but was undetectable on blood T cells. Spectratyping analysis of T cells from normal skin demonstrated a highly diverse T cell receptor repertoire with no apparent Vβ bias. To establish that lymphocytes produced in our system do not undergo phenotypic changes in culture, we cultured subsets of CLA$^+$ and CLA$^-$ T cells from peripheral blood in matrices colonized with skin cells for four weeks. Expression of CLA, CCR4, CCR7 and L-selectin were largely unchanged after culture, suggesting that the phenotype of cells we isolate likely represents the true phenotype of T cells homing to non-inflamed skin. In summary, we have isolated T cells from normal skin in meaningful numbers using novel isolation conditions. These cells expressed high levels of CLA, CCR4, and CCR6, supporting a role for these homing molecules in T cell trafficking to non-inflamed cutaneous sites. TCR spectratyping demonstrated that diverse populations of CLA$^+$ T cells migrate to and reside in the skin as a part of normal immunosurveillance. Explant cultures also supported long-term survival and maintenance of CLA phenotype in T cell subsets isolated from blood, suggesting that CLA expression neither increases nor decreases after T cell entry into skin.

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Positive selection, negative selection and generation of a diverse TCR repertoire in thymus-independent T cell development mediated by skin cells in an artificial matrix

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T cells require a period of development in the thymus. The complex requirements for T cell development have precluded the laboratory production of T cells that can be given to immunocompromised patients to treat infection or malignancies. Utilizing the natural similarities between skin and the thymus, we have used keratinocytes and fibroblasts in three-dimensional matrix to replicate the thymus microenvironment, and demonstrated the production of mature T cells from bone marrow hematopoietic stem cells. However, the potential utility of these T cells for use in patients was unknown. We report here that T cells developing in our thymus-independent skin constructs successfully undergo both positive and negative selection. Successful positive selection is an obligate requirement for the production of single-positive T cells from double-positive precursors. We observed transition of double-positive T cell precursors to single-positive, TREC$^+$ T cells, confirming that skin-derived epithelial cells can support positive selection. Mixed lymphocyte reactions using newly generated T cells demonstrated that these cells were non-reactive to dendritic cells derived from autologous bone marrow precursors but reacted normally to dendritic cells derived from allogeneic, unrelated donors. Spectratyping analyses of newly generated T cells demonstrated a remarkably diverse T cell repertoire with all Vβ families represented. Lastly, T cell production was augmented by inclusion of the Notch ligand Delta-1. In summary, we report that human skin cells can replicate the thymus microenvironment, supporting the development of functionally mature, self-tolerant T cells with diverse TCR repertoires. This system could be used to produce autologous T cells for use in patients using only samples of bone marrow and skin. T cells generated in this manner could be extremely valuable in the treatment of human immunodeficiencies, infections and malignancies

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The lipid of *Malassezia furfur* inhibits ligand activation of toll-like receptors 4 and 2

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Malassezia is a fungal species that is present as part of the normal cutaneous flora, but it can cause a wide range of cutaneous inflammatory diseases and even systemic infectious diseases in predisposed individuals . Recent studies have demonstrated that *Malassezia* are capable of suppressing cytokine release and down-regulating the phagocytic function of monocytes. It is thought that the lipid-rich layer on the yeast membrane plays a critical role in this suppression. However, the mechanism by which *Malassezia* activates the immune response is not well understood. We compared the immunomodulatory capacity of normal and lipid-depleted *Malassezia furfur* (*M. furfur*). The lipid-depleted yeasts induced significant cytokine release including TNF-α, IL-6, and IL-1β by primary human monocytes, while monocytes treated with non-lipid-depleted yeast did not produce significant levels of these cytokines. Cytokine production by lipid-depleted yeasts could be inhibited by the addition of an anti-Toll-like receptor 2 (TLR2) antibody but not with anti-Toll-like receptor 4 (TLR4) antibody, suggesting that *M. furfur* activation of cytokine production in human monocytes is at least partially dependent on TLR2. Furthermore, extracted lipid from *M. furfur* inhibited the effects of TLR4 and TLR2 ligands to induce cytokine induction in human monocytes. These results suggest that the lipid of *Malassezia* may prevent the yeasts from inducing inflammation and also inhibit the inflammatory effects of TLR2 and TLR4 ligands.

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Cross-reactivity between C.I. Disperse Blue 106 and paraphenylenediamine

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In this study, we investigated about cross reactivity between C.I. Disperse Blue 106 and paraphenylenediamine (PPD). Female Hartley strain albino guinea pigs were introduced at 4 weeks of age. We selected C.I. Disperse Blue 106 and PPD. We decided 5% aq. as intracutaneous and percutaneous sensitizing concentration and 1% aq. as challenge concentration to C.I. Disperse Blue 106. We decided 0.1% ac. as intracutaneous sensitization concentration, 0.1% ac. as percutaneous sensitizing concentration and 0.1% ac. as challenge concentration to PPD. The procedure of the Magnusson & Kligman's Guinea Pig Maximization Test was followed for induction. Ten animals were included in the C.I. Disperse Blue 106 sensitized group and the PPD sensitized group. Readings were made at 24, 48, 72 hours after the challenge, according to the Draize's criteria. A summation of numerical scoring of more than 2 was judged as a positive reaction. The positive reaction to C.I. Disperse Blue 106 was observed in 5 out of 10 animals. All five positive animals showed negative reaction to PPD. The positive reaction to PPD was observed in 8 out of 10 animals. Four out of 8 positive animals showed positive reaction to C.I. Disperse Blue 106. We concluded sensitized animals from C.I. Disperse Blue 106 did not cross-react to PPD, while sensitized animals from PPD cross-reacted to C.I. Disperse Blue 106.

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Roxithromycin suppression of keratinocyte Th2 chemokine production and Th2 cell chemokine receptor expression

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Recent studies have revealed that macrolide drugs exert a variety of bioactivities to allergic and inflammatory cells in addition to their antibiotic functions. We investigated the modulatory effects of roxithromycin (RXM) on keratinocyte production of chemokines and T-lymphocyte expression of chemokine receptors. Normal human epidermal keratinocytes (NHEK) and HaCaT cells were cultured for 72h with or without RXM at varying concentrations under stimulation with IFN-g and TNF-a. RXM at 10-4M significantly suppressed the production / expression of MDC and TARC but did not affect that of IP-10, as assessed by ELISA in the culture supernatants and RT-PCR in the extracted RNA. To examine the effect on chemokine receptors, Th2-rich peripheral blood mononuclear cells (PBMC) were isolated from patients with mycosis fungoides and cultured with or without RXM under stimulation with IL-2 or IL-4. By flow cytometric analysis, RXM down-modulated the expression of CCR4 on Th2 cells upon stimulation with IL-2 but did not alter the expression of CXCR3. Thus, RXM down-modulates both Th2 chemokine production and chemokine receptor expression in cutaneous immunity. These findings suggest that RXM has a beneficial therapeutic effect on Th2-mediated or allergic skin disorders by suppressing both chemokine production and ligand expression.

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Double-blind randomised trial evaluation of a new product containing MMP and PKC inhibitors in cutaneous contact hypersensitivity

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Matrix metalloproteases (MMPs) and PKC are implicated in several skin pathologies, especially inflammatory cutaneous disorders such as psoriasis and contact hypersensitivity. More over, several molecules regulate the migration of skin dendritic cells, which are potent antigen presenting cells implicated in the primary T-cell-mediated

immune response of the skin, among which MMPs and PKC. We have previously shown that MMP and/or PKC inhibitors are able to modulate the migration of skin dendritic cells both in vitro and in vivo. Thus the aim of this study was to evaluate the clinical efficiency of an innovative topical product containing both MMPs and PKC inhibitors (verum) on cutaneous contact hypersensitivity (CHS). A double-blind randomised trial was conducted by 12 dermatologists on 50 selected volunteers having repeated nickel allergy due to buckles. The panellists have applied products on both ears (placebo or verum) twice a day during 7 days, and then buckles until CHS appears (at least for 14 days, 8 to 12h per day). An individual board has to be filled by the panellist in order to assess the course and development of CHS. At day 14, a final examination was performed by the dermatologist. The first results showed that 75% of the panellists using the placebo developed CHS, while only 37% of the verum treated one were sensitised. This trial demonstrates that topical applications of both MMP and PKC inhibitor prior to nickel exposition are able to reduce by 50% the frequency of CHS in allergic patients.

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Pimecrolimus and tacrolimus show comparable potencies to inhibit the proliferation and cytokine synthesis of allergen-specific, atopic skin-derived T cell clones *in vitro*

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Pimecrolimus and tacrolimus are in clinical use for treatment of atopic dermatitis. Both compounds have been shown to share macrophilin-12 (FKBP-12) as intracellular receptor and to inhibit T cell activation via blockade of calcineurin activity. In this study, we compared the relative potencies of pimecrolimus and tacrolimus to inhibit the proliferation and synthesis of cytokines from house dust mite allergen-specific T cell clones derived from the skin of an atopic dermatitis patient. Following in vitro stimulation by specific antigen presented on the surface of dendritic cells, the IC_{50} values for the inhibition of T cell proliferation by pimecrolimus and tacrolimus differed by a factor of less than two. A ten- to twenty-fold higher IC_{50} value was obtained for cyclosporin A used as a reference compound. A similar picture emerged for the inhibition of cytokine synthesis (IL-2, IL-4, IL-5, IL-10, IFN-γ and TNF-α) by pimecrolimus or tacrolimus. The arithmetic means of the relative ratio IC_{50} pimecrolimus / IC_{50} tacrolimus were 1.53 (± 0.26) or 1.66 (± 0.36) when stimulating the T cells by anti-CD3 monoclonal antibody plus phorbol ester or by antigen-presenting dendritic cells, respectively. Taken together, these data demonstrate that pimecrolimus and tacrolimus have similar potencies to inhibit recall antigen responses of skin-derived T cell clones.

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The regulation of STAT-1 and NFKB DNA binding activity in human keratinocytes HaCaT cells

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Psoriasis is a chronic inflammatory skin disease where Th1 cytokines participate in the pathogenesis. We have previously shown that CXCR3 positivity was increased in CD4+ CD45RO+ T cells in psoriatic patients. STAT-1 and NFkB are transcriptional factors which regulate inflammatory cytokine production such as IL-8 and IP-10/ CXCL10. Immunohistochemical analysis reveals that the lesional skin of psoriatic patients shows STAT-1 positivity in epidermal keratinocytes. We examined STAT-1 and NFkB DNA binding activity using human keratinocytes cell lines, HaCaT cells using electrophoretic mobility shift assay. STAT-1 and NFkB DNA activity was enhanced by both TNF-a and IFN-g at maximum level at 1hr after the stimulation. Cyclosporine did not change the levels of STAT-1DNA binding activity. VitaminD3 analogue slightly decreased STAT-1 DNA binding activity (10-8M 92.4%, 10-10M 88.9%, 10-12M 98.7%). NFkB DNA binding activity was partially blocked by genistein and LY294002. These data suggest that STAT-1 and NFkB activity in keratinocytes was enhanced in inflammatory skin conditions and its regulation can be the target for the treatment of inflammatory skin diseases such as psoriasis.

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Shift in the profile of antibodies against desmoglein 3 and 1 with time in pemphigus vulgaris

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The clinical phenotype of pemphigus can shift from that of pemphigus vulgaris (PV) to that of pemphigus foliaceus (PF), and this shift is associated with a corresponding shift in the pattern of anti-dsg antibodies from that seen in PV to that seen in PF. How often this occurs is not known. To evaluate how often the change occurs, we examined the ratio of anti-dsg 3 to anti-dsg 1 antibodies over time in patients with PV. The study was conducted in 37 sequential patients with PV based on clinical, histological, and immunological criteria, who had intercellular antibodies at baseline, and in whom at least two sera specimen taken at different times were available. Antibodies against desmoglein 3 and 1 were measured by ELISA at baseline and periodically thereafter. At baseline, 29 (78%) of patients had anti-dsg 3 dominant disease, i.e. the level of anti-dsg 3 antibody was higher than that of anti-dsg 1; and 7 (19%) had anti-dsg 1 dominant disease. In 3 (8%) of patients, the ratio of anti-dsg 3 to anti-dsg 1 decreased over time, so that the disease became anti-dsg 1 as opposed to anti-dsg 3 dominant. The clinical manifestations of PV shifted in all three of these patients from the mucocutaneous form (oral and skin lesions present) to the cutaneous form, (skin but not oral lesions). By contrast, among the patients who had anti-dsg 3 dominant disease throughout the course of their illness, the clinical manifestations of this disease did not shift. In conclusion, serological transition from anti-dsg 3 dominant to anti-dsg 1 dominant disease occurs in pemphigus vulgaris, but is a relatively rare event. This shift can be associated with shifts in the clinical manifestation of the disease.

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Differential activation of mast cells by microbial peptides

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Mast cells (MCs) have been shown to play an important role in innate immunity against pathogens. This view is supported by recent findings showing that MCs express toll like receptors (TLRs) 2, 3, 4, 6, 8, and 9. Furthermore, some bacterial signals, i.e. Staph. aureus peptidoglycans (PGN), have been shown to activate MCs via TLR2. Here, we asked whether MCs can be activated by Mycoplasma-derived lipoprotein MALP-2, which requires signalling via TLR2/TLR6. While none of the examined MC populations (murine skin MCs, peritoneal MCs, C57 MCs) exhibited degranulation upon stimulation with PGN (via TLR2), LPS (TLR4), or MALP-2 (TLR2/TLR6), activation of MCs via TLR2 or TLR4 resulted in pronounced release of IL-6 and TNFα (IL-6: 1573 ± 328pg/ml [LPS], 1701 ± 1010 [PGN], TNFα: 140 ± 7.2pg/ml [LPS], 341.6 ± 11.5pg/ml [PGN]). However, stimulation of MCs with MALP-2 failed to induce cytokine release from MCs, suggesting impaired functions of TLR2/TLR6 in MCs. When we performed RT-PCR analyses for TLRs and MyD88, MCs were, as previously reported, found to express MyD88, TLR4, TLR6 and the extracellular domain of TLR2. However, MCs failed to express large parts of the TLR2 signalling domain, whereas macrophages showed full length transcripts for TLR2. These observations are supported by our in vivo findings showing that MALP-2 induces equally strong inflammatory reactions after intradermal injections in normal or genetically MC-deficient Kit^{W}/Kit^{W-v} mice (Kit^{W}/Kit^{W-v}, after 1h: 57.5 ± 12.8µm, vs. *Kit+/+*: 64.1 ± 11.4µm, p=0.72). These data indicate that MCs express a novel isoform of TLR2 which is able to exert effects mediated by selective TLR2 ligands, but, unlike other skin cell populations, does not respond to TLR2/TLR6 mediated stimulation. Since it has been shown that TLR2 has to be physically associated with TLR6 to exert TLR2/TLR6-mediated effects, this impaired response could possibly be due to a disturbed interaction of TLR2/TLR6. Further investigations are required to identify the unique structure of MC-TLR2 and its possible functions in MCs.

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Contrasting roles of TLR2 and MyD88 in an *in vivo* model of skin infection with *Staphylococcus aureus*

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The majority of skin infections in humans are due to *Staph*. aureus. Toll-like receptors (TLRs) have been shown to be important in host defense against bacteria and other microbial pathogens. However, to our knowledge, no study to date has evaluated the role of TLRs in skin infection *in vivo*. Here, we studied the role of TLR2, which is activated by bacterial peptidoglycan and lipoprotein, and MyD88, an important signaling molecule for TLRs. We induced skin ulceration in mice by subcutaneous inoculation of Staph. aureus. Mice deficient in TLR2 initially had smaller ulcers than wildtype mice (day 2: TLR2-/- 0.014 ± 0.008 cm^2 vs. WT 0.114 ± .04 cm^2 ; mean ± sem, p<0.05, n=7 per group), but healed at approximately the same time (~day 14). Mice deficient in TLR2 had smaller dermal inflammatory cell infiltrates than wildtype mice according to histologic analysis at 24 h. Similarly, MyD88 deficient mice initially had smaller ulcers than wildtype mice (day 3: MyD88-/- 0.02 ± 0.016 cm^2 vs. WT 0.07 ± .02 cm^2; mean ± sem, p<0.05, n=7 and 8, respectively). In contrast to TLR2 deficient mice, the mean size of ulcers in MyD88 deficient mice continued to increase in size to 5 times greater than that seen in wildtype mice (MyD88-/- 0.32 ± 0.075 cm^2 on day 26 vs. WT 0.07 ± 0.02 cm^2 on day 4, p<0.05, n=7 and 8, respectively). In addition, by day 30 only 2 of 7 MyD88 deficient mice healed their ulcers, whereas all wildytpe mice healed by day 14-18. These results suggest that both TLR2 and MyD88 may be important in initiating early inflammatory signals that produce skin ulceration and that MyD88 is a critical molecule for resolution of Staph. aureus induced skin ulceration.

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Identification of a unique population of regulatory T cells in psoriasis by elimination of transiently activated CD4+CD25+ cells

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CD4+CD25+ regulatory T cells are able to suppress autoreactive T cells and play a unique role in the control of autoimmune disease in murine models. We previously showed that psoriatic CD4+CD25+ regulatory T cells are functionally deficient in suppressing effector T cell responses. The goal of this study was to examine more closely the CD25+ regulatory T cell population in psoriatic blood. One challenge in working with regulatory T cells is to distinguish these CD25+ T cells from transiently activated T cells. Therefore, we used CD71, a transient activation marker with similar duration of expression as CD25. CD4+CD25+ regulatory T cells from normal and psoriatic blood were sorted by magnetic beads and analyzed by flow cytometry. Immediately upon isolation, CD4+CD25+ cells contained about 15% CD71+ cells in both normal and psoriatic blood; this decreased to less than 5% after overnight incubation in both groups. In psoriatic volunteers, however, CD4+ T cells contained a persistent population of CD25high+CD71- non-activated cells, which remained constitutively CD25high+ for more than 48 hours post-isolation. These cells represent a high proportion of total CD4+CD25+ T cells in the blood of psoriatics (12.44%± 0.52%), compared to the blood of normals (3.19%± 0.29%) (n=3, p⟨0.05). Thus, in humans, the CD4+CD25+ population immediately upon isolation contains significant numbers of activated T cells; upon resting *ex vivo*, the expression of CD71 in these cells diminishes, revealing a novel regulatory T cell subset in psoriasis. We identified a T cell population in psoriatic patients with constitutive and unusually high CD25 expression not linked to activation. This T cell subset may contribute to the pathogenesis of psoriasis.

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Synthetic granulysin peptides demonstrate antimicrobial and anti-inflammatory activity against *Propionibacterium acnes*

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Peptides based on the structure of human granulysin were synthesized and subsequently tested for their abilities to kill *P.acnes* and modulate the immune response to this microbe. Colony forming unit assays measured the peptides' ability to kill *P.acnes*. The peptides' effect on inflammation was assessed by stimulating primary human monocytes with *P.acnes* sonicate and measuring cytokine levels by ELISA. Furthermore, the peptides' effects on monocyte Toll-like receptor expression was determined by flow cytometry. A peptide possessing a helix-loop-helix motif, peptide 31-50, killed *P. acnes* while peptides lacking the motif, including peptide 1-20, were ineffective at killing *P.acnes*. Substituting a tryptophan for the valine at amino acid 44 of peptide 31-50 to increase its hydrophobic moment increased the antimicrobial activity of this peptide (31-50v44w). Furthermore, addition of two terminal arginines that may facilitate transport of peptides through the skin did not alter the antimicrobial activity of the resulting peptide, 31-50v44wRR. Peptides 31-50, 31-50v44w, and 31-50v44wRR also had anti-inflammatory effects, as demonstrated by the suppression of IL-12p40 production in monocytes stimulated with *P.acnes* sonicate. Peptide 31-50v44w also markedly inhibited the production of a wide array of inflammatory cytokines and chemokines, though the peptide did not effect TLR2 expression. Peptides 31-50 and 31-50v44w are not toxic to human monocytes or keratinocytes at concentrations up to 20µM and exhibit minimal toxicity at higher concentrations. Moreover, as evidenced by transmission EM, *P.acnes* develop vacuoles shortly after treatment with granulysin or peptide 31-50v44w, suggesting their antimicrobial activity may be due to membrane perturbation. Since the ideal treatment for acne vulgaris would be both antimicrobial and anti-inflammatory, these peptides have promise as topical therapeutic agents, providing an alternative to current acne therapies.

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DC-HIL (on antigen presenting cells) is an endogenous ligand of dectin-2 (on activated T cells) and their binding leads to inhibited CD3-dependent activation of $CD4^+$ T cells

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Previously, we showed that the C-type lectin, dectin-2, is expressed on mouse Langerhans cells (LC), dendritic cells (DC), and activated macrophages (MΦ), and that it recognizes hyphae by binding to mannan-like carbohydrates (exogenous ligand) on these fungi. Postulating that dectin-2 also has endogenous ligands, we screened leukocytes for binding to soluble dectin-2. By immunofluorescence, we showed dectin-2 to bind bone marrow DC and peritoneal and Raw MΦ (but not spleen T and B cells), indicating that these antigen presenting cells (APC) express dectin-2 ligands. Because mannan and EDTA were each capable of completely blocking binding of dectin-2 to hyphae, we tested their effect on binding of dectin-2 to Raw MΦ. By ^{125}I- binding assays, mannan and EDTA were 50% less-efficient blockers, suggesting that mechanisms of dectin-2 binding to exogenous vs. endogenous ligands are not identical. In testing other agents, we serendipitously considered a molecule we identified previously: DC-HIL, a cell adhesion receptor expressed by LC, DC and MΦ. Again using ^{125}I assays, we found that soluble DC-HIL (but not controls) completely blocked binding of dectin-2 to Raw MΦ, indicating that DC-HIL is an endogenous ligand of dectin-2. Meanwhile, by RT-PCR, we discovered that activation with anti-CD3 Ab can induce dectin-2 mRNA in T cells ($CD4^+ > CD8^+$). Using binding assays as before, we showed DC-HIL to bind activated (but not resting) T cells ($CD4^+ > CD8^+$). To assess function, we measured proliferation of $CD4^+$ T cells stimulated concurrently with immobilized anti-CD3 Ab and DC-HIL. At suboptimal doses of anti-CD3 Ab, DC-HIL (but not controls) blocked T-cell proliferation (91% inhibition). We conclude that DC-HIL on APC is an endogenous ligand of dectin-2 on activated T cells and their binding leads to inhibited $CD4^+$ T-cell proliferation. These novel interactions may constitute an important negative regulatory feedback between APC and T cells.

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Downregulation of dendritic cell-bound IgE/FcεRI in anti-IgE-treated patients with atopic dermatitis

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Omalizumab, a recombinant humanized monoclonal anti-IgE antibody is safe and effective in patients with severe allergic asthma and allergic rhinoconjunctivitis. This is the first study to investigate to what extent high IgE-levels play a pathogenetic role in the inflammatory skin reaction in atopic dermatitis (AD). 20 AD patients (13 omalizumab, 7 placebo) were enrolled into this 16-week double-blind placebo-controlled study. Omalizumab was dosed according to IgE levels and bodyweight of the patients. Levels of free and total serum IgE were monitored, and immunohistochemistry staining of skin biopsies before and after treatment was performed. Basophils, myeloid and plasmacytoid dendritic cells (DCs) and monocytes were investigated by Fluorescence activated cell sorter analysis (FACS) analysis for cell-bound IgE, IgE-binding capacity and FcεRI. Titrated skin prick tests were performed and clinical outcome was monitored. Serum levels of free IgE decreased significantly after the first administration of Omalizumab. Levels of cell-bound IgE and relative expression of FcεRI as well as IgE-binding capacity and FcεRI receptor saturation also decreased not only on basophils but also on DCs as measured by FACS analysis. Immunohistochemistry staining revealed almost complete disappearance of IgE in the dermis of patients of the omalizumab group but no difference was seen in quality and quantity of the inflammatory infiltrate. A reduction of patients sensitivity in titrated skin prick test could be demonstrated too, but no major clinical effect became apparent during the treatment period. Treatment of AD patients with omalizumab interrupts the binding of IgE to the surface of antigen-presenting cells. Although no immediate clinical benefits were seen, there may be long-term sequelae for the course of this disease.

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Topical imiquimod prevents UVB-induced tolerance to topical haptens by modulating cytokines in Langerhans cells

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Imiquimod (IMIQ) has direct effects on epidermal Langerhans cells (LC), causing their maturation and migration from the epidermis. To determine whether IMIQ could prevent the loss of contact hypersensitivity (CHS) induced by UVB, topical 5% IMIQ cream or vehicle was applied to normal mouse skin prior to UVB radiation (70mJ/cm2 daily x 4 days), then experimental mice were sensitized with DNFB. Topical IMIQ prior to sensitization prevented the loss of CHS to this hapten in UV-irradiated mice. Furthermore, only IMIQ treated, UVB irradiated mice were not tolerant to repeat sensitization and challenge with DNFB, and exhibited significantly higher anti-hapten IgG1 and IgG2a responses compared to tolerant mice. Although IMIQ prevented UVB-tolerance to hapten, it did not prevent UVB-induced depletion of epidermal LC, suggesting that IMIQ blunts the damaging effects of UV light on LC function. This hypothesis was addressed by studying XS52, a LC-like cell line which express TLR7 mRNA. XS52 respond to IMIQ by rapidly phosphorylating ERK and JNK, decreasing cytoplasmic IkB, translocation of NFkB to the nucleus and secreting IL-12 and TNF-a. IMIQ treatment (5 mg/ml) of XS52 prior to UVB radiation maintain their ability to secrete IL-12 and TNF-a, in contrast to untreated, UVB radiated XS52. Lastly, local lymph nodes from IMIQ treated, UVB irradiated mice contain LC that respond to LPS by vigorous secretion of IL-12; LC in lymph nodes of untreated or vehicle-treated UVB-irradiated mice respond poorly to LPS. These data suggest that topical IMIQ preserves CHS by modulating the deleterious effects of UVB on epidermal LC ability to secrete critical cytokines such as IL-12. This topical agent may play a role in preventing UVB immune suppression and subsequent skin cancer.

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Haptens' potential to activate dendritic cells correlates with their capacity to induce oxidative stress

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Using human monocyte-derived DCs (MoDCs), we demonstrated that purified MoDCs respond to haptens such as NiCl2 and DNCB, but not to irritants, by significantly augmenting their expression of CD54, CD86, and HLA-DR and by increasing their production of proinflammatory cytokines. In addition, recently we have also reported that, to induce these phenotypic and functional changes, 2,4-dinitrochlorobenzene (DNCB) preferentially stimulated p38 mitogen-activated protein kinase (MAPK), while NiCl2 activated ERK and NF-kB in addition to p38 MAPK. Although these studies suggested the crucial role of p38 MAPK in the activation of MoDCs by haptens, the upper streaming signals of p38 MAPK remain undetermined. In this study, we hypothesized that haptens induce oxidative stress in DCs, which subsequently stimulates p38 MAPK. So, we measured the ratio of the oxidized (GSSG) vs reduced (GSH) form of cellular glutathione. MoDCs were exposed for 2h to sublethal concentrations of haptens such as NiCl2, MnCl2, DNCB, diphenylcyclopropenone, thimerosal, and formaldehyde, and primary irritants such as sodium dodecyl sulfate, and benzalkonium chloride, with or without an antioxidant, N-acetyl-L-cysteine (NAC). GSH and GSSG were measured using colorimetric assays. Without NAC, all haptens lowered GSH-GSSG ratio, while primary irritants did not. In the presence of NAC, all haptens recovered GSH-GSSG ratio. Furthermore, NAC suppressed the activation of MoDCs by haptens. Using human monocyte cell-line, THP-1, instead of MoDCs, we could obtain the similar results. These studies revealed the crucial role of oxidative stress in the activation of DCs by haptens. Furthermore, it suggested that the decreased GSH-GSSG ratio in MoDCs or even in THP-1 stimulated by simple chemicals can predict the sensitizing potential of these chemicals

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Apoptotic cells and tumor cell lysate pulsed CD14+monocyte-derived dendritic cells from cutaneous T-cell lymphoma mature and induce allogenic T-cell proliferation *in vitro*

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Dendritic cells (DCs) are professional antigen-presenting cells that can capture antigens from apoptotic and necrotic tumor cells and induce MHC class I- and II- restricted responses. However, necrotic cells are generally considered to be immunogenic, while apoptotic cells are thought to be immunologically innocuous. In certain models, apoptotic cells are shown to be an attractive immunogenic antigenic source for the cross- priming of cytotoxic T lymphocytes (CTL). The heterogeneity seems to involve the ability of a given necrotic or apoptotic cell to provide signals that induce activate mature DCs. In this study, we examined the mature status of CD14+ monocyte-derived DCs from patients with cutaneous T-cell lymphoma (CTCL) that were pulsed with either UVB-induced apoptotic CTCL cells or tumor cell lysates. CD14+monocytes were isolated from peripheral blood mononuclear cells from Sezary syndrome patients, and immature DCs were generating after incubation with GM-CSF and IL-4; the typical morphology with cytoplasmic projections and immunophenotype (CD83low, CD80low, CD40low, CD86low, HLA-ABChigh, HLA-DRhigh and CD14low) was confirmed. Both apoptotic cells and tumor cell lysate pulsed DCs (Apop-DC and Lysate-DC) exhibited the typical morphology and immunophenotype of mature DCs (CD83high, CD80high, CD40high, CD86high, HLA-ABChigh, HLA-DRhigh and CD14low) after maturation cytokines [TNF-α, IL-1β, IL-6, and PGE2 (ITIP)] were added. Apop-DCs and Lysate-DCs both demonstrated the ability to stimulate allogenic T-cell proliferation in mixed leukocyte reactions compared to mature DCs alone. IL-12 secretion was induced when IFN-γ was added to the ITIP maturation cocktail. Our preliminary study indicated that Apop-DCs and Lysate-DCs might be used as therapeutic vaccines for CTCL. The induction of CTCL-specific CTLs by both Apop-DCs and Lysate-DCs is being investigated.

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Diesel exhaust particles and formaldehyde deviate human immune system to Th2-dominant pattern by affecting both T cells and dendritic cells

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There is growing evidence that environmental pollutants such as diesel exhaust particles (DEP) and formaldehyde can impact the immunologic processes, which lead to the development of allergic diseases whose hallmark is increased IgE production and promoted Th2 differentiation. Animal and human models have also indicated the effects of DEP in enhancing IgE production and promoting Th2 differentiation, which enhances allergic inflammation. In addition, the children exposed to formaldehyde at home or school have been reported to increase their risk of asthma. In this study, we examined the effects of DEP and formaldehyde on the cytokine production by MoDCs stimulated with CD40 ligand and IFN-g and on that by T-cells stimulated with anti-CD3/CD28 antibody. The cytokines we examined were TNF-a, IL-1b, IL-10, IL-12p35, and IL-12p40 produced by MoDCs or IFN-g, IL-4, IL-5, IL-8, and IL-10 produced by T-cells, which were evaluated by ELISA of the supernatants and by real-time PCR analysis for the expression of the mRNA. In MoDCs, DEP and formaldehyde significantly increased TNF-a and suppressed IL-12p40 in both the protein and mRNA level. On the other hand, in T cells, they suppressed both the production and mRNA expression of IFN-g and IL-10, while they did not affect IL-4, IL-5, or IL-8 production. These results show that Th1 cytokines such as IL-12 and IFN-g were significantly suppressed by DEP and formaldehyde although Th2 cytokines such as IL-4 and IL-5 were not suppressed by them. These data strongly support the crucial role of DEP and formaldehyde on deviation of the immune system to Th2-dominant pattern, which enhances allergic inflammation.

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Regulatory function of anergic T cells is dependent on $p27^{KIP1}$-induced cell cycle arrest controlled by activated MAP kinase p38

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Regulatory T cells are crucial components of the immune system and play an essential role in the control of self tolerance and processes of adaptive immunity. We previously described that human IL-10-treated dendritic cells (DC) induce anergic $CD4^+$ T cells with antigen-specific regulatory activity. Cell cycle analysis in these anergic T cells revealed high levels of cdk inhibitor $p27^{Kip1}$ in contrast to activated control T cells, which was responsible for cell cycle arrest in G_1. In this study, we further studied possible interactions of cell cycle regulation and signal transduction by MAP kinases in anergic T cells as compared to optimally stimulated T cells, cocultured with mature DC. Kinase-assays after immunoprecipitation showed a reduced activity of the MAP kinases JNK1/2 and ERK1/2 in anergic T cells compared to control T cells. Notably, we observed an enhanced and sustained activity of MAP kinase p38 during primary culture as well as after restimulation. The kinase MAPKAP2/3, the downstream substrate of p38, also showed significantly increased activity, indicating an important role of MAP kinase p38-signaling in anergic regulatory T cells. Blocking of p38 with the specific inhibitor SD203580 completely impaired the induction of anergy in these T cells, as demonstrated by proliferation assays, IL-2 production and cell cycle analysis. Furthermore, western blots demonstrated a downregulation of the G_1 phase specific cdk inhibitor $p27^{Kip1}$ after inhibition of MAP kinase p38, leading to cell cycle progression in regulatory T cells. Strikingly, after addition of p38-inhibitor SD203580 these T cells lost their antigen-specific suppressor function, demonstrating a critical role of p38 for the suppressor function of anergic T cells. In conclusion, our data indicate that activation of MAP kinase p38 is required for induction of the regulatory activity of anergic T cells mediated by cdk inhibitor $p27^{Kip1}$-controlled cell cycle arrest.

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TNF-a/TNF-receptor-2 signaling is required for low zone tolerance to contact allergens

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Low zone tolerance (LZT), induced by epicutaneous application of low doses of contact allergens, depends on the induction of regulatory $CD8^+$ T cells (Tprevs) that inhibit the development of contact hypersensitivity (CHS). Here, we used TNF-a-/- and TNF-a receptor-deficient mice to better characterize the role of TNF-a in the induction of LZT. Tolerizing doses of the contact allergen TNCB (0.45 or 4.5 µg per site, 5 times per mouse) were applied epicutaneously. Tolerance induction was assessed by measuring the inhibition of a contact hypersensitivity reaction (ear swelling). Importantly, we found that low zone tolerance is TNF-a-dependent as TNF-a-/- and receptor-double-KO-mice failed to develop LZT. Interestingly, TNFR-1-/- (p55-/-) mice showed normal LZT responses, whereas TNFR-2-/- (p75-/-) mice did not develop LZT. These findings indicate that TNF-a and its receptor p75 play an important role in LZT development. In addition, prevention of hapten-specific clonal expansion of lymph node cells and high levels of IL-4 and IL-10 as typical Tc2 cytokine pattern of LZT did not occur in TNF-a-/-, receptor-double-KO- and p75-/- mice after tolerization as compared to WT and p55-/- animals. The neutralization of TNF-a in WT by application of mAb inhibited the tolerance reaction revealing a direct function of TNF-a during LZT development. Adoptive transfer experiments of T cells from tolerized TNF-a-/- or WT mice injected vice versa into WT or TNF-a-/- animals demonstrated that TNF-a is not required for the generation of regulatory $CD8^+$ T cells of LZT (induction phase). Thus, our data demonstrate a critical role of TNF-a via TNFR-2 (p75) in the effector phase of LZT, where it inhibits the generation of CHS-promoting Tc1 cells and consequently the development of CHS.

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IgA antibodies to epidermal transglutaminase are elevated in patients with dermatitis herpetiformis compared to normals and patients with celiac disease

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Dermatitis herpetiformis (DH) is a cutaneous manifestation of celiac disease (CD) and is characterized by deposition of granular IgA in dermal papillae. A circulating antibody responsible for this deposition of IgA in DH skin has not been identified. Recently, IgA antibodies to epidermal transglutaminase (eTG) have been found in the serum of patients with DH. These antibodies were reported to be quantitatively the same as those seen in the serum of CD patients but qualitatively of higher affinity. eTG antigen was also found to co-localize with IgA in DH skin (Sardy, JEM, 2002). We evaluated IgA eTG and tissue transglutaminase antibody (tTG) levels in the serum of normals (n=57) as well as untreated DH (n=34), CD(n=34), and psoriasis (n=37) patients with ELISA techniques which utilize recombinant eTG and tTG in commercial kits. We confirmed our eTG results by indirect immunoflourescence using a separately cloned eukaryotic eTG transfected into HEK cells. DH, but not CD, sera had quantitatively higher levels of eTG antibodies compared to normals and psoriatics (p<.01). tTG antibody levels were elevated in both CD and DH sera compared to normals and psoriatics (p<.01). The independent indirect immunofluorescence assay confirmed the ELISA results. It demonstrated a cellular binding pattern in eTG-HEK cells that was seen using patient serum with high levels of eTG antibodies on ELISA, but not those with low levels of eTG antibodies on ELISA. This quantitative serologic difference in IgA eTG, but not tTG, antibodies differentiates patients with DH from patients with CD alone. IgA antibodies to eTG may be responsible for the cutaneous deposition of IgA in DH skin.

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Characterization of recombinant soluble mouse Langerin

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A range of C-type lectins have recently been shown to be expressed by antigen presenting cells (APC), including dendritic cells. Several of these C-type lectins recognize and bind pathogens while others may regulate cell signaling, mediate intercellular interactions or facilitate DC migration. In any case, C-type lectins likely play important roles in innate and adaptive immunity. Langerin is a novel type-II Ca2+-dependent C-type lectin with mannose-binding specificity that is thought to be expressed exclusively by Langerhans cells and related cells. Langerin is constitutively associated with Birbeck granules (BG) and induces BG formation when overexpressed in fibroblasts. The Langerin extracellular region is comprised of neck and mannan-binding carbohydrate recognition domains. We isolated Langerin cDNA from mouse fetal skin-derived DC (FSDDC) by subtractive cloning and rapid amplification of cDNA ends. When Langerin cDNA was transfected into fibroblasts, BG formation was induced. In order to further characterize Langerin, we expressed soluble epitope-tagged (Ni++- and S protein-binding) recombinant fragments of mouse Langerin with or without the neck domain in bacteria, and purified them using Ni++-column and mannan-agarose affinity chromatography. The neck domain of Langerin was required for efficient interaction of recombinant Langerin with mannan. Recombinant Langerin bound to mannan-agarose beads and mannose-rich yeast invertase in a calcium-dependent fashion. Langerin-invertase binding could be blocked with mannan. Recombinant Langerin did not bind to bulk peripheral lymph node cells, splenocytes, or thymocytes. However, Langerin did bind to mouse lung epithelial tumor (TC-1) cells, and binding was inhibited by EDTA. A soluble bacterial recombinant fragment of an unrelated mannan-binding lectin (T14) did not interact withTC-1 cells. Soluble recombinant Langerin may facilitate identification of additional physiologic Langerin ligands and further our understanding of Langerin function.

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Secretion and processing of cathelicidin antimicrobials as a paradigm for innate immune defense

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Antimicrobial peptides (AMPs) are an essential first line of immune defense against infection. Recently, the AMP cathelicidin was found in eccrine glands. To be functional, AMPs must be enzymatically processed from an inactive precursor protein to the active peptide. We sought to discover if novel forms of cathelicidin are processed in sweat and determine if other exocrine systems also produce cathelicidin. In human neutrophils, cathelicidin hCAP18 is processed to a 37 aa peptide known as LL-37. To identify novel skin AMPs, sweat was collected from healthy volunteers, separated by HPLC, and fractions tested by radial diffusion assay against S. aureus (mprF). Candidate fractions were analyzed by Western blot, MALDI TOF mass spec, and aa sequencing. Mammary and salivary glands were evaluated by in situ hybridization, RT-PCR, immunostaining and Western blot. Three novel processed forms of LL-37 were found by this approach, and cathelicidin was abundantly detected in both mammary and salivary systems. Synthetic versions of the novel sweat peptides were then tested for antimicrobial activity against S.aureus, E.coli, and Group A Streptococcus. Cytotoxicity and host immune stimulatory activity were also tested in human blood and keratinocytes. Surprisingly, the forms of cathelicidin in sweat differ from LL-37 in size, antimicrobial activity and immune stimulatory activity. Two sweat peptides derived from LL-37, RK-31 and KS-30, increase antimicrobial activity against multiple bacteria (eg. MIC against S. aureus is 8-16 uM compared to >64uM for LL-37). Conversely, sweat peptides decrease their ability to stimulate IL-8 release from normal human keratinocytes. These results demonstrate that cathelicidin production is a general exocrine phenomenon and that post-secretory processing has a critical role by modifying the balance of antimicrobial and inflammatory activity. Our findings suggest that peptide processing is an essential element to innate immune defense.

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Imiquimod induce increased IL-6 secretion of normal human mixed epidermal cells

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In order to better understand the mechanism of immune response regulation by imiquimod, we have investigated the effects of imiquimod on IL-6 secretion from normal human mixed epidermal cells. Single cell suspensions of normal human epidermal cells were prepared utilizing standard techniques, then cells were cultured with culture medium alone or with medium containing 5 ug/ml of imiquimod for 1, 2, 4, 8 and 12 hours, and then cell-free culture supernatants were harvested to assay. IL-6 contents of the supernatants were studied using commercially available ELISA kits. We found that a low level of IL-6 secreted from mixed epidermal cells treated with single culture medium, but when incubated with imiquimod, mixed epidermal cells secreted large amounts of IL-6, and at any time intervals IL-6 secreted from imiquimod treated groups was higher than that from control cells (p<0.01). IL-6 secretion reached its' peak at 8 hours, 245.1 pg/ml (imiquimod treated group) compared with untreated group (97.2 pg/ml).The results demonstrated that imiquimod can enhance the secretion of IL-6 from normal human mixed epidermal cells. Our data partially explain immune response regulatory mechanism of imiquimod.

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***In vivo* transfection of a cis element "decoy" against STAT6 binding site ameliorates IgE mediated third phase reaction in an TNP-IgE transgenic mice as an atopic dermatitis mouse model**

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We have recently established transgenic mice that carry the genes cording for heavy and light chain of TNP-IgE and demonstrated that not only immediate and late phase reaction but also third phase reaction infiltrated tremendous numbers of eosinophils, were elicited at 3-7 days after epicutaneous challenge in TNP-IgE-transgenic mice as a AD mouse model. We therefore hypothesized that synthetic double-stranded DNA with a high affinity for Signal Transducers and Activators of Transcription 6 (STAT6) could be introduced in vivo as a decoy cis elements to bind the transcriptional factor and to block the gene activation of contributing the onset and progression of AD, thus providing effective therapy for AD. Treatment by the transfection of STAT6 decoy oligodeoxynucleotides (ODN), but not scramble decoy ODN in the TNP-IgE transgenic mice,had a significantly inhibitory effect on not only STAT6 binding to nuclei but also on the third phase response(62% inhibition). A histological analysis revealed that both edema and the infiltration of eosinophils and degranulated mast cells significantly decreased in STAT6 decoy ODN transfected mice. To examine the mechanism of the in vivo effect of STAT6 decoy ODN, we employed an in vitro mast cells culture system. After IgE receptor engagement, mast cells transfected by STAT6 decoy ODN exhibited normal histamine release, but their cytokine release (TNF-a, IL-6) markedly decreased. We herein report the first successful in vivo transfer of STAT6 decoy ODN to reduce the third phase reaction in TNP-IgE transgenic mice, thereby providing a new therapeutic strategy for atopic dermatitis.

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Induction of β-defensin 3 through toll-like receptors 2 in keratinocyte

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Toll-like receptors (TLRs) play important roles in inate immunity and are expressed not only on the surface of monocytes but also on keratinocytes. It is well established that peptidoglycan(PG) and LPS are the ligands for TLR2 and TLR4 and that MyD88 is an adaptor molecule of TLRs. β-Defensins (BD) working in protection from microbial infection are products of varieties of epithelial cells including keratinocytes (KC). To examine whether the signals through TLRs - MyD88 regulate expression of BD, we isolated KC from newborn wild type (WT:C57/B6), TLR2 deficient (TLR2KO), TLR4deficient (TLR4KO) and MyD88 deficient (MyD88KO) mice. KC cultures were stimulated with LPS (100ug/ml) or PG (500ug/ml), and TNF-α in the supernatant was measured by ELISA. TNF-α increased in the WT KC cultures stimulated with LPS or PG, respectively. In contrast, neither TLR4KO KC stimulated with LPS nor TLR2KO stimulated by PG produced TNF-α. TNF-α was not detected in MyD88KO KC cultures stimulated by these ligands. These results indicate that KC recognize LPS through TLR4 and PG through TLR2 and that the production of TNF-α was mediated through TLRs-MyD88 pathway. Next, we studied the expression of mouse BD 3(mBD3) mRNA in KC after stimulation with LPS or PG by real-time RT-PCR. In WT KC, PG stimulation showed significant increase of mBD3 mRNA at 6hr. In contrast, LPS stimulation showed no increase. KC from TLR2KO and MyD88KO mice showed no increase of mBD3 after stimulation with PG. These results strongly suggest that PG induces the expression of mBD3 through TLR2-MyD88 pathway in KC. Finally, we studied biological roles of TLRs-MyD88 pathway in KC in vivo. S. aureus were inoculated on the back skin of WT and MyD88KO mice. The inoculated sites of WT mice showed marked inflammation, while MyD88KO mice showed slight inflammation. These results suggest that TLRs on KC have important roles for recognition of S.aureus in vivo.

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Age-dependent breakdown of peripheral tolerance to the epidermal autoantigen

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We established double transgenic (dTg) mice which carry both the transgene of ovalbumin (OVA) expressed selectively in the keratinocytes under the keratin 5 (K5) promoter (K5-mOVA) and the Kb-restricted OVA-specific T cell receptor transgene (OT-I). Thymus-derived CD4+CD25+ regulatory T cells (Treg) induced peripheral tolerance of OT-I cells to the epidermal autoantigen in euthymic dTg mice, while athymic dTg mice developed toxic epidermal necrolysis (TEN) as a consequence of lacking Treg. Unexpectedly, peripheral tolerance to the epidermal autoantigen was not a particular event in the skin lymph nodes (LNs). Transferring mesenteric LN cells devoid of OT-I cells from euthymic dTg mice prevented the skin disease in athymic dTg mice, and sorted OT-I cells from mesenteric LNs of euthymic dTg mice could induce the skin disease in athymic K5-mOVA mice. Here we demonstrate that peripheral tolerance to the epidermal autoantigen break down in age-dependent manner. OT-I cells in euthymic dTg mice was not activated until 6 months of age, however, naive OT-I cells and activated ($CD44^{high}$) OT-I cells coexisted in the skin LNs at 8 months of age. Activated OT-I cells were not found in the mesenteric LNs and emerged selectively in the skin LNs, although the ratio of OT-I to Treg showed no significant change between skin LNs and mesenteric LNs. Euthymic dTg mice developed white hair and mild hair loss over 6 months of age. Histological examination of the skin specimen showed apoptosis of some epidermal keratinocytes and the tissue damage deteriorated in age-dependent manner. These results suggest that peripheral tolerance regulating CD8+ T cells specific for epidermal autoantigens may break down in age-dependent manner.

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Lfa-1 function on dendritic cells is regulated by cytip

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The beta2 integrin LFA-1 is important for transendothelial migration of leukocytes as well as for T cell activation during antigen presentation. To determine the relevance of LFA-1 for antigen presentation of DC, we investigated bone marrow-derived DC (bmDC) from CD18-deficient (-/-) mice, which lack all functional beta2 integrins, in vitro. Surprisingly, antigen presentation of bmDC from CD18-/- mice is not impaired as determined by DC-induced T cell activation. T cell proliferation as well as cytokine production of T cells and DC are similar, when CD18 wt or CD18 (-/-) DC were used. To determine why LFA-1 does not seem to be active in this process we studied transendothelial migration and performed binding studies to ICAM-1. Migration of DC was dependent on ICAM, but not on LFA-1 expression. Also LFA-1 expressing mature DC did not bind to ICAM-1, and binding was not inducible by activation. The binding aviditiy of LFA-1 is regulated by the cytosolic proteins Cytohesin-1 and Cytip. In order to be active, Cytohesin-1 aggregates LFA-1 at the plasma membrane, resulting in enhanced LFA- avidity, whereas Cytip complexes Cytohesin-1 and removes the complex from the inner membrane, resulting in LFA-1 inactivation. In activated T cells, Cytohesin-1 is strongly colocalized with LFA-1 in T cells as determined by confocal microscopy, Cytip expression is not detectable and LFA-1 is active in these cells. In contrast, Cytip expression in DC increases during maturation and colocalized Cytip-Cytohesin-1 complexes accumulate in the cytosol, thereby rendering LFA-1 inactive. Thus, LFA-1 function in DC is regulated by Cytip expression.

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Effect of CpG-oligodeoxynucleotides in primary human keratinocytes and in the skin of NC/Nga mice

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Immunostimulatory activities of synthetic oligonucleotides containing CpG motifs (CpG-ODN) have been shown to be useful immunotherapetics due to the stimulating effect of innate immune response. Toll-like receptor (TLR 9) has been known to play an important role in CpG-ODN induced cellular responses. Although TLR 9 expression has been demonstrated in cultured primary human keratinocytes, the exact cellular mechanisms are still unknown. In this study, we examined the function of CpG-oligodeoxynucleotides (CpG-ODN) in human keratinocytes and NC/Nga mouse. CpG-ODNs stimulated NF-KB nuclear localization of human keratinocytes in a CpG-ODN dependent manner by indirect immunofluorescence assay using confocal microscopy. FITC-labeled CpG-ODN were applied to the back of NC/Nga mice and topical application of FITC-labeled CpG-ODN resulted in the accumulation of fluorescence deep in the skin, including in keratinocytes and more in dermal infiltrated cells and cells comprising hair follicles at day 1. The fluorescence was continued up to at least 5 days after the topical application, and disappeared with in 1 week after the topical application. Furthermore, the fluorescence was discovered in the draining lymph nodes. These results suggested the potential application of CpG-ODN as a modulator for inflammatory disorders of the skin.

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IFN-γ up-regulates the mouse complement Clr expression in mouse keratinocytes

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The complement system provides critical and multifaceted defense system against inflammation and infection. We have previously reported cloning of the mouse complement C1r mRNA. The present study analyzes the expression of the complement C1r in mouse keratinocytes by IFN-γ. Northern blot analysis showed that IFN-γ induced the increase of complement C1r mRNA. To better clarify the molecular basis of IFN-γ action, the 2200 bp 5'-flanking region of the complement Clr gene was isolated and characterized. The transcription start point of the complement Clr was identified by a rapid amplification of cDNA ends assay. This promoter region was ligated to a luciferase reporter vector to elucidate the molecular basis of the complement Clr up-regulation by IFN-γ. The transcriptional activity of the 2200 bp 5'-flanking region was analyzed by deletion constructs using a reporter vector transiently transfected into mouse keratinocytes. The results indicated that a -80 bp to +120 bp fragment including the transcription start point was sufficient for IFN-γ -induced promoter activity and the core promoter activity was not significantly changed by inclusion of upstream sequences as far as -2100 bp. Taken together, our results demonstrated that the complement C1r expression in mouse keratinocytes was regulated by the 200 bp proximal promoter region containing several transcriptional elements responsible for this observation.

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Plasmacytoid dendritic cells induce hapten-specific Tcell proliferation

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Plasmacytoid dendritic cells (pDC), also referred to as type I interferon-producing cells, are known to play a critical role in the innate and adaptive immune response against viruses. We have recently detected these cells in the epidermal and dermal infiltrate of allergic contact dermatitis elicited by epicutaneous patch tests (EPT). In the dermis they represented a substantial proportion of the entire DC infiltrate and were in close apposition to T-cells, suggesting interactions between pDC and infiltrating T-cells. In this study, we investigated the capacity of pDC to stimulate hapten specific T-cells. As a positive control, pDC were compared to $CD1c^{+}$ myeloid dendritic cells (mDC), which represent the major dendritic cell population in EPT lesions. Nickel-specific $CD4^{+}$ T-cell lines from nickel-allergic individuals were co-cultured with autologous blood-derived pDC and mDC for 72h. Before co-culture, both pDC and mDC were exposed to nickel and either left immature or matured by using TNF-α and IL-3 and TNF-α and GM-CSF, respectively. Nickel-pulsed mature, but not immature mDC acted as strong stimulators of hapten-specific T-cells, thus confirming previous observations. Importantly, nickel-pulsed mature pDC induced a nickel-specific T-cell response comparable in magnitude to that elicited by mDC. As expected, neither unpulsed mature nor immature pDC and mDC were able to stimulate hapten-specific T-cells. In conclusion, following exposure to nickel, mature pDC acquire the capacity of inducing a hapten-specific T-cell proliferation, indicating that pDC may act as prominent amplifiers of the adaptive immune response in allergic contact dermatitis.

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Expression of cytokines and chemokines by human monocyte-derived dendritic cells as valuable tool for discriminating potential allergens from non-allergenic compounds

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In industrial states, the proportion of persons developing contact hypersensitivity growths constantly. Dendritic cells (DCs), the most potent antigen-presenting cells of the immune system, play an important role in the induction phase of contact allergy and several cytokines and chemokines are implicated in this pathology. The aim of the present study was to evaluate the interest of monocyte-derived DCs (Mo-DCs) and their mediator expression as a predictive in vitro assay system for contact sensitization by molecules. We performed the evaluation of cytokines / chemokines expression in Mo-DCs exposed to known reference sensitizers vs irritant. Our results have shown that sensitizers can induce TARC and MDC chemokine expression. These mediators recruit Th2 lymphocytes, to the detriment of Th1, into the inflammation site. We have also shown that IL-12 (Th1 orientation), IFNg (Th1 cytokine) and IP-10 (Th1 recruitment) are inhibited suggesting an orientation towards a Th2 response. Ours present results allow to conclude that Mo-DCs represent a cell system well suited for screening the sensitizing potential of molecules.

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Molecular insights into infliximab's impact on gene expression profiles in psoriasis

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Psoriasis is a multifactorial, chronic inflammatory skin disease, with a prevalence of 2.6% in the US population. Microarray technology has allowed global gene expression studies to reveal disease-associated gene maps in psoriasis and provided insight into the disease pathogenesis at molecular level. A recent placebo-controlled clinical study using a chimeric monoclonal anti-TNFα antibody, infliximab, showed marked clinical efficacy in treating moderate to severe plaque psoriasis, which strongly supports the hypothesis that TNFα plays a pivotal role in the pathogenesis of psoriasis. The purpose of this study is to explore the role that TNFα plays in disease pathology in psoriasis at the molecular level. Skin biopsy samples obtained from the aforementioned clinical study with infliximab were analyzed for gene expression patterns using cDNA microarray gene chips. Gene expression profiles were compared between lesional or lesional and non-lesional sites prior to as well as after infliximab treatment. Differential gene expression data support the notion that aberrant proliferation of keratinocytes and an active inflammatory response are crucial pathways contributing to the maintenance of psoriatic plaques. Infliximab treatment resulted in a change of gene expression patterns favoring the reduction of inflammation as reflected by up-regulation of anti-inflammatory cytokines, down-regulation of pro-inflammatory mediators, repair promotion, and homeostasis restoration. Our study using microarray technology provides insights into the impact of infliximab on gene expression profiles in psoriasis. In addition, a variety of new therapeutic targets could potentially emerge from studies using this technology.

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Differential expression of B7-DC by Langerhans cells and keratinocytes—a duel role for B7-DC in skin immunity

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B7 family costimulatory molecules play an important role in the regulation of T cell activation. B7-DC/PD-L2 is a novel member of the B7 family. It shares an inhibitory receptor, PD-1, with B7-H1/PD-L1. Studies have demonstrated that B7-DC can bind a stimulatory non-PD-1 receptor as well. In this study, we sought to examine whether B7-DC is involved in skin immunity. First, we determined if murine Langerhans cells (LC) and keratinocytes (KC) express B7-DC, comparing with B7-H1. Immunolabeling of murine epidermal sheets showed that resting LC and KC did not express B7-H1 or B7-DC. However, they expressed significant levels of these molecules after activation. Ex vivo and in vivo migratory mature LC expressed B7-DC but not B7-H1, whereas IFN-γ-stimulated KCs expressed both B7-H1 and B7-DC. Second, we examined the in vivo functions of LC B7-DC using B7-DC-deficient mice. B7-DC-/- mice demonstrated significantly decreased contact hypersensitivity (CHS) response, supporting that B7-DC plays a stimulatory role in the initiation of primary immune response via a non-PD-1 pathway. Third, we examined the role of KC-derived B7-DC in regulating effector T cells through antibody blocking at challenge. Blockade of B7-DC slightly enhanced CHS, whereas B7-H1 blocking markedly enhanced CHS responses. However, joint blockade of B7-DC and B7-H1 could lead to a greater enhancement of CHS to an extent similar to PD-1 blocking, suggesting that B7-DC, synergetic with B7-H1, down-regulates effector T cell activities via the PD-1 receptor. Collectively, our studies demonstrate the LC and KC differentially express B7-DC after activation, which plays a duel role in T cell-mediated immune responses. LC B7-DC co-stimulates naive T cells via the non-PD-1 pathway, whereas KC B7-DC down-regulates effector T cell activities via the PD-1 pathway.

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Identification of CD3+CD4−CD8−T cells as potential regulatory cells in tolerant double transgenic mice

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We have reported that both single [K14-mOVA] transgenic (Tg) and double Tg [K14-mOVA x OT-I that have a T cell receptor (TCR) that recognizes OVA peptide in association with MHC class I] mice express membrane-associated ovalbumin (mOVA) under the control of a K14 promoter. When injected with CD8+ TCR Tg T (Vα2Vβ5+) cells from OT-I mice, single Tg mice develop graft vs. host disease (GvHD), whereas double Tg mice do not develop disease. This suggests that, in double Tg mice, regulatory mechanisms prevent infused OT-I cells from inducing GvHD. Since CD3+CD4-CD8-(double negative-DN) T cells have been reported in other systems to have regulatory functions (Nature Med 2000, p782; PNAS 2002, p2181), we determined whether CD3+DN T cells exist in these double Tg mice. In lymph nodes (LN) of double Tg mice >80% of cells were CD4-CD8-, whereas in single Tg mice only 25% of the cells were CD4-CD8-. 20% of these CD4-CD8- cells were CD3 positive in double Tg mice, whereas only 2.7% of CD4-CD8- cells were CD3 positive in single T g mice. To further characterize this cell population, we purified CD3+DN T cells from the LNs and spleen of double Tg mice. 95-99% of purified CD3+DN T cells expressed the same TCR as OT-I cells. These CD3+DN T cells proliferated in response to anti-CD3 mAb but they did not proliferate in response to antigen (OVA peptide). Strong proliferation (100X baseline) was observed in response to antigen when IL-2 was added to the cultures. No cytokine was detected in cultures that were stimulated by antigen in the absence of IL-2, however, when IL-2 was added, IFN-γ (116 pg/ml) was detected. Ten days after stimulation with anti-CD3 mAb, the cells retained the CD3+DN phenotype with partial down-regulation of Vα2Vβ5 TCR. The number of CD3+DN T cells expanded over 20-fold during 10 days of culture and the expanded population expressed CD62LlowCD25+CD44+CD69+ (activated) phenotypes whereas the initial phenotype was CD62LhighCD25-CD44-CD69- (naive). The isolation and expansion of these CD3+DN T cells should facilitate their use as potential regulatory cells in these double Tg mice.

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Impairment of epicutaneously induced Th2 responses in the absence of MyD88 or Toll-like receptor (TLR) 2

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We have shown that Th2 responses induced by exposure to inhaled (i.n.) ovalbumin (OVA) are dependent on signaling through Toll-like receptor (TLR) 4, while those induced by epicutaneous (e.c.) OVA exposure are TLR4 independent. To determine if other innate immune signals are involved in e.c. Th2 induction, we investigated the role of MyD88, an intracellular adaptor molecule important for signaling through most TLRs, in these responses. Wildtype (WT) and MyD88 -/- mice were exposed to e.c. OVA (100 μg) on day 0 and challenged 2 weeks later with i.n. OVA. Compared to WT, MyD88 -/- mice had decreased OVA-specific IgG1 (p=.01) and total IgE (p=.03) in serum and decreased airway eosinophils following i.n. OVA challenge (p<.001). To ensure this did not simply reflect defective recruitment into the lungs, skin draining lymph node (LN) cells were isolated 4 days after e.c. OVA exposure and restimulated in vitro with OVA for evaluation of cytokine production. Compared to WT, LN cells from MyD88 -/- mice produced significantly reduced levels of the Th2 cytokines IL-5 (p=.03) and IL-13 (p=.01) and no detectable IFN-γ. These data indicated that a member of the IL-1/TLR superfamily, other than TLR4, was required for e.c. Th2 induction. Given the known expression of TLR2 in skin and the potential role for TLR2 ligands (e.g., cell wall components of Gram+ bacteria) in atopic dermatitis, we next exposed WT or TLR2 -/- mice to e.c. OVA. Compared to WT, LN cells from TLR2 -/- mice also produced significantly less IL-5 (p=.01) and IL-13 (p=.01) and no detectable IFN-γ. Consistent with our previous findings, IL-5 and IL-13 production by LN cells following e.c. OVA exposure of TLR4 defective mice was equivalent to WT. Taken together, the data indicate that innate immune system signals are important for generation of Th2 responses in the cutaneous environment, with the TLR2/MyD88 pathway playing a major role. In contrast to its known role in Th1 generation, dependence on MyD88 for induction of Th2 type immune responses has not previously been reported.

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B7H1 co-stimulation mediated by dendritic cells inhibits the function of activated T cells and suppresses the development of T cell mediated immune responses

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A line of evidence indicates that PD-1, a molecule expressed by activated T cells, plays an important role in the down-regulation of immune responses. However, literature about the effect of its two defined ligand molecules, B7H1 (PD-L1) and B7-DC (PD-L2), is controversial. The current study has addressed the role of B7H1 in dendritic cell (DC) mediated regulation of contact hypersensitivity (CHS). The expression of B7H1 and B7-DC is induced on migratory hapten carrying DC following sensitization while PD-1 is only expressed by primed T cells, both CD4+ and CD8+ subpopulations. Addition of anti-B7H1 but not anti-B7-DC antibodies increases the cytokine production by primed T cells in cultures with hapten labeled DC. In vivo administration of anti-B7H1 antibody increases the magnitude of CHS responses. Accordingly, T cells from anti-B7H1 treated animals produce a higher level of inflammatory cytokines than those from control animals. The data suggest that B7H1 may play an inhibitory role in the regulation of CHS responses. To further define the effect of B7H1 in DC mediated regulation of immune responses, a DC line that expresses a high level of B7H1 molecules (B7H1/DC) has been generated. Culture with hapten labeled B7H1/DC inhibits the proliferation of primed T cells. IFN-γ and IL-2 production by both CD4+ and CD8+ T cells is inhibited. T cell proliferation in the culture with B7H1/DC can be restored by addition of recombinant IL-2, suggesting that the down-regulation of IL-2 may be a major mechanism for B7H1 mediated suppression. Further experiments demonstrate that administration of hapten labeled B7H1/DC in primed animals inhibits the elicitation of CHS responses in an antigen specific manner. Our studies have indicated that B7H1 co-stimulation plays inhibitory roles in DC mediated regulation of T cell activation and immune responses. The ability of B7H1/DC to inhibit the elicitation of immune responses may be exploited to develop new strategies for treatment of immune response mediated disorders.

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Differential Langerhans cell gene expression profiling during the induction of contact hypersensitivity

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To identify genes involved in the induction of contact hypersensitivity (CH), we examined epidermal gene expression after hapten (3% TNCB) painting of naive mice using array technology. Total RNA was prepared from the epidermis (either sheets or suspensions) of BALB/c mice two hours after either hapten or vehicle painting and differential gene expression was examined using the GeneChip®. The two hour time was chosen because of the lack of inflammatory cells in the epidermis. To confirm the chip findings results and to identify the cellular sources of hapten-induced genes, semi-quantitative RT-PCR was performed with or without magnetic bead cell separation. After hapten painting epidermal cell suspensions were depleted by either CD45 or MHC-class II beads and gene expression levels of these CD45- depleted (Langerhans cell [LC] and dendritic epidermal T cell depleted at <0.04% and <0.013%, respectively), MHC-class II-depleted (only LC depleted at <0.01%) and each positive fraction after depletion were examined together with non-treated, vehicle- and hapten- (non-separated) painted samples. 14 of the 15 genes that were consistently upregulated (3-fold increases) in two independent chip experiments were also shown to be upregulated by semi-quantitative RT-PCR. Among these, the combination of RT-PCR and the two cell separation techniques revealed that only two of these genes (Interleukin 1β [IL-1β] and Macrophage inflammatory protein-2 [MIP-2])were preferentially upregulated by LC. In keeping with prior studies a time-course study of IL-1β upregulation showed fold increases of 1.1, 52.8, 38.8, 25.1 and 6.3 at 1, 2, 4, 8, and 24 h after hapten painting. The time-course for the upregulation of MIP-2 showed fold changes of 3.2, 70.0, 77.5, 103.3 and 6.4 at the various times after hapten painting. In mice, MIP-2 has been shown to be abundant in mast cells and has been shown to be a chemoattractant for neutrophils in the elicitation phase of CH. Our finding that MIP-2 is derived from LC during the induction phase suggests its potential importance in the generation of the early events in CH.

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Engraftment of Langerhans cells, as compared to plasmacytoid- or lymphoid-dendritic cells, occurs in an interleukin 7 receptor-independent manner

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We have demonstrated that Langerhans cells (LC) can be derived from thymocytes suggesting that LC may have a lymphoid origin. To directly address the question of whether LC are of lymphoid origin, we used bone marrow (BM) cells from interleukin 7 receptor (IL-7R) knockout mice (-/-) (in which lymphopoiesis is impaired) as donor cells and used X-irradiated recipient mice. Ears of recipient were painted with TNCB to enhance LC repopulation. If LC were of lymphoid origin, engraftment would be negatively affected by the IL-7R defect of donor cells. Percent chimerism of epidermal LC, various dendritic cells (DC) and other cells was assessed in recipient spleen, lymph nodes (LN), thymus and BM by flow cytometry 4 weeks after cell transfer. Recipient BM was >90% chimerized when either $BM^{-/-}$or BM from wild type (BM^{wt}) cells were used. In contrast, in the thymus there was <10% chimerism when $BM^{-/-}$ cells were used, as opposed to >99% chimerism when BM^{wt} was used. With $BM^{-/-}$ transfer, myeloid (monocyte and neutrophil, roughly 83-99%), but not lymphoid (B and T lymphocyte, 0.4-3.5%) lineage cells were efficiently reconstituted in the spleen or LN as expected. In skin, there was no significant difference in epidermal LC engraftment between $BM^{-/-}$ (34.3±9.0%) and BM^{wt} (36.8±20.4%) transfer. Repopulation of splenic $CD8a^+DC$ (lymphoid) and $B220^+DC$ (plasmacytoid) was reduced in $BM^{-/-}$ (66.0±2.5, 48.1±7.0%, respectively) reconstituted, compared to BM^{wt} (83.8±8.0, 92.0±1.7%) reconstituted mice. These reductions were also observed in peripheral LNs (34.6±7.8, 79.8±8.6% with $BM^{-/-}$ compared to 61.7±2.8, 97.1±0.7% with BM^{wt}). Thus, lymphoid and plasmacytoid DC reconstitution is enhanced in LN and spleen in the presence of the IL-7R. $CD11b^+$ (myeloid) DC were chimerized in the spleen by >85% when eiher $BM^{-/-}$ or BM^{wt} were used. Taken together, the absence of IL-7R seems to affect development of several DC types depending on their phenotype and the location, but does not affect LC development. These findings suggest that LC originate from cells that do not require IL-7R-mediated lymphopoiesis.

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Early interleukin-4 administration promotes TH1-driven, protective anti-cancer immunity

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Recently, we found that the co-administration of the polycation poly-L-arginine (pR) and β-galactosidase (βgal; thereafter referred to as pR-PV) yields substantially higher numbers of specific, IFN-γ-secreting, CD8+ T cells than the s.c. application of βgal alone. In addition, there are studies showing that IL-4 is capable of promoting the activation of TH1 lymphocytes provided that it is applied early after the induction of the immune response and at high doses. Based on these observations, we asked whether IL-4 can be used to augment the immunological as well as clinical efficacy of the pR-PV. To test this hypothesis, BALB/c mice were injected s.c. with the pR-PV either alone or admixed with IL-4. Seven days later, CD8+ T cells producing either IFN-γ or IL-4 in response to βgal were quantified by ELISPOT analysis. Results obtained showed that co-administration of IL-4 significantly enhances the number of vaccine-induced, specific CD8+ T lymphocytes that produce IFN-γ. In addition, we found the IL-4 treatment used to shift the specific immune response towards a TH1 phenotype. To investigate the effect of IL-4 administration on the clinical efficacy of the pR-PV, BALB/c mice were injected on days 0 and 14 with pR-PV +/- IL-4 and challenged on day 24 by the s.c. inoculation bgal-expressing RENCA cells. None of the naive controls (n=6) rejected the tumor inoculum. S.c. administration of the pR-PV protected 3/6 (50%) animals. The highest protection rate (5/6; 83%) was obtained by the combined use of the pR-PV and IL-4. Together, these results demonstrate that IL-4 has the potential to act as a natural adjuvant capable of inducing a protective TH1-driven anti-cancer immune response.

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Inhibition of lymphotoxin signaling blocks the clinical expression of murine graft-versus-host skin disease by altering the microenvironment within secondary lymphoid organs

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Lymphotoxin (LT) signaling is essential for maintaining the organized structural microenvironment within secondary lymphoid organs (lymph nodes, spleen) that is required for the induction of effective adaptive immune responses to exogenous antigens. We have previously demonstrated that inhibition of LT signaling via treatment with a LTβ receptor-Fc Ig fusion protein (LTβR-Ig) can markedly ameliorate the development of murine graft-versus-host skin disease (GVHSD) *(JID: 121[#1], July, 2003, Abstract #0910; manuscript in press, J. Immunology)*. We have more recently questioned whether the LT signaling-mediated microenvironment of secondary lymphoid organs might play an important role in alloreactive T cell activation and proliferation observed in GVHSD. In the present study, we blocked LT signaling in a murine model of minor histocompatibility antigen (mHA) mismatch GVHSD (LP/J→ B6) by using LTβR-Ig. The number of dendritic cells (DCs) in the spleen and lymph nodes of LTβR-Ig treated recipient B6 mice was greatly reduced, especially for the CD11c(high),class II(high) DC subset. The reduced numbers of DCs in lymphoid tissues of LTβR-Ig treated-receipt B6 mice exhibited a decreased ability to stimulate allogenic T cells in vivo and in vitro. Passive transfer of splenocytes and bone marrow cells from LP/J→B6 chimeras displaying severe clinical GVH skin lesions into lethally irradiated naive B6 recipient mice resulted in severe GVH skin lesions on the posterior aspect of the neck within two weeks. Strikingly, the administration of LTβR-Ig prevented skin lesions resulting from such passively transferred primary alloreactive T cells. These data suggest that DCs play an important role in the LT signaling dependent microenvironment of secondary lymphoid organs that is critical for the development of GVHSD.

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Spontaneous *in vivo* presentation of a model melanoma antigen to CD8 T cells : a variable event occuring in the absence of CD4 T cell activation

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(a) Observations such as immune cell infiltration of tumors, recognition of tumor antigens by T lymphocytes and spontaneous clinical regression in cancer patients suggest the development of a spontaneous immunological response against tumor cells. This spontaneous immune response remains poorly characterized. (b) Here we analyzed in vivo the presentation of a model melanoma tumor antigen, ovalbumin (OVA), to CD8+ and CD4+ T lymphocytes. (c) In regional lymph nodes draining the tumor, OVA was spontaneously presented to CD8+ T lymphocytes in 65% of mice bearing subcutaneous B16 melanoma. In the remaining 35% of mice, OVA was not presented to CD8+ T cells, despite the fact that the investigated lymph nodes in these mice were tumor-draining (as visualized by intratumoral injection of evans blue). It is noteworthy that OVA expressed in the cytoplasm of melanoma cells was more potently presented to CD8+ T cells than a membrane or a secreted form as assessed by using B16 cells stably transfected with the appropriate constructs. When detected, spontaneous OVA presentation to T cells was shown to be dependent on host bone marrow-derived cells, indicating a cross-presentation mechanism. Importantly, the cross-presentation of melanoma-associated OVA to CD8+ T cells was achieved in the absence of any detectable presentation to CD4+ helper T cells. (d) Taken together, these data indicate that the spontaneous cross-presentation of melanoma antigens to T lymphocytes is a variable event that favors presentation on MHC class I molecules. The absence of MHC class II presentation and CD4+ T cell activation highly suggest that spontaneous recognition of melanoma tumor antigens may lead to CD8+ T cell tolerance.

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The balance between nuclear factor-kappaB and c-Jun N-terminal protein kinase activity controls dendritic cell life and death

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Survival and death of dendritic cells (DCs) must be delicately regulated for proper function of adaptive immunity. While a pathway signaling survival in DCs (i.e. NF-kB) has been identified, signals that actively mediate DC death remain enigmatic. To elucidate how decisions on the DCs fate are made at the molecular level we aimed at identifying transcriptional activators that mediate survival and death in human DCs. Triggering of TNF-R-superfamily members (TNFR-SF) on DCs (CD40, CD95, TRAIL-R) or cognate DC-T cell contact resulted in parallel NF-kB and c-Jun N-terminal kinase (JNK)-mediated AP-1 activation and strongly enhanced DC longevity. When NF-kB activation was experimentally blocked by endogenous inhibitors, JNK-dependent AP-1 activity was drastically augmented and TNFR-SF triggering and cognate T cell encounter resulted in cell death. Pharmacologic and genetic inhibition of the JNK-AP-1 pathway completely restored receptor and T cell-mediated death in NF-kB-deficient DCs. We conclude that the JNK-AP-1-dependent death pathway is subject to feedback inhibition by NF-kB transcriptional activity in DCs. Thus the relative strength and cross-talk of two transcriptional pathways following surface receptor triggering decide upon the fate of a DC.

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Epinephrine inhibits the migration of epidermal $I\text{-}A^+$ cells to draining lymph nodes

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We have previously shown that intradermal injection of epinephrine inhibits the induction of contact hypersensitivity. In accordance with this observation, in vitro exposure of purified LCs to epinephrine (EPI) or norepinephrine (NE) inhibits presentation of a defined antigen to a responsive T cell clone. Furthermore, in vitro exposure of epidermal cells enriched for LC content to EPI or NE inhibited the elicitation of delayed-type hypersensitivity by injection of antigen-pulsed LCs into previously sensitized mice in a β-adrenoceptor mediated manner. In an effort to delineate the mechanisms of action, we found that EPI downregulates LPS-induced tumor necrosis factor (TNF)-α expression by the LC-like cell line XS106 and purified LCs, as shown by real-time PCR and ELISA. At the protein level, this effect can be specifically blocked by a β-adrenergic antagonist. TNF-α has been shown to play an important role in the mobilization of LCs from human epidermis and, thus, we investigated whether EPI affects migration of LC. EPI was injected intradermally into BALB/c mice 15 min prior to epicutaneous sensitization with 1% FITC or diluent alone. Twenty-four hours later draining lymph nodes were harvested and gently disrupted. To enrich for DC, cell suspensions were layered onto a 14.5% Nycodenz gradient and separated by centrifugation. Cells at the interface were collected, stained with PE-conjugated anti-$I\text{-}A^d$ antibodies, and analyzed by flow cytometry. Cells labeled with both FITC and PE were quantified as migrated LC. In mice sensitized without EPI, 5.70% of cells were double-positive for FITC and I-A while 2.58% were $FITC^+/I\text{-}A^-$. Injection of EPI led to a marked reduction of $FITC^+$ positive cells (4.64% $FITC^+/I\text{-}A^+$; 1.34 $FITC^+/I\text{-}A^-$). These preliminary experiments suggest that intradermal injection of EPI reduces migration of epidermal LCs to draining lymph nodes.

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Lentiviral mediated transduction of dendritic cell progenitors does not alter the plasticity or antigen presentation function of dendritic cells

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Introduction of antigen encoding genes into dendritic cells (DCs) without skewing or limiting their intrinsic function remains a critical challenge for the design of immunotherapies for the treatment of and prevention of infection, neoplasms, and autoimmune diseases. We investigated the effect of lentiviral vector transduction on the phenotype and function of murine bone marrow derived DCs (BMDCs). We found that although maturing DCs could be transduced using lentiviral vectors, targeting DC progenitor or immature DCs resulted in improved transduction efficiency, reproducibly enabling 50% of transduction of resulting DC populations. Importantly, transduction of early immature DCs or DC progenitors with lentiviral vector did not affect their maturation and antigen presentation function. Significant amount of IL-12p70 could be produced by both lentiviral vector-transduced and mock-transduced BMDCs in response to the stimulation by CpG oligonucleotides. Furthermore, transduction did not affect the DCs capacity to stimulate T cells as reflected by allogenic T cell proliferation (MLR) and had no effect on skewing Th1/Th2 cytokines of effector cells. Transduced BMDCs efficiently processed and presented both MHC I and II restricted epitopes from expressed transgenic antigens. Animals vaccinated with transduced BMDCs developed CTL activity and were completely protected from tumor cell challenge. Our data suggest that lentiviral vector can effectively introduce antigen genes into DCs without interfering with their antigen presentation function and Th1/Th2 cytokine skewing capability.

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Keratinocytes express nucleotide-binding oligomerization domain (NOD) 1 and 2: implications for cutaneous innate immunity

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Recent evidence indicates that mammalian NOD proteins are intracelluar molecules that function as cytosolic sensors for the recognition and response to intracellular microorganisms. NOD proteins appear to function in a manner similar to Toll-like receptors which are typically located on the cell surface membrane. Previous reports indicate NOD1 is expressed in most adult tissues whereas NOD2 expression is detected principally on certain epithelial cells and myeloid lineage cells. The expression and biological function of NOD in keratinocytes has not been previously reported. In the current study, we examined the expression of NOD1 and NOD2 in normal human keratinocytes and in the murine keratinocyte cell line PAM212. Our results indicated that both NOD1 and NOD2 are constitutively expressed in human keratinocytes and PAM212 cells. We found that keratinocyte NOD1 and NOD2 mRNA expression was augmented by exposure to bacterial derived peptidoglycan (PGN) and this resulted in the induction of the proinflammatory cytokine IL-6. The results of these studies indicate for the first time that in addition to TLR, keratinocytes also express NOD which may function as an intracellular sensor of microbes and microbial derived products. We propose that keratinocyte NOD may be an important component of the cutaneous innate immune response to a variety of infectious agents in the skin.

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IL-7 promotes Th1 cell differentiation

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IL-7 is principally a product of non-lymphoid cells that regulates the expansion of lymphocytes prior to and during antigen receptor gene recombination. It promotes the proliferation of T cells, supports their effector functions, and may also regulate their homeostasis. IL-7 deficient (IL7KO) mice have dramatically reduced but not eliminated populations of lymphocytes (2-4 % of WT) that retain the ability to respond to antigens. Immunization of IL7KO mice with ovalbumin (OVA) in complete Freund's adjuvant (CFA) provokes reduced, but detectable levels of antigen-specific reactive T cells. However, in contrast to wild-type (WT) mice, which mount a response to antigens in the presence of fixed mycobacteria that is dominated by type 1 T helper cells (Th1), the responsive cells in IL7KO mice are primarily type 2 T helper (Th2) cells. Splenocytes from immunized WT mice cultured with antigen produce interferon-γ (IFN-γ) but very little IL-4 while those from immunized IL7KO mice cultured with antigen produce IL-4 and very little IFN-γ. To study the effect of IL-7 on Th1 and Th2 cell differentiation, lymphocytes from Balb/c mice carrying the DO11.10 anti-OVA T cell receptor transgene and a modified IL-4 gene in which green fluorescent protein (GFP) is expressed when the IL-4 gene is transcribed (4get) were introduced into WT and IL7KO Balb/c mice by adoptive transfer. Transferred T cells can be readily identified by flow cytometry using a clonotypic antibody and expression of the IL-4 gene in individual cells is measured as green fluorescence. Few T cells transferred to WT mice that were subsequently immunized with antigen in CFA had detectable GFP while a prominent population of T cells transferred to IL7KO mice expressed GFP. Remarkably, in the absence of immunization, T cells that have circulated in IL7KO mice for one week are predominately predisposed to express GFP in response to non-specific activation while half or fewer of those that circulated in WT mice express GFP. Thus it appears that an IL-7-mediated signal predisposes naive CD4 T cells to differentiate to Th1 cells while the absence of that signal enables them to differentiate to Th2 cells.

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Blockade of skin dendritic cell migration by topical application of MMP- and PKC-inhibitors is associated with inhibition of allergic contact dermatitis *in vivo*

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Epidermal langerhans cells (LC) and skin dendritic cells (DCs) are potent antigen presenting cell (APC) in the induction of primary T cell-mediated immune responses in the skin. They capture foreign antigens (Ags) and migrate to regional lymph nodes (LNs) to carry and present this antigen to naive T cell. This mechanism is critical in the initiation of different cutaneous pathology such as Psoriasis, Contact Dermatitis or Contact Hypersensitivity. Several molecules regulate the migration of skin DC, among which metalloproteinase (MMPs) and protein kinase C (PKC). We have previously shown that topical application of MMP or PKC inhibitors on the skin once a day for 4 consecutive days induced a dose-dependant reduction in the number of FITC+ migrating DC (around 30% of inhibition for the optimal dose), compared to placebo application. The aim of this study was to evaluate the effect of MMPs and PKC inhibitors in a in vivo model of primary ACD (1). Naive mice were sensitised by a single application of DNFB 0.3%. Ear thickness was measured each day after sensitisation until day 10. Topical application of MMP and/or PKC inhibitors on the ear once a day for 3 consecutive days before sensitisation induced a significant reduction of the ear swelling response to hapten. These data suggest that inhibition of PKC/MMP could be of value in the treatment of skin inflammatory diseases which are mediated by DC-T cell interactions.

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Immunology of the human nail apparatus: evidence that the infantile nail matrix is a site of relative immune privilege

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The nail apparatus is prominently exposed to environmental damage and infection, and thus requires a well−functioning immune system. However, our knowledge of nail immunology is still extremely fragmentary, and it is essentially unknown how the nail immune system differs from the skin or hair follicle immune system. Therefore, we have investigated the normal nail immune system of human infants by immunohistology (3 males, 3 months). Surprisingly, HLA−A/B/C expression is prominently downregulated on both keratinocytes and melanocytes of the proximal nail matrix (PNM), compared to other regions of the nail epithelium. The PNM also is HLA−G+, has unusually few CD1a+, CD4+ or CD8+ cells, shows strong immunoreactivity for locally generated immunosuppressants such as TGF−β1, α−MSH and ACTH and for inhibitors of NK cell activity (MIF). Dendritic cells in/around the human nail apparatus show reduced MHC class II expression. This suggests that the nail immune system strikingly differs from the skin immune system, but shows intriguing similarities to the hair follicle immune system, including the establishment of an area of relative immune privilege in the PNM. The latter may explain the notorious chronicity and therapy−resistance of nail infections.

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***Listeria monocytogenes* expressing murine TRP2 induces anti-melanoma protection that is enhanced by topical imiquimod**

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Mechanisms maintaining tolerance to 'self' antigens represent a major obstacle to developing immunotherapies targeting nonmutated, tumor-associated antigens. One potential approach to activate tumor-specific T cells is to use live bacterial vectors that deliver heterologous antigens in a highly immunostimulatory context. We constructed a recombinant strain of *Listeria monocytogenes* (rLM) expressing murine tyrosinase-related-protein 2 (TRP2), a nonmutated, melanocyte-derived differentiation antigen highly expressed in melanomas. We used this rLM-TRP2 strain to immunize mice and then challenged the mice with B16 melanoma cells with or without adjuvant topical imiquimod. Immunization of C57Bl/6 mice with rLM-TRP2 efficiently primed CD8 T cells to recognize the MHC class I-restricted $TRP2_{180-188}$ epitope and express IFN-γ upon *in vitro* peptide stimulation. Peptide-loaded target cells were lysed *in vitro* by TRP2-specific T cells in cytotoxicity assays. As evidence of the *in vivo* functionality of these T cells, mice immunized and boosted with rLM-TRP2 were protected from subcutaneous challenge with B16 melanoma cells. To further explore the protective efficacy of rLM-TRP2, we adapted an optical imaging system to monitor bioluminescent tumor cells growing *in vivo*. In addition to protecting against subcutaneous challenge, rLM-TRP2 vaccination also protected against B16 lung metastases following systemic challenge. Lastly, daily application of the topical immunomodulator imiquimod to subcutaneous tumor sites greatly enhanced protection induced by rLM-TRP2. These results indicate that murine TRP2-expressing rLM can stimulate anti-tumor immunity directed against an endogenously expressed, nonmutated tumor antigen. They also suggest that direct tumoral stimulation with the toll-like receptor 7 (TLR-7) agonist imiquimod may enhance anti-tumor immunity after vaccination against tumor specific antigens.

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Combination of benzo[a]pyrene and ultraviolet A promotes tumor propagation

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We previously reported that benzo[a]pyrene (BaP) and ultraviolet A (UVA) irradiation synergistically increased oxidative DNA damage *in vitro* and tumorigenesis *in vivo*. It has been reported that BaP and UV radiation can both affect humural and cellular immunity in several animal species. The present study has investigated the effect of BaP and UVA on immune surveillance in mice. SKH1 mice were treated with subcarcinogenic dose of BaP (8 nmol/mouse) and UVA (20 KJ/m^2) for 30 weeks. The tumor-free mice were subcutaneously inoculated with 5 x 10^5 of B16 murine melanoma cells. The tumor in BaP plus UVA treated mice showed approximately 4-fold larger than untreated and UVA treated groups. In addition, *in vitro* experiments demonstrated that BaP plus UVA was much more cytotoxic to lymphocyte cell lines than BaP or UVA alone, probably through the apoptotic pathway. The present study suggests that BaP and UVA may synergistically promote tumor cell propagation by inducing immunosuppression and compromising immunosurveillance.

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Identification of novel genes regulated by alpha-melanocyte-stimulating hormone in murine bone marrow-derived dendritic cells

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Many strains of evidence indicate that alpha-melanocyte-stimulating hormone (alpha-MSH) elicits its immunomodulatory activity via binding to melanocortin receptors (MC-Rs) expressed on monocytes and dendritic cells. In order to identify novel target genes regulated by alpha-MSH in these cells, we prepared bone marrow-derived dendritic cell precursors from Balb/c mice and treated them with GM-CSF and IL-4 for 6 days. The MC-R profile on these immature dendritic cells was first determined by quantitative RT-PCR. Both transcripts for MC-1R and MC-5R were detected in these cells. Cells were subsequently stimulated with dinitrobenzene sulfonic acid (DNBS), alpha-MSH or both substances for 2 or 16 hours. After RNA preparation, cDNA synthesis and in vitro transcripton hybridization of biotinylated cRNA samples was performed on MG U74A Affymetrix gene chips. Data evaluation, cleansing, extraction and analysis of the more than 12 000 cloned genes and expressed sequence tags were performed using the Gene Data Analyst vs.1 Expressionist software. Filter criteria included a minimum threshold of 100, normalization by the logarithmic mean and a quality setting of p<0,04. Changes with a change factor of >2 were regarded as significant. As expected, stimulation with DNBS resulted in induction or upregulation of genes encoding proinflammatory cytokines, growth factors, signal transduction intermediates and transcription factors. Treatment with alpha-MSH blocked the DNBS-driven upregulation of several known genes such as IL-1 or CD86. On the other hand, alpha-MSH modulated the expression of several novel genes implicated in immunomodulation, e. g. IL-1beta converting enzyme, IFN-gamma receptor, FK506 binding proteins or several neuropeptides and their receptors. These data indicate novel molecular targets by which alpha-MSH exerts its immunomodulatory activities in immunocompetent cells.

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Reinforcement of innate host defense via induction of anti-microbial peptides

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Anti-microbial peptides are an important weapon of the innate immune response arsenal. The large surface area of the skin provides an easy target for microbial colonization. Keratinocytes monitor the surface of the skin for potential hazards. When a potential pathogen is encountered, keratinocytes release anti-microbial peptides. We investigated a battery of substances for inducing NHEKs to release anti-microbial peptides. Human β-defensin-2 mRNA levels were monitored by real-time RT-PCR. Treatment of NHEK monolayers with proinflammatory cytokines up-regulates hBD-2 mRNA levels after 24hrs. Polysaccharides up-regulate hBD-2 mRNA levels after 48hrs. *Staphyloccocus aureus*, *Pseudomonas aeriginosa* and *Lactobacillus plantarum* stimulated NHEK monolayers to synthesize hBD-2 mRNA after 48hrs. Studies with *Lactobacillus*, an accepted probiotic for gastrointestinal infections, were extended to the skin. Clinical studies investigated whether heat-killed *Lactobacillus* alters several skin parameters. An increase in barrier strength and barrier repair was observed. A reduction in microbial growth was also observed. One mechanism accounting for the improvements in skin quality is a reduction in bacterial colonization resulting from keratinocytes releasing anti-microbial peptides.

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Cathelicidin antimicrobial peptides modulate antigen presentation. Innate immune regulation of the adaptive allergic response

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Antimicrobial peptides (AMPs) are essential to innate immunity through their action as natural antibiotics but can also alter host behaviors such as chemotaxis and cell proliferation. Cathelicidin (cath) AMPs are released in proximity to dendritic cells (DC) in the skin. To study if caths directly influence DC function we treated bone marrow-derived DC with cath peptides for up to 2hr, then removed the peptide prior to stimulation. Cath was applied at physiologic concentrations (<20uM) and was not toxic to DC as determined by LDH release. Cath blocked the response to LPS by inhibiting 100% of cytokine release (IL-6, TNF-a, IL-8, IL-10) but not (GMCSF, IL-4, IL-5, INF-g) and blocked an increase in CD40 expression at the cell surface by at least 75%. Cath at 1uM also inhibited antigen presentation of KLH by epidermal cells to a specific TH1 clone as seen by a 75% decrease in g-INF release. In mice, intradermal injection of cath inhibited sensitization with DNCB as measured by a 50% decrease ear swelling and diminished inflammatory infiltrate. Further support for the ability of cath to suppress antigen presentation was obtained with cath knock-out mice that showed an significant increase (p<0.05) in ear swelling following DNCB sensitization compared to wild-type littermates. These data suggest that the presence of cath inhibits DC maturation and the ability to present antigen. This mechanism suggests that innate immune effectors simultaneously kill pathogens while inhibiting development of an unnecessary allergic response to superficial microbial antigens. Speculatively, the lack of cath expression by patients with atopic dermatitis could partially explain the enhanced inflammation observed in this disease.

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Real-time, multi-photon confocal imaging of epidermal Langerhans cells

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The biology of Langerhans cells (LC) has been studied in fixed tissues or with LC preparations isolated from skin or generated in vitro from their progenitors. These studies, however, have provided no insights into dynamic behaviors of LC in living tissues. By reconstituting the corneal LC with EGFP-transgenic bone marrow cells, we have recently reported 4D images of EGFP+ corneal LC. Because these EGFP bone marrow chimeric mice showed only partial reconstitution of LC networks in the epidermis, we employed in this study the I-A/EGFP knock-in mice in which the endogenous MHC class II molecule is replaced by an EGFP-tagged version (Nature 418:983,2002). Multi-photon confocal microscopy (Leica SP2/Coherent Ti:Sapphire femtosecond pulsed laser) revealed the presence of large numbers of EGFP+ LC within the epidermal compartment (as defined by the lack of collagen-generated second harmonic signals). As has been observed in fixed tissues, LC were found to extend long, branched dendritic processes both laterally and apically up toward the surface in living skin. To study their in situ behaviors, we placed freshly harvested skin samples in a temperature-controlled chamber with circulation of aerated culture media and recorded 3D multi-photon confocal images of EGFP+ LC every 2 min for up to 4 hr. 4D movies were then reconstructed from these data sets. Although no lateral or axial movement of cell bodies was detected, EGFP+ LC exhibited a unique behavior, termed dendrite surveillance extension and retraction cycling habitude (dSEARCH), characterized by rhythmic and repetitive extension and retraction cycles of dendritic processes. All EGFP+ LC observed in three independent experiments showed dSEARCH exercise, primarily in the secondary branched dendrites. Although the physiological function of this behavior remains unknown, it is tempting to speculate that dSEARCH may facilitate two important tasks of LC at the environmental interface, i.e., antigen sampling and detection of microbial and inflammatory signals. This is the first report showing in situ behaviors of epidermal LC in intact skin.

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T cells in patients with Netherton syndrome and atopic dermatitis show a Th-2 bias

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Netherton syndrome (NS) is a rare skin disorder characterised by ichthyosis erythroderma, trichorrhexis invaginata, atopy with universally high IgE level and severe infections. Mutations in *SPINK5* on chromosome 5q32, encoding for LEKTI (Lympho-epithelial Kazal type serine protease inhibitor), are associated with NS. LEKTI is expressed in the skin and thymus. Genetic polymorphisms in *SPINK5* are associated with atopic dermatitis (AD). T-cells in AD were previously shown to have a Th2 bias. In view of the high IgE and atopic features, T-cells in NS were also thought likely to have a Th2 profile. Blood from children of similar age distribution with NS with confirmed SPINK5 mutations (n=6), AD (n=10) and controls (n=14) was stimulated with Phorbol 12-myristate 13-acetate (PMA), Calcium ionophore and Brefeldin A for 5h. T-cells were then stained for CD3, CD4 or CD8, fixed and permeabilized and the intracellular cytokines IL-4, IFN-γ and TNF-α measured by flow-cytometry. Results from NS and AD were compared to controls using the Mann-Whitney non-parametric statistical analysis. CD4 T-cells from NS and AD produced significantly higher IL-4 compared to controls (p=0.03 and p=0.05). CD8 T-cells from NS but not AD also produced higher IFN-γ (p= 0.05). All other cytokines did not show significant differences between patients and controls. Our data firstly confirmed the previously observed Th2 bias in AD (increased CD4 IL4 production). Interestingly, T-cells from NS not only produced more Th2 cytokines when compared to controls but also to AD. This may account for the extremely high IgE level and severe atopy in NS. With the recent localisation of LEKTI to the skin and thymus and its genetic association with AD, our data suggests that LEKTI may have an important role in the pathogenesis of atopic diseases. The significantly higher CD8 IFNγ in NS but not AD may reflect more severe ongoing infections. Our data show that T-cells from both AD and NS have a Th-2 bias.

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Modification of IL-7 to enhance bioavailability

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Interleukin-7 (IL-7) is an essential regulator of lymphocyte development and homeostasis that also enhances certain immune responses. As such IL-7 is being investigated as a therapeutic agent for the management of lymphoid deficiencies and as an immunostimulant for the treatment of cancers and other diseases. However, a major factor limiting the therapeutic development of IL-7 and certain other cytokines is their rapid clearance from circulation. Optimal biological activity in animals requires continuous infusions or frequent injections of large doses of IL-7. Previous studies have demonstrated that the half-lives of various proteins can be greatly increased by attaching them to the constant region of an immunoglobulin molecule (Fc). In an effort to enhance the bioactivity of IL-7 by increasing its half-life, we have created a fusion protein consisting of Fc and IL-7. A cDNA encoding the fusion protein was constitutively expressed at high levels in CHO cells. Fc-IL7 protein produced in this system was purified and characterized in vitro and shown to be comparable with native IL-7. In vivo experiments to compare the bioavailability of the fusion protein with native IL-7 are currently underway.

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Activated ras downregulates Fas expression in mouse melanoma with H-ras^{G12V} transgene

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Malignant melanoma grows and progresses despite the fact that the immune system can recognize melanocyte/melanoma differentiation antigens expressed by melanoma cells. In order to elucidate the mechanisms of immune escape in malignant melanoma, we analyzed host-melanoma interaction using genetically engineered mice with melanocyte-specific H-ras^{G12V} expression on an Ink4a/Arf-deficient background that mimics the gene alterations in human melanoma. Our transgenic mice developed multiple spontaneous melanomas around several months of age despite the fact that tumors expressed all melanocyte differentiation antigens (tyrosinase, TRP-1, TRP-2, MART-1, and gp100). Immunohistochemical study showed significantly reduced Bcl-2 and undetectable levels of Bax and Fas expression in tumor cells. Further analysis of the melanoma cell lines from our transgenic mice showed that activated ras downregulated Fas expression at the transcriptional level and that the downregulation of Fas was reversed by the treatment with FTI-277, a farnesylation inhibitor. The transcript of Fas L was not detected in the melanoma cell lines and was not affected by FTI-277. The effect of FTI-277 on Fas upregulation was not observed in B16/F10 murine melanoma cells, suggesting that the downregulation or loss of Fas expression in B16/F10 melanoma cells was not regulated by farnesylated proteins. These results suggest that activated ras downregulates Fas expression in malignant melanomas with ras mutation, contributing to the tumor escape from the immune system.

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Variable expression of the B7 costimulatory molecule family on normal human keratinocytes

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Interactions between costimulatory molecules and their receptors on T cells play a critical role in growth, differentiation, and death of T cells. During an inflammatory response, optimal T cell activation requires a costimulatory signal beyond antigen-MHC II recognition by the T cell receptor (TCR). B7 signaling is one of the best-characterized T cell co-stimulatory pathways. The B7 family has expanded in recent years and now includes the B7-1, B7-2, B7-H1, B7-DC, B7-H2 (ICOS-L), B7-H3 and B7-H4 family members. These proteins are expressed on professional APCs, as well as on multiple cells not classically thought to have immune functions, such as epidermal keratinocytes. Growing evidence suggests that keratinocytes play a key role in the pathogenesis of inflammatory and T cell-mediated dermatoses. Keratinocytes can produce inflammatory cytokines, and express both MHC II molecules and ICAM-1. But, little is known about the expression of co-stimulatory molecules on keratinocytes. This study investigated the expression of B7 costimulatory molecules on normal human keratinocytes. We found variable transcription of the B7 genes with different stimuli. Following UVB irradiation (20 mJ/cm^2), keratinocytes demonstrated induction of B7-H1 and B7-H2; whereas stimulation with IFN-γ had minor effect on these molecules. Our findings are consistent with the immunosuppressive effects of UVB, and further support the role for B7-H1 and B7-H2 in dampening the T cell immune response. Altered expression of B7 molecules on keratinocytes may suggest the potential for further peripherial immunomodulatory functions of epidermal keratinocytes.

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Novel yeast-based immunotherapeutic vaccines expressing human MART-1 elicit anti-melanoma immunity in mice

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Despite recent advances in molecular and cellular immunology, the development of therapeutic cancer vaccines has proven to be an enormous challenge. Whole recombinant yeast represents a platform as a vehicle with the ability to deliver melanoma antigens to antigen-presenting cells and with immunostimulatory and adjuvant features needed as an immunotherapeutic product to circumvent obstacles seen in previous vaccines. In previous work, we demonstrated that the whole recombinant yeast expressing chicken ovalbumin (OVA) mediated DC maturation (i.e. IL-12 production), efficient priming of MHC class I- and class II- restricted antigen-specific responses, and protection from OVA-expressing tumor challenge. In this study, human MART-1 (melanocyte/melanoma antigen recognized by T cells) was engineered for expression as a tumor antigen in Saccharomyces cervisiae yeast (yeast-based human MART-1-immunotherapeutic, or hMART-IT) and was tested for protective immune responses against mouse MART-1-expressing B16F10 melanoma cells. Immunization of naive C57BL/6 mice with the yeast-based MART-IT triggered protection against challenge with a lethal dose of B16F10 tumor cells implanted subcutaneously (4/5 mice are tumor-free and alive), compared to PBS-immunized animals (1/5 is tumor-free and alive). Cytokine analysis of spleens showed induction of IFN-gamma in some hMART-IT-immunized mice. These results validate that human MART-1 serves as an appropriate antigen for eliciting protective cell-mediated immune responses, even against mouse MART-1 expressing tumor cells. These results indicate that the yeast-based strategy has the potential for providing immunotherapeutic protection against melanoma.

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Langerhans cells are susceptible to apoptosis via Type II signaling pathways in response to antigen-specific T cell interaction

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Antigen presenting cells (APC) susceptibility to apoptotic signals remains controversial. We demonstrate that one APC member, the skin derived Langerhans cell (LC), is exquisitely sensitive to apoptotic stimuli, significantly, in the presence of antigen-specific T cells. Two models of activation-induced apoptosis exists utilizing different pathways of caspase activation, revealing Type I (extrinsic pathway signaling via caspase 8) or Type II cell responses (requiring both extrinsic and intrinsic pathways, via caspases 8, 9 & Bid). The nature of the caspase activation pathways used in LC was examined. We determined that primary LC as well as XS106 cells are highly susceptible to apoptosis by Fas cross-linking or by antigen-specific interaction with T cells. Apoptosis occurred when XS106 were cultured for 20 hours in the presence of the model antigen hen egg lysozyme (HEL) and HEL-specific 3A9 T cells. To test the role of caspase activation in regulating LC death, caspase-specific peptide inhibitors were utilized. XS106-GFP clones exhibited resistance to antigen-specific T cell-mediated apoptosis in the presence of inhibitor peptides for caspase-3, caspase-8 or caspase-9, each resulting in 60%-80% inhibition. Western blot analysis demonstrated that Bid protein levels were elevated (2-fold) in XS106 cells as compared to T cell lines or splenocytes and was cleaved in response to apoptotic stimuli. These data indicate that LC utilizes the Type II apoptosis signaling pathway.

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Mechanisms of immune evasion in malignant melanoma: reduced expression of E-selectin and ICAM-1 in melanoma vasculature

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Despite aggressive therapy, survival of patients with metastatic malignant melanoma remains poor. Both the presence of tumor infiltrating T cells and the observation of occasional striking tumor regression suggest that host immune responses may influence the course; however, attempts at exploiting these responses via vaccination have yielded limited success. T cell recruitment and emigration into tissues is mediated by the interaction of leukocyte homing receptors with ligands expressed on tissue endothelia. In this study we sought to establish whether dysregulation of leukocyte adhesion to melanoma vasculature may be responsible, in part, for the ineffective immune response to this tumor. Tissue (n=34) and blood (n=14) specimens were obtained from patients undergoing surgery for malignant melanoma. Control tissue and blood specimens were obtained from normal donors. Immunochemical staining of melanoma metastases showed little or no E-selectin expression on CD31-positive vessels within the tumor mass, despite strong E-selectin expression on vessels within adjacent tissue. Melanoma metastases showed diffuse ICAM-1 staining; however, no vascular staining of ICAM-1 was observed within the tumors. Flow cytometry of peripheral blood T cells, in contrast, showed no significant shift in the percentage of skin-homing phenotype T cells or in the naive/memory T cell distribution of patients compared with normal controls. Interestingly, tumor infiltrating T cells expressed surface receptor phenotypes similar to T cells isolated from comparable normal tissues (skin, lymph node, tonsil), despite the lack of counter-ligands on the local endothelia, suggesting these cells may have been recruited in adjacent tissue sites and migrated into the tumors or that alternate recruitment pathways exist. We hypothesize that the lack of E-selectin and ICAM-1 expression on tumor vasculature may reflect a block to tissue specific leukocyte homing. Intervention to increase expression of endothelial homing components could augment existing tumor vaccination strategies.

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The transcriptome of psoriatic epidermis is suggestive of an innate rather than an acquired immune response

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Studies comparing non-lesional and lesional psoriatic epidermis using oligonucleotide microarrays have defined a consistent transcriptome profile of established psoriatic plaques. However, the contribution of various cytokines known to be implicated in psoriasis to this transcriptome remains to be established. To expand these studies, we were interested to explore the relative contribution of cytokines implicated in the innate (IL-1α) and acquired (IFN-γ) immune responses in the development of psoriatic plaques by in vitro modeling utilizing cytokine-stimulated keratinocytes. Second passage human keratinocytes derived from unrelated, non-psoriatic adult donors were stimulated with either IL-1α (100 ng/ml) or IFN-γ (20 ng/ml) for 24 hours prior to isolation of total RNA and preparation of biotinylated cRNA (n=3). Samples were subsequently hybridized to Affymetrix U133A human microarrays (containing oligonucleotides representing approximately 22,000 transcripts), scanned and analyzed. Consistent strong induction (>5 fold) of a number of chemokines (e.g. CXCL9, CXCL10, CXCL11 and fractalkine) and class II MHC genes (e.g. DRα, DRβ5 and DPα1) was observed following stimulation with IFN-γ, a product of activated T-helper cells and associated with acquired immune responses. By contrast, the keratinocyte transcriptome resulting from IL-1α stimulation demonstrated induction of a distinct transcript set including IL-8, S100 proteins (most notably S100 A7, psoriasin), the proteinase inhibitors skin-derived antileukoproteinase (SKALP) and PI13, and β2 defensin. Thus the keratinocyte IL-1 transcriptome was markedly different from that associated with IFN-γ stimulation and closely mirrored that previously identified in samples derived from psoriatic lesional skin. These findings demonstrate a marked difference in the transcriptome of keratinocytes stimulated with two functionally distinct cytokines and suggest that the inflammatory milieu in psoriatic epidermis is more dependent upon an innate rather than an acquired type immune response.

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Chemokine MRNA expression in acne vulgaris

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Chemokines play important roles in the different activation, effector function, migration and localisation of cell types that mediate inflammatory reactions and immune responses. However, despite this, no study has previously been undertaken to investigate comprehensively the expression of chemokines in acne vulgaris. Recent immunohistochemical studies have indicated that inflammatory acne is a $CD4^+$ lymphocyte/macrophage led response. We have investigated the expression of chemokine mRNA in acne skin lesions using a highly sensitive and specific multi-probe ribonuclease protection assay (RPA). Skin biopsies of early inflamed papular lesions from 8 acne patients were examined for the expression of the chemokines; interferon-inducible protein-10 (IP-10), macrophage inflammatory protein-1 alpha and 1 beta (MIP-1α and MIP-1β) regulated on activation, normal T-cell expressed and secreted (RANTES), Lymphotactin (Ltn), Interleukin (IL)-8, I-309 and monocyte chemotacic protein (MCP-1) and compared to normal skin from 12 normal donors. Briefly, a mixture of [^{32}P] UTP—labeled antisense riboprobes were generated from a cDNA template set. Tissue RNA was hybridized with ^{32}P antisense riboprobe and after purification, the resulting protected probes were visualized following denaturing gel electrophoresis by autoradiography. The mRNA level of each chemokine was represented as a percentage of GAPDH. Levels of MIP-1B, MCP-1, IL-8 and I-309 ($P < 0.01$) and to a lesser extent RANTES, IP-10 and MIP-1α ($P < 0.05$) were elevated significantly in acne skin compared to normal skin, but there was no significant difference in the expression of mRNA for Ltn. IL-8 was the most dominant chemokine expressed in acne skin. These data suggest that the above chemokines play an important part in the immunopathogenesis of acne vulgaris and may account for the large $CD4^+$/macrophage presence in early inflamed acne lesions.

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E-selectin ligand up-regulation on CD8+ T cells encountering antigen in skin-draining lymph nodes

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The aim of this study was to test the hypothesis that T cells activated by antigen in lymph nodes draining skin are induced to express homing molecules that influence subsequent trafficking to skin. We analyzed the induction of skin-homing molecules on memory CD8+ T cells in an in vivo model of cutaneous autoimmunity. In this model, CFSE-loaded naive CD8+ T cells specific for ovalbumin (OVA) derived from OT-1 transgenic mice were transferred to mice expressing a membrane-bound form of OVA under the control of the keratin-14 promoter (K14mOVA). Transferred T cells distributed rapidly to secondary lymphoid organs and proliferated within the skin-draining lymph nodes (SDLNs). Proliferating T cells adopted an activated/memory phenotype, upregulating CD69, CD44, and CD43 levels, while down-regulating L-selectin expression. Surprisingly, proliferating OT-1 CD8+ T cells were also observed in non-skin draining lymph nodes and spleens in K14mOVA mice. However, levels of E-selectin ligand activity (a murine surrogate of CLA expression) appeared earlier and were significantly higher on OT-1 cells in SDLNs than on OT-1 T cells in noncutaneous secondary lymphoid organs. When antigen expression was limited to small grafts of K14mOVA skin placed on the flanks of WT mice, E-selectin ligand activity increased progressively as a function of division in the ipsilateral lymph node draining that skin region and not in the contralateral nodes or those draining other tissues. Our data demonstrate that while CD8+ T cells are induced to express E-selectin ligand activity in SDLNs, measurable levels of E selectin ligand can be generated in noncutaneous secondary lymphoid organs, suggesting a significant degree of overlap of homing capacity exists between T cells encountering antigen in anatomically distinct regions.

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Decreased TRECS in cutaneous T cell lymphoma

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We recently reported that there is a dramatic reduction in the complexity of the T cell repertoire in patients with all stages of CTCL. However, absolute numbers of T cells generally remain normal. Because of this, we hypothesized that nonmalignant T cells proliferate to fill the empty space created by this depletion of T cells. We here test this hypothesis by examining T cell receptor excision circles (TRECs). TRECs are DNA episomes which are formed during recombination of the T cell receptor gene complex in the thymus, and do not replicate during T cell division. The number of TRECs in a population of T cells is therefore an index of how many naive T cells are present. We reasoned that TRECs should be lower in peripheral blood of CTCL patients, as they would be diluted out as surviving T cells proliferate and expand. We examined TRECs from purified peripheral blood T cells of 62 patients with CTCL. They included patients with Stage 1 (n=37), Stage II (n=6), Stage III (n=12), and Stage IV (n=7) disease. In control experiments, TRECs decreased with PHA expansion in cell culture and also decreased with age, as expected. We found a significant decrease in TRECs with increasing stage of CTCL for stages I, III, and IV ($P<0.05$), and furthermore found a significant correlation between decreased TRECs and diminished complexity of the T cell repertoire, as judged by CDR3 spectratyping ($P<0.0001$). In patients with Sezary syndrome, when the dominant clone cells were excluded from the analysis, the remaining T cells also had significantly fewer TRECs than controls ($p<0.05$). This decrease in TRECs in CTCL is consistent with our hypothesis that normal T cells that are not deleted in CTCL expand to fill the space created by the diminished T cell repertoire.

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Characterization and evaluation of CD34+ generated Langerhans cells for tissue engineering

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Specialized antigen-presenting cells, particularly Langerhans cells (LC), residing in the skin, mucosa, and lymphoid tissues play a key role in sensitization and other immunological reactions of the body. However, difficulty in harvesting, short survival time in culture, and variability in cytokine production of cultured LC has prevented researchers from widespread use of LC for allergenicity and immunogenicity studies. Likewise, these problems have hampered the development of in vitro tissue models containing LC. Recently, we have developed a new culture method to generate large numbers of LC from umbilical cord blood (UCB). UCB derived CD34+ progenitor cells are expanded to produce up to 200 million LC per 60 ml UCB sample. The generated LC express CD1a, HLA-DR, CD209 (DC SIGN), and CD206 (mannose receptor), all characteristic of LC. Transmission electron microscopy shows the presence of Birbeck granules, a key ultrastructural marker of LC. The lifespan of these cells has been extended to 41 days with very little change in surface marker expression or phenotype. Upon stimulation with lipopolysaccharide (LPS) and phorbol-12-myristate-13-acetate (PMA), the LC show a reproducible, high level release of IL-12, MIP-1α, MIP-3α, IL-6, RANTES, and IL-6. In addition, recent experiments demonstrate that these cells can be incorporated into MatTek's reconstructed skin model, EpiDerm™, and its ectocervical-vaginal tissue model, EpiVaginal™. LC within these tissue constructs are detected by immunostaining for HLA DR antigens. These new tissue models and the isolated LC alone will likely be useful in a variety of studies related to: 1) allergenicity, 2) microbial infection, 3) neutralizing antibodies, 4) antigen presentation, and 5) immuno-therapy, amongst others.

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Mouse beta defensin-3 is strongly induced in a mouse model with a skin barrier defect

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We have demonstrated that a defect in skin barrier function resulting from the loss of loricrin, a major cornified envelope (CE) protein, induces a compensatory response that activates the expression of known and novel CE components. A recent analysis of cDNA microarray data has shown that beta defensin-3 expression is also dramatically increased in the epidermis of newborn loricrin null mice. Defensins are major antimicrobial peptides, synthesized by epidermal keratinocytes, that are important components of the innate host response to microbial infections. To determine whether beta defensin-3 induction is a general response to skin barrier defects or specific to loricrin null mice, we tested a second mutant mouse line with a skin barrier defect; RAR 403 transgenic mice express a dominant-negative RAR receptor and show a severe skin barrier defect at birth due to defects in the lipid component of the CE. Surprisingly, we did not detect up-regulation of beta defensin-3 in these mice, suggesting that this response is specific to loricrin null mice. Interestingly, we have previously demonstrated that the compensatory response which re-establishes skin barrier function of loricrin null mice begins in utero. The up-regulation of beta defensin-3 was only observed in the epidermis of newborn loricrin mutants, not in utero. It has been shown that beta defensin-3 is regulated by the NF kappa B pathway. Factors that activate NF-kappa B include TNF, IL-1 and Toll-like receptors. Current studies are directed toward identifying the signaling pathway responsible for induction of beta defensin-3 in loricrin null epidermis.

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CCR7+ central memory T cells significantly express organ-specific coding molecules including cutaneous lymphocyte antigen

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Central memory T cells are defined as CD45RO+ memory T cells expressing CD62L, CCR7, and LFA-1, allowing them to access lymph nodes from blood through high endothelial venules. It was originally proposed that these cells lacked peripheral non-lymphoid tissue homing molecules. In the present study, we investigated expression of skin- and gut-homing receptors on highly purified peripheral blood central memory T cells by flow cytometry. CCR7+ T cells were further isolated by magnetic cell sorting. These cells were stained with specific antibodies to CD45RO, CD45RA, cutaneous lymphocyte antigen (CLA), chemokine receptors including CCR4, andβ7 integrin as well as other T cell-related markers such as CD4, CD8, and CD25. Purity of central memory T cells was more than 97%, and they co-expressed L -selectin/CD62L. Central memory T cells purified this way were almost exclusively CD4+ T cells (>95%), and 48% (range 39-63%) co-expressed CD25. This result suggests that a significant fraction of the pool of central memory T cells are in fact CD4/CD25+ T cells, a population recently associated with immunoregulation. We further showed clear cut expression of peripheral tissue trafficking molecules on central memory T cells. We found that 47% (range 39- 56) of the central memory T cells expressed CLA, 75% (range 64-83) expressed CCR4, and 30% (range 19-40) expressed β7 integrin. Our results indicate that many cells with the phenotype of regulatory T cells reside within the central memory population, and that, contrary to current dogma, these cells have the requisite cell surface molecules to enter skin and GI tract from blood. A model where these cells mediate tolerance to peripheral tissues is consistent with these findings.

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Major histocompatibility class I processing of recombinant yeast antigens by dendritic cells

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We have previously demonstrated the vaccine potential of recombinant yeast in mediating MHC class I-resricted CD8+ cytotoxic T lymphocyte (CTL) responses. DCs may process exogenous antigen for MHC class I presentation via a transporter associated with antigen processing (TAP)-dependent cytosolic pathway, or a vesicular TAP-independent pathway. Since differences in these pathways might qualitatively and quantitatively effect the subsequent CTL response, we sought to determine the relative use made by DCs of each pathway in the MHC class I processing of recombinant yeast-derived antigens, and the effect of processing via each pathway on priming naive, versus activation of memory CD8+ T cells. Naive T cell priming was investigated using naive MHC class I-restricted antigen-specific T cells obtained from OT-I transgenic mice, whereas an antigen-specific MHC class I-restricted T cell hybridoma served as a surrogate memory T cell. T cells were co-cultured with recombinant yeast-pulsed DCs from normal or TAP-deficient mice in the presence or absence of various inhibitors of either the cytosolic or vesicular pathways. The relative ability to stimulate T cell activity was measured as a function of T cell proliferation (naive T cell priming),or IFN-γ secretion memory T cell activation). We show that DCs employ both the cytosolic TAP-dependent, and vesicular TAP-independent pathways in processing yeast-derived recombinant Ag for presentation via the MHC class I pathway, although in both cases the cytosolic TAP-dependent route is dominant. However, since the kinetics of MHC class I processing via the TAP-independent vesicular pathway is considerably faster than the cytosolic TAP-dependent pathway, and occurs prior to DC maturation and co-stimulatory potential, we hypothesize that Ag processing via the vesicular TAP-independent route might represent a conserved mechanism for rapid activation of memory T cells that are less dependent on co-stimulation than naive T cells.

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CXCR6 and its ligand are constitutively expressed in skin

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Chemokine signaling is an essential component of T cell homing to tissues. BONZO/CXCR6 expressing lymphocytes have been currently identified as a subset of T cells specialized in homing to inflamed tissues, such as rheumatoid joint and inflamed liver. However, the expression of CXCR6 and its ligand CXCL16 in the skin has not been characterized. In this study we analyzed the expression of BONZO/CXCR6 on T cells in the peripheral blood and normal uninflamed skin. Our data showed that in the peripheral blood only a very small subset (3%) of T cells expressed Bonzo, and 19% of this small CXCR6+ T cell subset expressed CLA. In contrast, approximately 50% of T cells in the skin were CXCR6 positive, and more than half of these Bonzo/CXCR6+ T cell expressed high levels of CLA. By immunohistochemistry, normal skin showed abundant expression of CXCL16, its chemokine ligand, by epidermal cells and endothelial cells. These data suggest that the role of this chemokine receptor-ligand pair is not restricted to inflamed tissue. Whether CXCR6/CXCL16 interactions play a role in constitutive T cell trafficking to the skin and normal immune surveillance remains to be determined, as does its expression in various inflammatory skin diseases.

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Ultraviolet B irradiation impairs immune response to epicutaneous protein immunization via antigen-specific CD4 /CD25 suppressor T cells

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In a murine model, repeated cutaneous exposure to low doses of ultraviolet B impairs the induction of contact hypersensitivity response to haptens and induces hapten-specific suppressor T lymphocytes. This study investigates whether exposure to UVB can impair the T cell response to peptide or protein antigen. Using an adoptive transfer model, we have determined that UVB induces suppression of antigen-specific CD8 T cell proliferation and IFNg production in response to OVA protein immunization in C57BL/6 mice. Epidermal sheet staining of mouse skin after UV irradiation shows depletion of IA Langerhans cells in the basal epidermis. Nevertheless, UVB failed to induce suppression of CD8 T cell priming in mice immunized only with the immunodominant MHC I restricted OVA peptide suggesting that priming of CD8 T cells in the lymph nodes was unaffected by UVB in the absence of antigen-related CD4 T cell epitopes. Suppression of protein antigen-specific CD8 T cell priming was mediated by CD4 T cells since transfer of CD4 T cells from UVB irradiated and OVA-immunized mice can induce the suppression of response to OVA immunization in a non-irradiated mouse. Functional analysis of CD4 T cell subsets using adoptive transfer reveals that the suppressor effect of CD4 /CD25 T cells is dominant and depletion of this subset, abrogates suppression. Conversely, adoptive transfer of this subset mediates suppression. We suggest that UVB promotes the induction of antigen-specific CD4 /CD25 suppressor T cells. These in turn inhibit protein antigen-specific CD8 T cell priming. The ability to generate antigen-specific suppression using UVB to limit CD8 T cell responses may be of therapeutic value to prevent CD8 T cell mediated autoimmune disease.

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Itraconazole suppresses an elicitation phase of a contact hypersensitivity reaction

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Contact dermatitis is caused by epicutaneous exposure to environmemtally and/or industrially-derived allergens. Since the exposure is unavoidable for many instances, therepeutic suppression of allergic inflammation appears to be of clinical relvance. Recently, it is reported that itraconazole, an antifungal agent, may be of therapeutic impact on allergic skin diseases. Therefore, we were interested to study the effect of itracinazole on contact hypersensitivity. Mice(C3H/HeN or Balb/c) were administered with itraconazole orally before sensitization or challenge with haptens (dinitrofluorobenzene or oxazolon). Consequently, the administration of itraconazole before challenge but not before sensitization, significantly suppressed the reaction. Intriguingly, itraconazole failed to suppress the irritant dermatitis induced by croton oil or benzalkonium chloride, suggesting that it may affect molecule(s) rather selectively involved in the elicitation of contact hypersensitivity. To further analyze mechanisms involved, splenic T cells obtained from sensitized or naive mice were stimulated with plate-bound anti-CD3 in the presence or the absence of itraconozale and release of cytokines was tested by ELISA assays. T cells from hapten-immunized mice produced a significant amount of IFN-γ, which was merkedly suppressed by itraconazole. Together, the present study demonstrates that itraconazole rather selectively suppresses the elicitation phase of contact hypersensitivity possibly via down-modulation of IFN-γ.

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Intradermal administration of a purinergic agonist augments the induction of immunity in mice

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ATP signals through cell surface receptors that are either ionotrophic (P2X) or G-protein linked (P2Y) and has been shown to modulate aspects of dendritic cell function. We have recently shown that the murine Langerhans cell (LC)-like cell line XS106 responds to purinergic agonists with increased expression of I-A, CD80 and C86. Also, secretion of IL-1β and IL-12 stimulated by GM-CSF/LPS was augmented by a purinergic agonist while IL-10 secretion was decreased. Additionally, the hydrolysis-resistant ATP analogue ATPγS significantly augmented the ability of BALB/c (H-2^d) epidermal cells to present antigen (KLH) to a responsive T cell clone [HDK-1 (H-2^d)]. We have now examined the ability of ATPγS to enhance vaccination of mice against tumor antigens and bacteria. CAF_1 mice (H-2$^{a/d}$) were injected intradermally with a soluble extract of the S1509a spindle cell tumor (H-2^a) (TAA) mixed with 700 nanomoles of ATPγS or medium alone 3 times at 7-day intervals. One week after the last immunization a hind footpad of each mouse was injected with TAA and 24-hour swelling assessed as a measure of delayed-type hypersensitivity (DTH). ATPγS significantly increased the DTH response. BALB/c mice were injected intradermally with 10^8 killed (freeze-thaw x 4) group A streptococcus (GAS) mixed with 700 nanomoles of ATPγS or medium alone 3 times at 7-day intervals. One week after the last immunization each mouse was challenged on the dorsum with live GAS and lesion size scored over time. In preliminary experiments GAS alone led to the development of larger lesions while immunization with ATPγS resulted in greatly decreased lesion size. Purinergic agonists hold great promise as a new class of immunologic adjuvants.

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Langerhans cells apoptosis mediated by CD4 T cells requires antigen specific cell contact

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Antigen presenting cells (APC) susceptibility to apoptotic signals remains controversial. We demonstrate that one APC member, the skin derived Langerhans cell (LC), is exquisitely sensitive to apoptotic stimuli, significantly, in the presence of antigen-specific T cells. Apoptosis, detected by annexin V and 7-AAD staining, occurred when murine LC or the LC line XS106 cells were cultured for 20 hours in the presence of the model antigen hen egg lysozyme (HEL) and HEL-specific 3A9 T cell hybridoma lines but not other T cell lines. Apoptosis of HEL-pulsed LC was also observed in the presence of naive CD4 T cells from 3A9 TCR transgenic mice and occurred concomitant with T cell activation, as determined by blast formation, CD69 expression and IL-2 production. Apoptosis of LC was preceded by LC activation as detected by increased surface staining for I-A, CD40 and CD69. To test the requirement of LC-T cell interaction in mediating LC apoptosis, we cultured cells in the presence of blocking antibodies to CD18 (LFA-1) and MHC-II. XS106-GFP clones exhibited resistance to antigen-specific T cell-mediated apoptosis in the presence of inhibitor antibodies to LFA-1 and MCH II, resulting in 60%-90% inhibition. Significant levels of inhibition was also observed after DC pre-treatment with blocking antibodies, however anti-I-A antibody pretreatment was not sufficient to block apoptosis of LC by 3A9 T cells when cultured with LPS. To test the role of soluble or membrane bound cytotoxic factors in mediating T cell-dependent LC apoptosis, specific antagonists for Fas or Trail were added during LC-T cell culture but had no effect. These data suggest that LC-T cell contact and antigen-specific interaction is necessary and sufficient for inducing LC maturation and subsequent LC apoptosis.

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Activation of liver X receptors inhibits the production of inflammatory cytokines by monocytes in response to *Propionibacterium acnes*

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Propionibacterium acnes contributes to the pathogenesis of acne vulgaris, in part, by inducing monocytes/macrophages to secrete proinflammatory cytokines. Previous studies have demonstrated that P. acnes induces monocyte activation through NF-KB and that P. acnes induction of monocyte cytokine production occurs through Toll-like receptor (TLR)2. Several therapeutic agents used in acne are thought to act through anti-inflammatory mechanisms. Liver X receptors (LXRs) are nuclear receptors activated by oxysterols, and their ligation has been shown to decrease the production of NF-KB-induced proinflammatory cytokines in macrophages. Agonists of LXR have been shown to effective in decreasing inflammation in murine models of contact dermatitis. We undertook studies to determine whether activation of LXR diminishes inflammatory cytokine production induced by P. acnes. Adherent human peripheral blood mononuclear cells cultured in the presence of P. acnes produced decreased amounts of proinflammatory cytokines (e.g. IL-12) following pre-treatment with the LXR agonist GW3965. These findings suggest a potential novel class of therapeutic agents for acne.

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Targeting Notch in Kaposi's sarcoma inhibits tumorigenesis

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Kaposi's sarcoma (KS) is a common neoplasm in HIV-infected individuals causing significant morbidity and mortality. The etiologic agent in this tumor is KSHV (KS-associated herpesvirus); however, it is currently unknown how KSHV causes KS. Notch proteins are a family of evolutionarily conserved transmembrane receptors that play a fundamental role in cell fate decisions including proliferation, differentiation and apoptosis. It is, therefore, not surprising that Notch proteins have been implicated in tumorigenesis and appear to function as either oncogenes or tumor suppressors. In this report, we demonstrate elevated expression of activated Notch-1, -2 and -4 in KS cells *in vivo* and/or *in vitro* compared to normal endothelial cells (ECs), the precursor of KS cells. Notch activation was confirmed through detection of Hes-1 (a primary target of the Notch pathway) in nuclei of KS cells *in vivo*. KSHV infection may be partially responsible for altered Notch expression in KS as expression of the KSHV-encoded latency associated nuclear antigen (LANA) in ECs resulted in a 3-fold increase in Notch-1 and -2 protein, but had no effect on Notch-4. To determine if Notch activation was critical to KS cell proliferation, various immortalized KS cells were treated in culture with GSI-I (gamma-secretase inhibitor-I), a small molecule inhibitor blocking Notch activation. GSI-I resulted in either G_2/M growth arrest or apoptosis depending on the KS cell line. Similar studies injecting GSI-I into tumors composed of immortalized KS cells established on nude mice (n=7) demonstrated a 58.7% reduction in tumor size (average: 2.2±0.4 cm^3 reduced to 0.9±0.2 cm^3; p=0.02). This growth inhibition was characterized by the induction of apoptosis (detection of activated caspase 3 and TUNEL positive cells) in treated, but not control tumors. We conclude: a) KS cells overexpress activated Notch compared to ECs, b) KSHV-LANA can enhance Notch expression, and c) interrupting Notch signaling inhibits KS cell growth *in vitro* and *in vivo*. Thus, targeting Notch signaling may be of therapeutic value in KS patients.

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Construction of full-length cDNA differential display libraries for genital warts based on long template PCR and full-length cDNA suppression subtractive hybridization

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FL-cDNA-SSH was performed between genital warts and normal skin. In the forward subtraction, genital warts was used as tester and normal skin as driver for genes expressed highly or specifically in genital warts lesions, whereas in the reverse subtraction, genital warts was the driver and normal skin the tester for normal genes of high suppression because of HPV infection. The total RNA was extracted and first-strand cDNA was synthesized directly from 10 µg total RNA. And the second-strand cDNA was generated with Klenow fragment and T4 DNA ligase. The dscDNA was ligated to the linker which included site digested EcoR I. Two tester populations were created with different adaptors, but no adaptors with driver; the adaptors were used for subsequent PCR amplification. The first hybridization was carried out with each tester population and the driver. After this first hybridization, the two samples were combined and a fresh portion of heat-denatured driver was added. The second hybridization generates templates for PCR amplification from differentially expressed sequences between tester and driver. The products of secondary PCR were digested by EcoR I and Sal I. This process produced DNA with 5'- EcoR I sticky end and 3'- Sal I sticky end, which was directionally ligated with the EcoR I- Xho I arms of λ ZAP II vector followed by DNA packaging and infecting host bacterial strain XL1-Blue MRF. According to plate plaques blue/clear selection in presence of IPTG and X-gal, the percentage of recombinant plaques in both forward and reverse differential display libraries of genital warts is 100%, while the cloning efficiency is high too: 8.1×10^7 and 1.28×10^8, respectively. We used primer set of λ ZAP II 01 and λ ZAP II 02 on λ ZAP II arm several bases outer the insert position by PCR. About 20 positive plaques which were selected randomly have PCR products, varying from 1200bp to 7000bp.

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Cloning and expression of E6 and E7 gene of human papilloma virus type II in *E. coli*

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Human papilloma virus (HPV) has been implicated in various types of human skin disorders. Emerging evidence indicates that infection with HPV is a necessary risk factor for the development of cervical cancer. However, the molecular mechanisms through which HPV induces skin disorders or cancer remain to be elucidated. This project was designed to clone and express the genes encoding E6 and E7 protein of human papilloma virus type II (HPV II). The gene encoding for E6 and E7 of HPV II was amplified by PCR from condyloma accuminatun samples, cloned into vector PGEX-6P-1, pET-32a respectfully to form recombinant plasmid PGEX-6P-1/E7, pET-32a/E6, and then two recombinant plasmids were transfected into E. coli BL21. The glutathione S-transferase-E7 (GST-E7) and thioredoxin-E6 (Trx-E6) fusion protein was expressed when induced by IPTG and purified with Glutathione Sepharose 4B affinity column and 3S NTA resin affinity column. SDS-PAGE and Western blotting were used to detect the expressed protein. The recombinant plasmid was identified and confirmed with enzyme digestion and sequencing. A high level expression of GST-E7, Trx-E6 fusion protein was obtained and purified successfully. The results from SDS-PAGE and Western Blotting analysis suggest that molecular weights of E6 and E7 are 18 and 11 kDa respectively. We conclude that E6, E7 protein of HPV II could be expressed with high efficiency in prokaryotic expression system and could be applied for characterization of HPV II and are useful for further understanding of the mechanism of induction of skin disorders and cancer.

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***Malassezia* laccase melanization, a novel mechanism for seborrhoeic dermatitis pathogenesis.**

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Involvement of *Malassezia* yeasts in the pathogenesis of seborrhoeic dermatitis is well established but no melanin associated mechanism has ever been proposed before. The purpose of the study was to investigate the existence of laccase controlled melanization in *Malassezia* species by conventional and molecular methods. Pigment production by *Malassezia* yeasts was assessed in 67 strains of *M. furfur, M. sympodialis, M. slooffiae, M. globosa, M. restricta, M. dermatis, M. obtusa* and *M. pachydermatis* in lipid supplemented Staib agar with or without KH_2PO_4 and creatine and L-DOPA agar at 28°C, 32°C and 35°C. PCR amplification of sequences coding for laccase was performed with *M. sympodialis* CBS 8741 reference strain DNA. The PCR amplification product was sequenced, aligned and compared with laccase gene from the phylogenetically close *Filobasidiella (Cryptococcus) neoformans*. L-DOPA agar was the most suitable medium detecting melanization at 35°C. Different melanization rates were detected in all *Malassezia* species, with *M. furfur* colonies producing less pigment while *M. dermatis* colonies produced the most prominent brown color effect after 6 days incubation at 35°C. Melanization is a well studied virulence factor in *F. neoformans*. The close homology of the amplified gene sequences with those of *F. neoformans* denotes similar virulence and pathogenetic laccase-associated mechanisms. Triggering of the immune response in seborrhoeic dermatitis by *Malassezia* yeasts is well established although the exact pathogenetic mechanism has not been defined. *Malassezia* melanization is a candidate mechanism through the ability of fungal melanins to activate complement and to assist immune system evasion.

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Analysis of the prevalence and genotyping of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks demonstrate for the first time the presence of B. valaisiana in Austria

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(i) To determine the prevalence of Borrelia burgdorferi (Bb) sensu lato, the causative agent of Lyme borreliosis (LB), in Ixodes ricinus tick vectors in an endemic area in Austria. (ii) To identify the Bb genotypes present in those ticks. 518 ticks were collected in the summers of 2002 and 2003 by the flagging method in a woodland recreation area of the city of Graz, Austria. All ticks were placed into humidified tubes and stored at 4dgrC until separate DNA extraction for each tick with a commercial preparation kit (QIAamp DNA mini Kit; Qiagen, Hilden, GER). Extracted DNA was analyzed using a two-step semi-nested polymerase chain reaction (PCR) to detect infection of ticks. Primers were designed to amplify an 818bp fragment of the Bb ospA gene. PCR was followed by restriction fragment length polymorphism (RFLP) analysis with 5 endonucleases (SfuI, SspI, BglII, HindIII, Roche, Penzberg, GER; Kpn2I, Fermentas, St. Leon-Rot, GER) for genotype identification of positive samples. 133/518 (26%) ticks were found to be positive for Bb, 65 (49%) of which were nymphs and 68 (51%) were adults. RFLP analysis revealed 96 (72%) ticks to be infected with the genotype B.afzelii, 20 (15%) with Bb sensu stricto (ss), 8 (6%) with B.garinii, and 3 (2%) with B.valaisiana. 6 ticks (5%) were coinfected with B.afzelii and Bbss (5) or B.valaisiana (1), respectively. We conclude that (i) a high percentage of ticks is infected with Bb, indicating a considerable risk to contract LB after a tick bite in the study area. (ii) Tick nymphs may transmit Bb, which is of practical importance as they are hardly visible by the naked eye. (iii) B.afzelii, the main pathogen of dermatoborrelioses, is the most prevalent genotype in ticks, consistent with the predominance of dermatological manifestations of LB in Europe. (iv) B.valaisiana, which may have the potential to cause LB, was detected for the first time in Austria in this study.

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Studies on skin vaccination via proteomic profiling of murine skin

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We have established a reference proteome map of murine skin allowing the resolution of greater than five hundred protein spots in a single two-dimensional polyacrylamide gel. Forty-four protein spots, corresponding to 28 different cutaneous proteins, were identified using MALDI-TOF MS and the Mascot online database searching algorithm. Five molecular chaperones including ER60, GRP78, HSP60, HSP70, and HSP27 were also identified. Of these, HSP27 expression was confined mainly to the epidermis. We have demonstrated that HSP27 in mice skin was up-regulated and phosphorylatd after epicutaneous application of an adenovirus vector encoding the tetanus toxin C fragment, suggesting that HSP27 may be involved in the adenovirus-mediated skin vaccination.

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Mosquito salivary gland extracts induce EBV-infected NK cell oncogenesis via CD4 T cells in patients with hypersensitivity to mosquito bite

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Severe hypersensitivity to mosquito bites (HMB) is characterized by intense local skin reactions and systemic symptoms such as high fever, lymphadenopathy, and hepatosplenomegaly. The patients with HMB often have NK cell lymphocytosis associated with Epstein-Barr virus (EBV) infection. We previously demonstrated that CD4 T cells of the patients proliferated well in response to mosquito salivary gland extracts and played a key role in EBV reactivation from latent state in NK cells. Here we investigated whether mosquito antigen-stimulated CD4 T cells have any influence on oncogenesis of EBV-infected NK cells in the patients. Purified CD4 T and NK cells of HMB patients were cocultured in the presence of mosquito salivary gland extracts (4ug/ml), and the expression of viral oncogenes (LMP1, EBNA2) was sequentially assessed by RT-PCR and immunostaining. The expression of LMP1 mRNA remarkably increased at 12-168 hrs, and LMP1 antigen expression was also enhanced from day 3 to day 7. In contrast, another viral oncogene EBNA2 expression was not detected. To investigate whether mosquito antigen-stimulated CD4 T cells can induce NK cell lymphocytosis, PBMCs were stimulated with mosquito salivary gland extracts once per week and the NK cell number was counted sequentially. In result, NK cell number on day 14 increased one and a half times as many as the number at the beginning of the culture, and the number on day 28 was equivalent to the number at the beginning. In contrast, NK cell number reduced to almost zero on day 28 in culture without mosquito antigen or in culture without CD4 T cells. Furthermore, to know whether viral oncogene was actually expressed in the mosquito bite sites, we examined mRNA expression of LMP1 in the skin lesions. In result, LMP1 mRNA was detected in the biopsy specimens taken from the mosquito bite sites. These results suggest that CD4 T cells stimulated with mosquito salivary gland extracts can induce expression of viral oncogene LMP1 in the NK cells and is involved in NK cell oncogenesis.

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SOCS-1 negative feedback mechanism of STAT1 activation is a key pathway in the dsRNA-induced innate immune response of human keratinocytes

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Toll-like receptor 3 recognizes virus-associated double-stranded RNA (dsRNA) and mediates innate immune response in virus infection. dsRNA stimulates two intracellular signaling pathways: the MyD88-dependent and -independent pathways. Previously, we showed that keratinocytes produce various chemokines in response to dsRNA. However, the mechanism regulating the excessive innate immune reaction is still unknown. We hypothesized that a signal transducer and activator of transcription (STATs)-suppressors of cytokine signaling (SOCSs) systems regulate this innate immune response in normal human keratinocytes, as STATs are key mediators in the MyD88-independent pathway. Exposure to 10 mg/ml poly(I;C) induced normal human keratinocytes to produce MIP-1α, MIP-1β, TNFα, IL-15, and IL-6. Since poly(I;C) phosphorylated STAT1 and STAT3, we studied whether STAT1 or STAT3 are involved in this response using the dominant negative forms of STAT1 (STAT1F) and STAT3 (STAT3F). Transfection of STAT1F using adenovirus vector significantly reduced the induction of these genes by poly(I;C), while STAT3F had a minimal effect. This indicates that STAT1 primarily mediates the induction of these genes. Since SOCS-1 is a negative feedback regulator of STAT1 signaling and poly(I;C) enhanced SOCS-1 expression, SOCS-1 may negatively regulate this poly(I;C)-induced gene expression. Transfection of SOCS1 inhibited the phosphorylation of STAT1 and STAT3, and significantly reduced the induction of these genes by poly(I;C). However, transfection of SOCS1 did not affect the activation of NF-kB by poly(I;C), indicating that the inhibitory effect of SOCS-1 is not via NF-kB. Although poly(I;C) activated p38 and JNK, inhibitors of these pathways had no effects on the induction of these genes. In conclusion, the SOCS-1 negative feedback mechanism of STAT1 activation is a key pathway in dsRNA-induced MIP-1α, MIP-1β, TNFα, IL-15, and IL-6 production via the MyD88-independent pathway in normal human keratinocytes.

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***In vivo* activity of soluble factor/s by *Candida albicans* that inhibit macrophagic IL-12 production**

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We have previously demonstrated that culture supernatants of C.albicans (CA) can effectively inhibit IL-12 production by human monocytes induced by lipopolysaccharide (LPS) and interferon γ (γ-IFN) in vitro, although monocyte MCP-1, IL-1β, TNFα and IL-10 were induced. The IL-12 inhibition is mediated through stimulation of ERK MAPK and inhibition of p38. We next investigated the in vivo impact of CA supernatants on macrophage IL-12 production. To determine the in vivo consequence of secreted immunomodulatory CA factor, we transferred to a murine system. Macrophages were prepared from C57BL/6 mice elicited by thioglycollate and IL-12 production induced by LPS and γ-IFN. CA culture supernatants inhibited IL-12p70 (ELISA) by 32 % from 571 pg/ml to 387 pg/ml ($p<0.05$, n=3) .This indicated that macrophage in addition to human monocyte was attributed to IL-12 inhibition by CA supernatants. IL-12 production in vivo was optimized using C57BL/6 mice pretreated for 5 days with thioglycollate peritoneally (ip) and combining a priming ip injection with 100 ng LPS with an iv injection of 1μg LPS 1 h later . CA supernatant was injected in prior to LPS, and six hours later, serum was collected. Our data showed that the levels of IL-12 detected in the sera of mice with CA culture supernatant were significantly inhibited from 216pg/ml to 81pg/ml ($p<0.005$). In conclusion, our findings demonstrated that the CA soluble factors are an important IL-12 inhibitor both in vitro and in vivo, and may modulate immune responsiveness of already immunocompromised hosts at sites distant from the priming inhibition as well as locally.

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Lymphatic reprogramming of blood vascular endothelial cells by Kaposi's sarcoma-associated herpes virus

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Kaposi's sarcoma (KS) is the most frequent cancers in HIV positive patients. KS-associated herpes virus (KSHV) is essential for the tumor formation and development of KS. KSHV infected cells are associated with slit-like vessels that often contain red blood cells. KS is generally considered as a neoplasm of KSHV-infected lymphatic endothelium, due to the expression of lymphatic markers such as VEGFR-3 and podoplanin by the tumor cells. However, KS cells also express some blood vascular markers and, therefore, the histogenetic origin of KS remained unknown. Previously we defined the transcription factor Prox1, essential for the lymphatic vessel development from embryonic veins, as a master control gene that mediates lymphatic endothelial differentiation. Ectopic expression of Prox1 in blood vascular endothelium resulted in re-programming of these cells to adopt lymphatic endothelial cell differentiation. This switch was associated with the inhibition of blood vessel-specific markers and with the induction of lymphatic markers, including LYVE-1, VEGFR-3 and podoplanin. Importantly, we found that infection of cultured human cutaneous blood vascular endothelial cells with KSHV re-programs these cells to express lymphatic endothelial markers, including Prox1, LYVE-1, VEGFR-3 and podoplanin. Immunocytochemical study shows that more than 90% vascular endothelial cells positive for KSHV latency associated nuclear antigen (LANA) strongly express Prox1. These results were confirmed by immunostains of KS tissue samples. Furthermore, expressions of some blood vessel specific markers, such as endoglin and collagen type IV, are still maintained in KSHV-infected blood vascular endothelial cells in vitro as well as in tumor biopsy samples. Based on these findings, we hypothesize that KSHV induces lymphatic re-programming of blood vascular endothelial cells in KS tumor lesions.

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Skin microenvironment dictates susceptibility of bacteria to antimicrobial peptides

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A contradiction exists between in vivo and in vitro observations of antimicrobial peptide (AMP) activity. In bacterial culture media AMPs are inactivated in the presence of 125mM NaCl and 10% serum, nevertheless they function well in vivo. Expression of AMP correlates with innate antibiotic activity in plants, insects and mammals. To understand how human AMPs can function in vivo, we investigated bacteriacidal action in a culture environment resembling mammalian skin and found that AMPs remain active in the presence of NaCl and serum. Bacteria such as Staph. aureus and MRSA, previously reported to be resistant to the human cathelicidin LL-37, become highly susceptible to LL-37 in this environment. Sequential analysis revealed that carbonate anion is an essential antimicrobial enhancing factor (AEF) normally present in the host microenvironment. AEF increases the susceptibility of many Gram negative and positive bacteria and Candida albicans to multiple AMPs, including cathelicidins, alpha and beta defensins. This occurs at physiological pH and at physiologically relevant concentrations of carbonate, but not with other anions or buffers. Bacterial susceptibility persists for at least 2 hrs after removal of AEF, indicating that the effect of AEF is on the target bacteria and not peptide structure. Kinetic analysis of killing and electron microscopy confirmed these observations. Membrane permeability assays and gene chip analysis demonstrated that AEF causes an increase in the permeability of the E. coli inner membranes and influences expression of the regulatory gene, Bar-A. This controls OmpR, a global transcription factor critical for stress response. The alteration in expression of some or all of these genes is therefore an important determinant in bacterial resistance AMPs. These findings illustrate how AMPs, molecules that have evolved to kill potential pathogens, have done this in the unique mammalian microenvironment. Such a strategy inactivates natural AMPs outside of the host and minimizes pressure for the microbe to develop resistance.

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Staphylococcal lipotechoic acid inhibits delayed type hypersensitivy reactions via the platelet activating factor receptor: a novel mechanism by which staphylococcal infections can worsen atopic dermatitis

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Bacterial skin infections with Staphylococcus aureus cause significant morbidity and can even result in mortality. Skin infection or even colonization with staphylococcus aureus is a known trigger for inflammatory skin diseases, especially atopic dermatitis. Thus, the mechanisms by which staphylococcal infections can modulate immune responses are an active area of study. Recent studies have suggested that staphylococcal lipotechoic acid, a constituent of the cell wall of this Gram-positive bacteria, can act as an agonist for the receptor for the lipid mediator platelet-activating factor (PAF-R). The objective of the present studies was to use model systems consisting of PAF-R-positive and negative cells and mice deficient in PAF-Rs to demonstrate that lipotechoic acid is a potent PAF-R agonist. Treatment of the PAF-R-negative human epithelial cell line KB transduced with PAF-R (KBP) cells with lipotechoic acid resulted in an intracellular calcium mobilization and enhanced cytokine (IL-8, TNF) production over retroviral control transduced (KBM) cells. Similarly, lipotechoic acid-mediated signaling in the PAF-R-positive keratinocyte-derived cell line HaCaT was inhibited by pretreatment with PAF-R antagonists. Intradermal injection of lipotechoic acid resulted in an urticarial reaction in wild-type C57BL6 but not PAF-R-/- mice. Because the PAF-R upregulates a number of immunomodulatory compounds, including interleukin-10 and prostaglandin E2, the hypothesis that lipotechoic acid could inhibit delayed type hypersensitivity (DTH) reactions in vivo was assessed. Exposure to a PAF-R agonist or lipotechoic acid suppressed DTH reactions due to topical DNFB in wild-type, but not in PAF-R-/- mice. We propose that lipotechoic acid exerts inflammatory and immunomodulatory effects via the PAF-R. This finding could potentially provide a novel mechanism for how staphylococcal infections can inhibit TH1 reactions and thus worsen TH2 skin diseases such as atopic dermatitis.

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Subsets of murine blood monocytes—Trojan horses in infection

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In some infections phagocytic cells transport microbes through the blood and enable their entry into certain organs. The exact identity of these phagocytes is not known. Different monocyte subsets have been characterized in the human, but not yet in the murine system. Our goal was to identify monocyte subsets in the mouse, to analyze their differeniation and their role in infections with Listeria monoctyogenes or L.major. We found that differential expression of Ly-6C divides mouse monocytes into three subsets, i.e. into Ly-6Chi / med / low. In addition, these subsets also show differential expression of CD43, CD11c and CD62L. By cell sorting we revealed that these subsets all express MCSF-R (CD115), and that they can develop into both macrophages or dendritic cells, depending on added growth factors. By eliminating blood monocytes with clodronate-loaded liposomes and monitoring their repopulation we showed that monocytes reappearing in circulation were all of the immature Ly-6Chi subset which presented the subset released from bone marrow. In circulation their expression of Ly-6C was downregulated. Chronic infection with L. major and acute infection with Listeria monocytogenes resulted in significant increase of immature Ly-6Chi monocytes, similar to the inflammatory “left shift” of granulocytes. We found that Ly-6Cmed/hi monocytes were the ones preferentially recruited to inflamed tissue sites. In blood this subset also harbored the majority of Listeria and, unexpectedly, sometimes even L. major. Importantly, in Listeriosis it was this subset that transported bacteria into the brain. There are distinct developmental subsets of murine blood monocytes of which Ly-6Chi monocytes are preferentially recruited to inflammatory sites; These subtypes harbor certain pathogens during experimental infection; The Ly-6Chi subset is a Trojan horse which carries L. monocytogenes into the brain.

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Virus replication begins in Langerhans cells and not in dermal dendritic cells during transmission of HIV from skin emigrants to T cells

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Mucosal Langerhans cells (LC) or sub-mucosal dendritic cells (DC) are suspected to be the initial cells that support HIV replication following sexual exposure to virus. Recently, novel C-type lectins, Langerin and DC-SIGN expressed on LC and DC respectively, have been shown to bind HIV and facilitate viral infection of resting T cells in trans. Therefore, we studied relevant host molecular targets utilized for acquisition of HIV infection using a skin explant model system. HIV_{BaL} (a prototypic CCR5-using isolate) was applied to the abraded epidermal surface of skin explants. After 3 days, emigrant cells from skin explants were co-cultured with allogeneic T cells. HIV p24 levels in culture supernatants were monitored by ELISA. Interestingly, pre-incubation of skin explants with PSC-RANTES, a CCR5 inhibitor, completely blocked subsequent HIV transmission from emigrant cells to T cells, whereas anti-DC-SIGN mAb, or mannan, a potent C-type lectin inhibitor, did not. Strikingly, when infection levels of single LC or DC within emigrant cells were determined by flow cytometry 3 days following HIV exposure, HIV p24+ cells were detected only in LC, but not in DC. To further test whether HIV could replicate within LC, skin explants were exposed to HIV variants that were engineered to express GFP during productive infection of cells. Interestingly, HLA-DR+/ Langerin+/ GFP+ emigrated cells were detected in LC-T cell conjugates, when a CCR5-using HIV isolate (NLCSFV3EGFP), but not a CXCR4-using HIV isolate (NL43EGFP), was applied to skin. Thus, CCR5-mediated productive HIV infection of LC, and not C-type lectin-mediated capture of virus by DC, may provide a biologic basis for understanding susceptibility to initial infection by CCR5-using strains of HIV in humans.

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Expression of Toll-like receptors in human and mouse keratinocyte cell lines and in normal and diseased skin

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Toll-like receptors (TLRs) recognize conserved molecular structures in microbes called pathogen-associated molecular patterns and play critical roles in the induction of antimicrobial responses against microbial invasion in host cells. TLRs are abundantly expressed on monocytes/macrophages and neutrophils. Keratinocytes, the major constituent of the epidermis, interact directly with pathogenic bacteria and their constituents in the external environment. Recently, human keratinocytes have been suggested to participate in innate host responses to bacterial products and invasive bacteria. However, expression of TLRs in keratinocytes and in normal and diseased skin still is not well defined. By using the human (U937) and mouse (RAW264.7) macrophage cell lines as positive controls, expression of TLRs was investigated in human (CL-22) and mouse (Balbc/MK) keratinocyte cell lines and in whole normal and diseased skin. Using RT-PCR, U937 cells expressed TLR1,2,4,5,6 mRNAs. CL-22 cells expressed TLR1,2,3,5,6 mRNAs. RAW264.7 and Balbc/MK cells expressed all 6 TLR mRNAs. Whole normal human skin expressed TLR1,2,4,5,6 mRNAs that were mostly expressed by the epidermis. Immunohistochemistry of normal skin showed expression of TLR1,2,3,5,6 in the epidermis with different staining patterns. In addition to the epidermis, eccrine sweat glands expressed TLR1,2,4,5 and follicular structures expressed TLR1,2,3,5,6. Psoriatic and S. aureus-infected skin showed increased expression of TLR1,2,5,6 compared to normal skin. These results help support the notion that keratinocytes have a role in innate immune host responses of the skin.

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Susceptibility in BALB/c mice is not the result of diminished recruitment of $CD4^+$ or $CD8^+$ T-cells to *Leishmania* lesions

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Healing in leishmaniasis is associated with IFNγ-production by $CD4^+$/Th1-cells. Recently, the induction of $CD8^+$ cells during infection and thus their physiological relevance in resistant C57BL/6 was shown. We previously described that fusion proteins composed of HIV-1 TAT and *Leishmania*-antigen LACK efficiently vaccinate against progressive disease in otherwise Th2-prone BALB/c mice by facilitating MHC class I-antigen presentation. Thus, priming of *Leishmania*-specific $CD8^+$ cells is beneficial for protective immunity. In contrast to $CD4^+$ cells, the role of $CD8^+$ cells in infected BALB/c mice is not known. Therefore, we now investigated the frequency of $CD8^+$ T-cells in *L. major* lesions. BALB/c or C57BL/6 ear skin was infected with $2x10^5$ promastigotes. At multiple time points, lesional inflammatory cells were isolated and characterized by FACS. As described previously, BALB/c mice showed progressive disease from wk3 on, whereas lesions in C57BL/6 mice peaked at wk4 and resolved by wk7. BALB/c lesions contained significantly more total cells than C57BL/6 ears from wk3 on (e.g. 12±1 vs. $5\pm1x10^6$, wk5, $p<0.002$) with most dramatic differences in the number of neutrophils. Furthermore, an increase in $CD4^+$ cells was observed starting at wk3 reaching $1x10^5$ cells. In contrast, the number of $CD8^+$ cells was significantly lower (30±5 vs. $100\pm17x10^3$, wk4, $p<0.05$) and immigration of $CD8^+$ cells was delayed (starting at wk5 vs. wk3). No strain-specific differences were found regarding the numbers of recruited T-cells. In contrast, accumulation of $CD11c^+$ DC in BALB/c skin was higher than in C57BL/6 lesions (e.g. 3±0.2 vs. $0.5\pm0.2x10^6$, wk6, $p<0.002$) confirming our previous finding that DC alone are not sufficient for mediating protection. In summary, our results demonstrate that, although BALB/c mice are unable to heal, $CD4^+$ and $CD8^+$ T-cell recruitment to lesional skin is not different from C57BL/6 mice. Thus, further studies have to explore the nature of these $CD8^+$ cells (Tc1 vs. Tc2) in BALB/c mice and their potential use for vaccination strategies.

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An important role of IL-27 for the development of protective Th1-immunity against *Leishmania major*

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Leishmania major-infected dendritic cells (DC) release IL-12 and facilitate the development of protective immunity against *L. major* infections, which is strictly dependent on IFNγ-producing $CD4^+$ Th1 or $CD8^+$ Tc1 cells. Resolution of cutaneous lesions in leishmaniasis is mediated by IFNγ-activated macrophages that eliminate the intracellular parasite via NO^{2-}. A novel IL-12 family member, IL-27, is a heterodimer composed of the p40-related protein Epstein-Barr virus-induced gene 3 (EBI3) and p35-related protein p28. IL-27 binds to the WSX-1 receptor on Th0/Th1 cells and is produced by activated DC. Here, we utilized EBI3-deficient (C57BL/6) mice to investigate the role of IL-27 in cutaneous leishmaniasis. We used the more physiologically relevant low dose infection model (1,000 infectious stage metacyclic promastigotes injected i.d.) that closely mimics natural transmissions by the sandfly. Lesions in EBI3-/- mice were significantly larger between wk 3-10 post infection, most dramatic differences were found between wk 6-8 reaching ~3-fold increased lesion volumes compared to wild type (WT) controls (34±4 vs. 12±1 mm3 at wk 6, n≥10, p<0.002). The lesions of EBI3-/- mice also contained greater numbers of parasites reaching a peak load of $9x10^6$ parasites/ear at wk 6 (WT mice: $1x10^5$ parasites/ear, n=10, p<0.05). In accordance with WT C57BL/6 mice, lesions in EBI3-/- mice resolved after 10-12 wks. Antigen-specific restimulation of draining lymph node cells revealed that in the early phase of the infection (up to 6 wks post infection) EBI3-/- mice showed impaired IFNγ production (0.3±0.1 vs. 1.7±0.6 ng/ml at wk 2, n=10, p<0.05) and increased levels of IL-4 (195±47 vs. 57±7 pg/ml) as compared to WT controls. This was restored to the WT level at 8 wks post infection. In summary, our data suggest that – in addition to IL-12 – IL-27 plays a critical role for the establishment of Th1 immunity in this model of an important human infectious disease.

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Transgenic delivery of an antimicrobial propeptide protects against bacterial skin infection: making Mighty Mouse

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Cathelicidin antimicrobial peptides are essential for skin defense against microbial pathogens. However, unlike other mammals, humans and mice have only one cathelicidin gene. We hypothesized that supplementing the native human or mouse cathelicidins (LL-37 and mCRAMP respectively) with a xenobiotic cathelicidin (pig PR-39) would enhance resistance against infection. In bacterial culture, PR-39 was found to act synergistically with mCRAMP to kill Group A Streptococcus (GAS). In cells, human keratinocytes normally producing LL-37 were engineered to also express PR-39 by lentivirus and killed 1000X more GAS than vector controls. In mice, transgenes were engineered to constitutively express either PR-39 or mCRAMP in basal keratinocytes using a K14 promoter. These constructs were designed to express the inactive precursor form of PR-39 or mCRAMP since injection of PR-39 in mouse skin induced a leukocytic infiltrate when applied in its mature form. Transgenic mice were evaluated by Southern blot, RT-PCR, immunohistochemistry and Western blot, and 2 independent lines were selected for analysis of each construct. Both K14-mCRAMP and K14-PR-39 transgenic mice were indistinguishable from wild-type littermates when housed under sterile conditions. K14-PR-39 transgenic mice processed the precursor to the mature form and showed increased resistance to skin infection when inoculated with GAS (50% smaller necrotic ulcers and 90% fewer surviving bacteria in the skin). By comparison, K14-mCRAMP transgenic mice did not show increased resistance to infection. Therefore, we show that targeted gene transfer of an inactive xenobiotic cathelicidin precursor confers resistance against invasive bacterial infection. This approach enhances immune defense by adopting the innate defense strategy of requiring proteolytic processing for activation and mimicking the synergistic expression of antimicrobial peptides that has evolved in some mammals.

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Bacterial lipopolysaccharide induces human β defensin 2 expression in cultured human dermal endothelial cells

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Skin is the first line of defense against the invasion of microbial agents. A number of endogenous antimicrobial peptides, including β defensins, are produced by keratinocytes, and play an integral part in innate skin immunity. Once the epidermis is breached after injury, infiltrating leukocytes (neutrophils and macrophages) act against this invasion using anti-microbial agents. In addition, resident dermal cells, including the dermal microvasculature endothelial cells, may play an important role in the innate cutaneous immunity. The aim of this study is to investigate whether human dermal microvasculature endothelial cells play a role in the defense against microbial agents, by producing any antimicrobial peptides, such as the β defensins. Immortalized human dermal microvasculature endothelial cells (HMVEC) were cultured with various concentrations of stimulatory reagents (lipopolysaccharide (LPS), human β defensins 2 and 3). At different time points, total RNAs were prepared from the samples, and the expression of target mRNA was analyzed by RT-PCR. In addition, HMVEC were cultured on type I collagen coated slide glasses with or without various concentrations of LPS. The expression of β defensin 2 was analyzed at different time points by fluorescence immunohistochemistry using a confocal microscope. LPS induced β defensin 2 HMVEC mRNA expression, at a maximum of 24 hours after stimulation. All of the tested stimuli failed to induce β defensin 3 mRNA expression in HMVECs. HMVEC produced β defensin 2 peptide that was detected as early as 24 hours after LPS stimulation. In conclusion, human dermal microvasculature endothelial cells may play a role in the defense against microbial agents, by producing the antimicrobial peptide, β defensin 2.

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Introduction to beta-defensin-3 to epidermal keratinocytes

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Defensins are small cationic proteins that directly kill a broad spectrum of microbes. In the epidermis, 3 types of human beta-defensins (HBD), HBD1-3, have been well characterized so far. HBD1 and HBD2 show antimicrobial activity predominantly against Gram-negative bacteria, while HDB3 kills both Gram-negative and Gram-positive bacteria. An increase of resistant bacteria against conventional antibiotics requests to develop a new strategy for anti-infection therapies, and antimicrobial gene therapy using defensin genes can be one of potential choices in the near future. In this study we first examined the localization of HBD3 in the normal skin. Immunohistochemical staining found HBD3 in granular and horny layers, and further immnoelectron microscopy detected HBD3 in the lamellar bodies and the intercellular space. Next, we introduced the HBD3 gene to cultured HaCaT cells to generate a keratinocyte cell line stably expressing HBD3. The transduced cells produced detectable HBD3, which showed strong antimicrobial activity in bacterial colony-forming unit analysis. Furthermore, we then grafted those HBD3-engineered HaCaT cells to skin defect of the nude athymic rats, and then applied bacteria onto the HaCaT-cell stratified epidermis. The number of recovering bacteria in the skin with the HBD3 gene was significantly lower than that in control skin. These findings suggest that introduction of the HBD3 gene to keratinocytes is great potential for controlling bacterial infections in the skin.

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Small interfering RNA (siRNA) inhibition of Kaposi's sarcoma-associated herpes virus (KSHV) latency-associated nuclear antigen (LANA)

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KSHV is a human γ-herpesvirus that is found in all clinical forms of Kaposi's sarcoma (KS) as well as primary effusion cell lymphoma (PEL) and the plasmablastic variant of Castleman's disease. LANA is a KSHV gene that is expressed in virtually all cells latently infected with KSHV, including all KS tumor cells. LANA is likely to be important, if not critical, in KS pathogenesis since it binds to and interferes functionally with both p53 and pRb. In addition, LANA tethers episomal KSHV DNA to chromosomal DNA, thus ensuring viral propagation into daughter cells during mitosis. The objectives of this study were to 1) inhibit LANA by siRNA and 2) to examine the viral and biologic consequences of inhibiting LANA in KSHV-infected cells. We designed a number of double-stranded 21-nucleotide RNA oligomers targeting LANA and assessed their silencing effects in an in vitro test system. siRNA nucleotides were transfected into BHK cells together with a plasmid encoding an EGFP-LANA fusion protein (pEGFP-LANA). Using flow cytometry to detect EGFP+ cells, we discovered up to 80% silencing of LANA compared to cells that were either transfected with pEGFP-LANA alone or with pEGFP-LANA and a commercially available non-specific control siRNA. To further assess the specificity of our siRNAs, we designed control siRNAs with 1 or 3 base pair mutations located at or around position #11 and assessed these siRNAs for their gene silencing effects. Dramatically, 1 or 3 base pair mutations in the target sequence of the siRNA led to a 63-94% reduction of silencing compared to the unaltered siRNAs. Next, we established optimal electroporation conditions for transfection of BCBL-1, a PEL cell line latently infected with KSHV, using pEGFP-C1 (a plasmid encoding EGFP). Currently, experiments are underway to silence endogenous expression of LANA in BCBL-1. Assessing the virologic and biologic effects of inhibiting LANA in KSHV-infected cells should allow us to determine whether LANA represents an attractive therapeutic target for patients with KS and other KSHV-related diseases.

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Control of skin infection with *Pseudomonas aeruginosa* is mast cells dependent

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We and others have previously shown that mast cells (MC) are essential for initiating efficient innate immune response in murine sepsis. Here we asked whether MC are involved in controlling bacterial skin infections to *Pseudomonas aeruginosa* (PA). Genetically MC-deficient Kit^{w}/Kit^{w-v} mice and normal *Kit+/+* mice were subjected to infections with PA by s.c. injection of $6.5x10^6$ colony forming units (CFU) (0.1 ml, shaved lower back). Developing skin lesions characterized by infiltration and subsequent necrosis were markedly larger (up to 2 fold as assesed by planimetric analysis) in Kit^{w}/Kit^{w-v} mice than in *Kit+/+* mice at all time points studied (every 2-6h, for 72h). Kit^{w}/Kit^{w-v} mice skin lesion size reached a maximum at 36h after infection (1.8 cm^2 vs. 1.0cm^2 in *Kit+/+* mice). Local MC-reconstitution of Kit^{w}/Kit^{w-v} mice resulted in normally controlled PA infections that were virtually indistinguishable from those in *Kit+/+* mice, indicating that immune responses to PA are MC-dependent. Furthermore, skin infection sites of more than 50% of *Kit+/+* mice and MC-reconstituted Kit^{w}/Kit^{w-v} mice were found to be cleared from PA 24h after infection, whereas large numbers of PA (up to 10.000 CFU/cm^2) were detected in skin infection sites in nearly all Kit^{w}/Kit^{w-v} mice tested. By histomorphometric analysis we found significantly increased numbers of degranulated MC at sites of infection. To test whether activated MC can recruit neutrophils to sites of PA infection we assessed myeloperoxidase (MPO) levels in PA infected skin of *Kit+/+* and Kit^{w}/Kit^{w-v} mice. MPO levels were increased in both groups of mice 3 hours after infection, but levels of MPO in *Kit+/+* mice were significantly higher (up to 2 fold) than in Kit^{w}/Kit^{w-v} mice. Our data suggest that activated MC induce neutrophil influx, control skin lesion size and promote containment of bacteria at sites of skin infections with PA.

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Papillomavirus-like particle amyloid beta vaccine induces strong antibody response

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The non-infectious virus-like particles (VLP) composed of major capsid protein L1 of papillomaviruses are successfully being tested in humans as vaccine to prevent HPV infection and associated neoplasia. In addition, chimeric VLP have been generated that incorporate foreign or self-antigens, and immunizations have induced high levels of (auto-)antibodies (ab), whereas immunizations with linear peptides were less effective. Ab were long-lasting and functionally active in experimental animal models. The neuro-degenerative disorder Alzheimer`s disease (AD), is characterized by neurofibrillary tangles and amyloid plaques, consisting of amyloid-beta (Abeta) fibrils. There is no effective therapy to prevent/cure AD. Recent studies have focused on anti-Abeta ab to inhibit fibril formation and/or to resolve Abeta-aggregates. Using the baculovirus system, chimeric bovine papillomavirus (BPV1) L1 (Abeta-VLP) was generated inserting 9 amino acid (aa) N-terminal peptide of Abeta into an immunogenic VLP surface loop. Electron micrographs demonstrated efficient self-assembly into capsomeric VLP. Expression of inserted Abeta epitope was verified by Western blot. This peptide shows aa sequence identity in human and rabbit representing a self-antigen for both species. To examine whether Abeta-VLP are capable to break B-cell tolerance, NZW rabbits were immunized with Abeta-VLP using Freunds adjuvans. After five months no signs of toxicity were observed. By ELISA, rabbit inoculated with Abeta-VLP developed strong ab response (titer>10.000) to Abeta peptide 1-9, whereas control rabbit did not. Reactivity was specific for the Abeta epitope and blocked by homologous, but not control peptide. Immune sera recognized amyloid plaques in post mortem brains sections of AD patients. These data support VLP as a tool to overcome tolerance to self-antigens with potential of inducing therapeutically useful auto-ab responses in humans. This hypothesis is currently evaluated by in vitro and in vivo models of AD.

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Mast cells control and contain infection with *Leishmania major*

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Mast cells (MC) initiate protective innate immune responses against bacteria. Here, we asked whether MC are also involved in the control of skin infections by the intracellular protozoan parasite *Leishmania major* (*L. major*). Injection of *L. major* ($2x10^5$ promastigotes) into the ears of C57BL/6 mice resulted in increased numbers of degranulated MC as soon as 6 hours after inoculation. Genetically MC-deficient Kit^W/Kit^{W-v}-mice subjected to skin infections with *L. major* developed markedly larger skin lesions as compared to normal *Kit*+/+ mice (~3-fold). Local and selective adoptive transfer of *in vitro*-derived MC to the ear skin of Kit^W/Kit^{W-v}-mice repaired their MC deficiency and resulted in the normalization of skin lesion development after *L. major* infections. Notably, Kit^W/Kit^{W-v} lesions contained significantly more parasites as compared to *Kit*+/+ mice (27 vs. $3 x10^3$/ear 5 wks post infection, p<0.01). In addition, *L. major* infections in Kit^W/Kit^{W-v}-mice resulted in enhanced systemic disease, i.e. spreading of parasites to the spleens, whereas *Kit*+/+ mice exhibited only occasional and transient systemic infections. After antigen-specific restimulation, IFNγ release of lymph node cells obtained from infected Kit^W/Kit^{W-v} mice was markedly reduced (e.g. 4.7±0.9 vs. 11.9±3.3 ng/ml at wk 1, n=8, p<0.05) and IL-4 levels were increased as compared to *Kit*+/+ mice (e.g. 143±29 vs. 86±13 pg/ml at wk 1, n=8). This indicates that MC-mediated control and containment of *L. major* infections are not limited to the induction of local inflammation and recruitment of proinflammatory cells to sites of *L. major* inoculation. These findings extend the view of MC as salient sentinels to innate host defense reactions against intracellular pathogens.

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Specification of *Staphylococcus aureus* infection requirements for TNF-α induction

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Tumor necrosis factor- alpha (TNF-α) is a pleiotropic cytokine and is responsible for a diverse range of biological processes including the inflammatory immune response, apoptosis and tumor necrosis. We have shown that S. aureus induces TNF-α rapidly (2h) and robustly (55x) and is one of the first cytokines induced, suggesting direct activation by S. aureus infection. To further understand the mechanism by which S. aureus elicits the TNF-α response, we examined infection properties, such as bacterial invasion. To determine whether invasion is required for TNF-α induction, keratinocytes were treated with cytochalasin D, an inhibitor of actin polymerization and S. aureus invasion. Cytochalasin D treatment failed to block TNF-α induction. To determine whether bacterial components on the cell surface are sufficient to induce TNF-α expression, we infected keratinocytes with live versus dead bacteria. UV- and heat-killed bacteria were incubated for 4 hours with keratinocytes at 1000-fold higher concentrations than live bacteria. We found that only live, not dead, bacteria induced TNF-α. As a control, we observed that dead bacteria were capable of keratinocyte invasion, indicating that structural integrity was maintained after UV and heat treatments. To test whether de novo bacterial protein synthesis is required for S. aureus-mediated TNF-α induction, gentamycin, an inhibitor of protein translation, was pre-incubated with S. aureus at concentrations sufficient to prevent bacterial growth but not to cause bacterial death. Gentamycin treatment of S. aureus also failed to induce TNF-α induction. Taken together, these data indicate that S. aureus-mediated TNF-α requires bacterial adherence (not invasion) and live bacteria to carry out de novo bacterial protein synthesis during the course of keratinocyte infection. These findings demonstrate that TNF-α-induction, in vitro, is blocked by selective and robust antibiotic treatment. Studies, like these, to understand the mechanism of TNF-α induction will ultimately lead to more effective treatment modalities for TNF-α-based inflammatory reactions.

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Oncostatin M-stimulated endothelial cells, endothelial cells infected with Kaposi's sarcoma-associated herpesvirus, and KS tumor cells express CCL21

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CCL21, also known as secondary lymphoid chemokine, is a ligand for CCR7 and is expressed by lymphatics, high endothelial venules, and T cell areas of lymphoid organs. This chemokine plays a critical role in the homing of CCR7+ leukocytes to lymphoid tissues, but little is known about regulation of *CCL21* expression. Since KS tumor cells express markers specific for lymphatic endothelial cells, we examined CCL21 expression in KS tumor cells and in endothelial cells relevant to KS pathogenesis. We first cultured human dermal microvascular endothelial cells (HDMEC) with oncostatin M (OSM), a major growth factor for KS. By real-time RT-PCR, OSM markedly increased CCL21 mRNA (max.15-fold increase) in HDMEC in a dose-dependent manner (0.01-100 ng/ml). Phosphorylated (p)-STAT3 was localized to both cytoplasm and nucleus in unstimulated HDMEC, yet became detectable only within nuclei of HDMEC stimulated for 30 min by 100 ng/ml of OSM. AG490, a JAK2/STAT3 inhibitor, blocked nuclear transport of p-STAT3 and inhibited up-regulation of CCL21 mRNA by OSM. We then compared CCL21 expression levels in uninfected versus KSHV-infected HDMEC. KSHV infection of HDMEC *in vitro* led to a 5-fold induction of CCL21 mRNA within 5 days of infection. Lastly, using immunohistochemistry, we discovered that KS tumor cells were strongly positive for CCL21 protein. In summary, KS tumor cells and endothelial cells highly relevant to KS pathogenesis expressed CCL21, the latter via a STAT3-dependent pathway. CCL21 expression by KS tumor cells lends further support to the hypothesis that these cells are of lymphatic origin. In addition, tumor-derived CCL21 may be an important mechanism by which KS tumors chemoattract CCR7+ inflammatory cells, which could be a rich source of pro-inflammatory cytokines necessary to promote tumor maintenance and growth.

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IL-12p40 homodimer, the natural IL-12 antagonist, released from *L. major*-infected DC contributes to susceptibility in cutaneous leishmaniasis

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Protection against *Leishmania major* (e.g. in resistant C57BL/6 mice) is the result of Th1-immunity, whereas susceptible BALB/c mice develop Th2-responses. IL-12 release of *L. major*-infected dendritic cells (DC) is critically involved in Th1-education. Bioactive IL-12p70 is a heterodimer composed of p35 and p40, whereas p40 alone – generally produced in excess to p70 – can be released as inactive monomeric p40 or as p40 homodimer $(p40)_2$ with inhibitory activity on the IL-12 receptor. We now analysed strain-dependent differences in the release of IL-12 by DC in more detail. As described previously, in the presence of IL-4, *L. major*-infected or LPS-stimulated bone marrow-derived DC (generated with GM-CSF) from both resistant and susceptible mice released comparable amounts of IL-12p70 and p40. However, without IL-4 (representing more physiological conditions), BALB/c-derived DC released significantly more p40 than C57BL/6-DC (LPS: 24±0.4 vs. 9.3±2.7 ng/ml, n=3, p<0.005; *L. major*: 6.3±2.1 vs. 3.3±1.5 ng/ml, n=4), whereas no differences in p70 production were found. We next determined, whether IL-12 was released as inactive monomeric p40 or in the form of the natural inhibitor $(p40)_2$. Interestingly, in western blot analyses of DC-supernatants a large proportion of anti-IL-12p40 reactivity was detected at 80kDa. We found substantially more $(p40)_2$ in BALB/c DC-supernatants suggesting that BALB/c-DC produce more homodimer than C57BL/6-DC. To determine the physiological role of IL-12$(p40)_2$ in cutaneous leishmaniasis, C57BL/6 and BALB/c mice were treated locally with 1 μg recombinant $(p40)_2$ or PBS intradermally during T-cell priming (d1-3 post infection with $2x10^5$ *L. major*). Lesions in $(p40)_2$-treated groups were significantly larger in both mouse strains compared to PBS-treated controls from wk3 on (e.g. BALB/c mice: 125±20 vs. 63±16 mm^3 at wk4, n≥7, p<0.01). In summary, genetical differences in the production of IL-12p40 homodimer from infected DC may contribute to disease outcome in cutaneous leishmaniasis.

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Immobilized molecular beacon bosensors for normal and methicillin-resistant *Staphylococcus aureus*

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Molecular Beacons, or DNA hairpin probes bearing a fluorescent group at one end and a quenching group at the other end, have become widely used in solution-phase genetic assays. In part, this is because the molecular beacon is a label free assay: in the absence of the target DNA strand, the molecular beacon probe is non-fluorescent, because of the proximity of the quenching group and fluorophore. We have developed an immobilized version of the molecular beacon concept, using a thin gold film both as underlying substrate and as the quenching group. This allows for the very simple preparation of immobilized hairpins, or biosensors, for a broad range of targets. In particular, we designed two molecular beacon probes for the FemA and mecR (methicillin resistance) genes of Staphylococcus aureus, each bearing a 5-prime thiol and a 3-prime fluorophore (tetramethylrhodamine). After immobilizing these individually on gold films, we observed very little fluorescence. Addition of the complementary DNA strand in either case resulted in as much as a 20-fold increase in fluorescence. Importantly, one chip exhibits a 6:1 selectivity for the fully complementary sequence over a single base mismatch, without increasing the stringency of the assay. We have also designed molecular beacon probes for methicillin-sensitive staphylococcus, and have demonstrated that the immobilized versions of these probes are effective biosensors for staphylococcus DNA.

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Specific toll receptor agonists mediate opposing regulatory effects on cutaneous infection with *Leishmania major* in susceptible BALB/c mice

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Activation of innate immunity can regulate the extent and cytokine phenotype of developing adaptive T cells responding to microbial infection. Although the parasitic pathogen, *Leishmania major*, is not known to possess toll agonists, we confirmed that a single intralesional injection with 100 µg of CpG oligonucleotide eliminates cutaneous infection in normally susceptible BALB/c mice. In contrast, injection of 40 µg of either TLR4 or TLR2 agonists, lipopolysaccharide (*Salmonella enteriditis*) and bacterial lipopeptide (Pam3CysSerLys4), significantly accelerated lesion development. Furthermore, single-dose injection of LPS completely reversed the curative effect of rFlt3L pretreatment in infected BALB/c mice. Whereas CpG promotes cure in association with enhanced Th1 and reduced Th2 cytokine production, LPS and Pam3Cys failed to prevent lymph node Th2 development as measured by IL-4 ELISPOT. LPS and Pam3Cys, but not CpG, deplete splenic DC in normal mice and reduce IL-12 p70 production, suggesting a possible mechanism of action. We confirmed that rIL-12 treatment restores curative immunity in *L. major*-infected BALB/c mice that were also co-injected with LPS. Addition of LPS to CpG injection of infected mice failed to reverse the protective effects of CpG, indicating a response to CpG that exceeds LPS effects. These findings show that different TLR agonists promote dramatically different developmental outcomes for Th1 or Th2 antimicrobial T cell responses. This has potential therapeutic relevance, but also highlights the distinct biological role of different toll receptor mediated responses despite the sharing of common transduction pathways.

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***Staphylococcus aureus* induced apoptosis in primary human keratinocytes**

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Infection of human keratinocytes by S. aureus leads to activation of a variety of immune-mediated responses, which are often followed by tissue invasion, cell damage and cell death. Cell death by apoptosis is widely recognized as an important control mechanism in the maintenance of tissue homeostasis and in the elimination of cells damaged by mutagens or infectious agents. S. Aureus-mediated apoptosis in primary keratinocytes has been observed, but the induction and characteristics of this process are poorly understood. In this study, the effects of S. aureus as an inducer of apoptosis in primary human keratinocytes were tested and compared to Camptothecin (CAM), a classic chemical inducer of apoptosis. Keratinocytes were infected with S. aureus at a multiplicity of infection (MOI) of 10:1 and 100:1 (bacteria: keratinocytes) for a range of times (t = 0, 6, 12, 18 and 24 h). Genomic DNA was extracted, and assayed by agarose gel electrophoresis for DNA fragmentation. This fragmentation is a classic feature of apoptosis, and appeared at 18 and 24 hours post-infection. Furthermore, DNA fragmentation was prevented by pretreatment of keratinocytes with a general caspase inhibitor (Z-VAD-FMK), indicating that apoptotic, effector molecules are activated upon S. aureus infection. Similarly DNA fragmentation was observed as a result of CAM treatment. Immunofluorescent microscopy of fluorescein-tagged TdT (TUNEL assay) to detect DNA fragments revealed that the extent of apoptosis occurring in 24 hours in S. aureus infected and CAM treated cells was about 25%. Viability assays performed with trypan blue exclusion dye gave results consistent with those from immunofluorescent experiments. Taken together, S. aureus caused apoptotic morphological changes in keratinocytes, similar to those produced by CAM. These findings indicate that apoptosis plays an important part of the keratinocyte response to S. aureus infection. Future studies will test whether elevated rates of apoptosis is one mechanism by which S. aureus infection leads to skin disease.

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Swimming pool flotation devices: a possible source of molluscum contagiosum infection

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The current research was designed to assess whether fomites (inanimate objects capable of transmitting infections between individuals) in swimming pool water could harbor and transmit Molluscum contagiosum virus (MCV). Molluscum contagiosum (MC) outbreaks have been linked to the use of public and school swimming pools, however the exact source of infection is unknown. Clinical observations at the Department of Dermatology, Royal Melbourne Hospital have noted patterns of MC infection consistent with the use of swimming pool flotation devices. Creation of in-house and quantitative real-time polymerase chain reaction (PCR) assays provided a diagnostic tool for detection of MCV. Probes were designed for specificity, and MCV was cloned and used as a standard for quantification. Fomites were spiked and exposed to a variety of chlorine dilutions simulating swimming pool water. MCV DNA was recovered in every instance. These results suggest that swimming pool fomites could be a common source of MCV infection. Given the popularity of swimming pool use among young people — and the fact that MCV is the most common poxvirus affecting humans — MC is a significant public health issue. Better understanding its mode of transmission will guide the improvement of best practices in swimming pool management.

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Infection of microvascular endothelium by *Anaplasma phagocytophilum*; an important first step in pathogenesis and persistence of anaplasmosis

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The skin is the major interface between vertebrates and their environment, and thus regulates interaction with ectoparasites as well as the pathogens they transmit. Blood-feeding ticks create an intradermal lesion, an inflammatory focus which attracts first-line immune responders such as neutrophil granulocytes. The human granulocytic anaplasmosis agent, *Anaplasma phagocytophilum*, is a tick-borne emerging rickettsial pathogen in the U.S. and Europe that utilizes PSGL-1, a receptor found on neutrophils, to enter human neutrophils. We present *in vitro* and *in vivo* evidence that infection of microvascular endothelium may be an important step during early pathogenesis and persistence of anaplasmosis. *A. phagocytophilum* invades human and primate primary cell cultures and immortal cell lines of microvascular endothelium. These microbes replicate to form large inclusions (morulae) that eventually rupture to infect other cells. Morulae may contain only a few or large numbers of bacteria of widely differing morphology and size. Infected endothelial cells upregulate CD54 and uptake of acLDL-containing cholesterol, an essential component of the anaplasma membrane. Uninfected neutrophils or HL-60 cells avidly adhere to infected endothelial cells, and the microbes are rapidly transferred to them. We hypothesize that this involves engagement of selectins and integrins that mediate neutrophil-endothelium interactions. In mice infected with *A. phagocytophilum* for 6 weeks, microvascular endothelium of heart muscle and liver contained morulae as demonstrated by colocalization of antibody to an endothelial marker (von Willebrand factor VIII) and to a major surface protein (MSP2/p44) of *A. phagocytophilum*. Likely, endothelial cells play a role in maintaining chronic infections, both as host cells, and as antigen presenting cells.

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Design of porous silicon biosensors for optical detection of pathogenic organisms causing skin infection

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We are developing a relatively new and innovative label-free optical biosensor technology based on porous silicon microcavities. This technology is intended for use as a non-invasive rapid detect sensor to aid in the clinical diagnosis of species specific pathogenic organisms that invade skin and cause infection. Current research has focused mainly on the material science aspects of designing sensors. Our preliminary research on developing a proteomic sensor for enteropathogenic strain of E. coli has provided a high a level of expertise in device fabrication and has enabled us to explore the relationships between the porous 3D microstructure, device sensitivity and sensor operation. Additionally, we have developed a solid phase immobilize enzyme assay using Glutathione-S-Transfersase (GST) to probe the porous microstructure. This work has enhanced our understanding of pore infiltration and results corroborate optical measurements pointing to the practical limits of utilizing porous silicon microcavities as a diagnostic devices in dermatological medicine. Our current focus is to develop a sensor for the detection of Candida, which is an opportunistic organism, and the causative agent of dermatological disease ranging from annoying or painful cutaneous mycoses to serious systemic disease. Prototype devices will utilize recombinant human antibody single-chain variable fragments (svFv) specific for hyphae to differentiate Candida albicans and Candida dubliniensis.

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Dendritic cell mediated killing of HPV E6 and E7 expressing keratinocytes

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Immature dendritic cells (DCs) are thought to have cytotoxic effector functions targeting HPV expressing tumors. These effector functions may also be important to combat genital warts caused by Human Papilloma Virus (HPV). To investigate cytotoxic efficacy against HPV infected cells, we analyzed infiltration of HPV expressing mouse tumors by CD3+, CD11c+, S-100+, CD14+ and CD68+ cells. Results were correlated to treatment of the mice with effective (encoding HPV-derived proteins) or ineffective (encoding GFP) vaccines (n=5). Immunostaining to detect these markers demonstrated that tumor shrinkage correlated mostly with infiltration by CD11c and S100 expressing cells. Similarly, tissue from human genital warts infected with HPV type 6 as shown by in situ hybridization displayed little infiltration by T cells. It is possible that the DCs present in HPV infected tissue contribute to killing of HPV infected cells. To study this process in vitro, the E6 and/or E7 genes of HPV 16 were introduced into keratinocytes by retroviral infection and effector functions of monocyte-derived immature DCs or DCs activated by exposure to heat shock proteins or IFN-gamma cells was measured in JAM assays. Results showed that DC mediated killing was most effective towards cells that express both E6 and E7, whereas normal keratinocytes were not killed. Also, DC effector functions were enhanced by exposure to IFN-gamma or HSPs, which elevated membrane TRAIL expression as shown by FACS analysis. Interestingly, keratinocytes expressing both E6 and E7 displayed reduced expression of decoy receptors for TRAIL, correlating with enhanced sensitivity to DC mediated killing. These studies demonstrate that immortalized keratinocytes expressing HPV encoded E6 and E7 genes are sensitized to dendritic cell mediated killing. It is possible that this mechanism can be exploited for shrinkage of warts and HPV expressing tumors.

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Confocal microscopy study of the effects of UVA radiation on the skin

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Skin exposure to UVA (320 - 400 nm) results in various biological responses, cutaneous tanning being the major one. When human skin is exposed to UVA, immediate pigment darkening (IPD) reaction occurs soon after exposure. In the case of high dose-UVA exposure, IPD seems to turn into a delayed pigment darkening reaction. For a better understanding of the UVA tanning mechanisms, we performed the present human study in 27 healthy volunteers with Fitzpatrick's skin phototype 2 to 5. Inner forearms from the subjects were exposed to 15- and 25-J/cm^2 UVA. Skin sites were photographed by a digital camera and imaged using reflectance-mode confocal microscopy (RCM) focusing on melanin distribution at the baseline and at several time points (from 0-30 min to 1 week) after UVA exposure. Two 3-mm punch biopsies were also taken from the UVA-exposed or non-exposed sites, either immediately or 1 week post-exposure. Furthermore, in order to investigate the effects of blood flow on IPD, intradermal anesthesia with or without the epinephrine was administered. Skin sections were stained with Fontana-Masson and assayed by a sensitive tyrosinase assay, for comparison with RCM images. Our results showed that the IPD response was present in the subjects of skin phototype 3 to 5. Skin phototype 2, however, showed only erythema. By CM image analysis, dendritic melanocytes were clearly visible at 1 week after UVA exposure in subjects of skin phototype 5, while no morphologic changes were observed immediately after exposure. The Fontana-Masson stained sections correlated well with CM images. A tyrosinase activity peak was also observed 1 week after the exposure. On the other hand, the epinephrine-induced reductions of blood flow as seen by CM *in vivo* led to a partially or completely diminished IPD response. These results indicate that blood flow may be an important factor for the development of the IPD response.

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Combined multiphoton excitation, polarization and second harmonic generation for imaging of biological specimens

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Multiphoton microscopy is a powerful tool for real-time noninvasive imaging of biological specimens. In this work, we demonstrate the application of multiphoton microscopy to analyzing the ultrastructures of biological specimens, especially the alignments of lipid bilayers of stratum corneum, the dermal fibers and temperature-dependent structural changes of collagen. Combined multiphoton excitation, polarization and second harmonic generqation were employed for imaging of the structures in ex-vivo human skin and bovine tendons. Stratum corneum was labeled with laurdan and bovine tendon was incubated in water of various temperatures prior to the imaging process. Our results show that both the excitation and emission dipoles of laurdan molecules are preferentially oriented perpendicular to the surface of corneocytes. Because the excitation dipole moment of laurdan is perpendicular to the lipid bilayer, the results are consistant with the molecular orientation of the lipid molecules in the intercellular space of stratum corneum. Furthermore, our results show that the fibrous structures in the dermis generate emission along the excitation polarization. The second harmonic signals decrease sharply when bovine tendens are heated up to 65oC, indicating that the molecular organization of collagen fibers was changed above this temperature. This work shows that combined multiphoton excitation, polarization and second harmonic generation can be a powerful imaging tool in monitoring structural orientation and molecular organization in skin and other biological specimens.

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EGCG inhibits UV-induced NF-κ B nuclear translocation and IL-6 secretion in cultured human keratinocytes

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Ultraviolet (UV) radiation from the sun is widely considered as a major cause of human skin photoaging and skin cancer. UV radiation-induced proinflammatory cytokines mediated by NF-κ B reportedly play important roles in human skin photoaging and skin cancer. NF-κ B and cytokines have been thus perceived as molecular targets for pharmacological intervention. With increasing amount of knowledge of the actions of green tea at cellular and molecular level, the beneficial effect of drinking green tea has become well recognized if not completely accepted. The components in green tea have even been added to skin care products unregulated, while the molecular mechanisms of the actions of those components on human skin are being unraveled. Using cultured human keratinocytes, we investigated the effects of (-)-epigallocatechin-3-gallate (EGCG), a major polyphenolic constituent in green tea, on UV-induced activation of transcription factor NF-κ B and proinflammatory pathway by measuring nuclear translocation of NF- κB and IL-6 secretion in vitro. p65, one of the subunits of NF-κ B was determined by immunohistochemical and Western blot analysis. IL-6 protein level in cell culture medium was measured by ELISA. The results showed that both nuclear p65 and secreted IL-6 were significantly ($p<0.05$) induced by UVB irradiation (20, 30 mJ/cm^2) and UVA irradiation (10, 20 J/cm^2). IL-6 secretion and NF-κ B nuclear translocation induced by UVB and UVA was dramatically inhibited by pretreatment of EGCG (0.15 and 0.3 mM) ($p<0.05$). Taken together, we conclude that EGCG inhibits UVB- and UVA-induced proinflammatory pathway in vitro and may be added to cosmetic or skin care products for prevention from UV-induced skin photoaging and skin cancer if this activity can be further confirmed and no cytotoxicity is reported in human skin in vivo.

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Aloin attenuates UVA-induced AP1 activation and MMP expression in cultured human skin fibroblasts

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Mounting evidence has indicated that excessive exposure of human skin to UVA radiation causes photoaging, leading to skin cancer if unattended. Cellular and molecular studies have demonstrated that UVA radiation induces generation of reactive oxygen species at high concentration leading to skin cell damage and at sub-cellular level resulting in activation of cell signaling components and consequently degeneration of collagen. Aloin, as a major antioxidant and widely used in skin care products, has been shown to have photo protective properties, and yet the molecular mechanisms of its action were only sporadically reported. Using cultured human skin fibroblasts we investigated the protective effects of Aloin on UVA-induced activation of the key components of AP1 transcription factor, C-Jun and C-Fos and expression of matrix metalloproteinases (MMPs) that are responsible for the degradation of collagen knowingly leading to cutaneous aging. Western blot analysis revealed that C-Jun protein increased 1.6, 2.3, 3.1 folds following UVA irradiation (10, 20 and 30 KJ/m^2), while C-Fos protein expression did not change significantly. The results by RT-PCR and ELISA utilized to measure mRNA and protein expression of MMP1 and MMP3 showed that UVA treatment induced mRNA and protein expression of both MMP1 and MMP3 in a time and dose dependent manner in cultured human fibroblasts. Pretreatment with Aloin (10^{-5}M) prior to UVA irradiation (30 KJ/m^2) reduced C-Jun expression to basal level and accordingly decreased mRNA and protein expression of MMP1 and MMP3 induced by UVA radiation. The protective effects of Aloin (10^{-6} to 10^{-4} M) were dose dependent. Collectively, our data indicate that Aloin can be applied to attenuate UVA-induced skin fibroblasts damage due to its effect on reduction of C-Jun protein and inhibition of MMP1 and MMP3 expression.

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***In vivo* and *in vitro* evaluation of lycopene as antioxidant substance**

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The aim of the present investigation was to study antioxidant activity of lycopene in vivo and on cell system. The in-vivo activity was studied on 3 preparations containing respectively: a) lycopene 0.03% (Lycopersicon Esculentum extract); b) alpha-tocopherol acetate (0.5%) and ascorbic acid (1%); c) a mixture of the 3 substances at the above concentrations. Photostimulation was performed on ten healthy volunteers with phototype II or III with a solar simulator (Multiport Solar UV Simulator) on the flexural surface of forearm with doses ranging from 20 to 80 mJ/cm^2. The cutaneous response was evaluated instrumentally, measuring erythema and trans epidermal water loss before UV exposure and after 24 and 48 hours. Erythema was measured with tristimulus colorimeter (Chroma Meter Minolta). The in-vitro investigation was performed on HaCat cell cultures, that were grown for 48 hrs until 75-80% confluence. Cells were incubated 30 min in PBS containing different concentrations of Vitamin E, Vitamin C, Lycopene as extract and Lycopene as pure substance (Sigma), then exposed to 150 mJ/cm^2 of UV light (Solar Simulator, Thermo-Oriel). After irradiation, cells were again incubated with fresh medium and maintained at 37^{0}C until further analysis. The formation of intracellular ROS was measured using 2',7'-dichlorofluorescin diacetate (DCFH-DA). The cells were analyzed with a FACSCalibur flow cytometer (Becton Dickinson) equipped with an excitation laser line at 488 nm and Cell Quest software (Becton Dickinson). The DCF (green fluorescence) was collected in a log scale through a 530±20 band pass filter. Monoparametric histograms of the fluorescence distribution were plotted for the estimation of ROS production. The in-vivo investigation demonstrated that lycopene-based preparations had a higher protective ability than the products containing the mixture of vitamins. In vitro experiments showed that lycopene in able to protect HaCat cell only when employed as extract. Further investigation is required to clarify such data.

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Infusion of apoptotic cells induced by experimental photopheresis induces antigen specific regulatory cells

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The basis of extracorporeal photopheresis is the reinfusion of leukocytes, which have been exposed to 8-methoxypsoralen (8-MOP) and UVA. This treatment induces annexin V+ apoptosis of nearly all leukocytes. Photopheresis is approved for the treatment of CTCL and has shown evidence of benefit for the treatment of autoimmune diseases, organ transplant rejection, atopic dermatitis and GvHD. However, the underlying mechanism remains unresolved. Since UV radiation of the skin causes immune tolerance via induction of regulatory T cells, we studied whether photopheresis exerts a similar effect extracorporeally. Thus, we utilized a murine model of contact hypersensitivity (CHS). Splenocytes and lymph node cells of mice which were sensitized with dinitrofluorobenzene (DNFB) were exposed to 8-MOP plus UVA in vitro. 8-MOP/UVA-treated cells were injected i.v. into naive mice which were subsequently sensitized with DNFB and ear thickening measured following ear challenge. Animals which had received 8-MOP/UVA-treated cells were suppressed in their CHS response. In contrast, mice which received cells which were untreated or exposed to UVA or 8-MOP alone were not suppressed. Induction of suppression was lost when lymph node cells were depleted of CD11c+ cells. Suppression was cell-mediated and antigen specific as demonstrated by the ability to transfer tolerance to naive animals which could, however, properly respond to the unrelated hapten oxazalone. Transfer of tolerance was lost when cells were depleted of CD4+ or CD25+ subpopulations. As few as 100,000 unfractionated cells could protect a naive animal. The generation of increased number and activity of regulatory cells was evident when animals were boosted with antigen and this protection was dose dependent. Together these data suggest that infusion of experimental ECP induced apoptotic cells produces antigen specific regulatory cells. Further studies are underway to better understand the nature of these regulatory cells and their mechanism of induction.

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Reducing p53 in human keratinocytes using siRNA delays onset of senescence, but increases susceptibility to apoptosis in response to UV-light

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Mice in which p53 is eliminated or enhanced reveal important roles in mediating apoptosis and senescence, respectively. While many mechanisms are evoked to explain these roles for p53, a pro-survival role for p53 in human keratinocytes (KCs) remains undefined. Rather than eliminating p53, we used siRNA to knockdown p53 levels in normal KCs. Compared to scrambled control (SC) treated KCs, p53 siRNA treated proliferating KCs expressed 70% lower constitutive p53 levels as determined by Western blots, and exhibited reduced transcriptional activity using promoter-reporter assays for p53 target genes (*p21, GADD45*). When SC-treated KCs reached confluency, they up-regulated CDKI (p21) and underwent premature senescence. By contrast, p53 siRNA treated KCs had reduced CDKI expression at confluency and underwent additional (6-8) population doublings, following confluency, consistent with delay in onset of senescence. As demonstrated, confluent/senescent KCs are relatively resistant to UV-light induced apoptosis compared to proliferating KCs, a potential role for p53 in regulating KC death was examined. When proliferating KCs were exposed to 15 mJ/cm^2 or 30 mJ/cm^2 (18 hrs, UV-B), siRNA treated KCs were approximately 2-fold more sensitive to induction of apoptosis than SC treated cells (using Annexin staining, or PI staining and Sub-G_0 DNA content by FACS). This enhanced apoptotic response was even greater (4-fold) when cultures became confluent. To determine the mechanism by which lower p53 bearing KCs underwent greater, rather than reduced apoptotic responses, Western blots revealed enhanced E2F1 levels in confluent siRNA treated versus SC-treated KCs. Since E2F1 can promote apoptosis, KCs with lower E2F1 levels may be less vulnerable to apoptosis. In conclusion: a) siRNA can be used to target specific genes in human KCs, b) caution is necessary in extrapolating results using knockout mouse models in which all p53 is abolished, c) p53 functions not only to promote senescence and apoptosis, but survival pathways are also regulated by p53.

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The osmolyte taurine is critically involved in photoprotection against ultraviolet B radiation-induced immunosuppression

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Uptake of osmolytes such as taurine could be part of the stress response of human epidermal keratinocytes induced by ultraviolet (UV) B radiation. This hypothesis is based on the observation that (i) ultraviolet (UV) B radiation induced the expression of TAUT, the specific transporter for taurine, in longterm cultured normal human keratinocytes (HNK) and that (ii) this upregulation was associated with an increased uptake of taurine into irradiated cells. In support of this hypothesis we now report that taurine uptake protects human skin cells against UVB radiation-induced immunosuppressive effects. Accordingly, preloading of HNK in-vitro with taurine completely prevented both hyperosmotic stress- and UVB radiation-induced gene expression including intercellular adhesion molecule-1 (ICAM-1). These studies indicated that taurine uptake might be critical in determining the susceptibility towards UVB radiation-induced immunosuppression. Moreover, we used TAUT deficient mice, which had been generated through disruption of exon 1 of the murine TAUT gene, to further test this possibility. We compared UVB radiation-induced immunosuppression in wildtype mice versus heterozygous versus homozygous TAUT knockout mice. Their response in a contact hypersensitivity assay was measured after exposure to increasing doses of UVB radiation. The result showed that homozygous, but not heterozygous TAUT knockout mice were more susceptible towards UVB radiation-induced immunosuppression than wildtype mice. Taken together these studies suggest that taurine uptake, similar to DNA repair and pigmentation, is critically involved in endogenous photoprotection of skin cells against UVB radiation-induced detrimental effects.

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Increase of dermal collagen fibrils diameter with uvb exposure: an optical and ultrastructural study in albino Balb/c mice

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Cutaneous aging is a complex biological phenomenon which depends not only of innate or intrinsic process (biologic clock) but also of extrinsic elements, primarily chronic sun exposure (photoaging). In order to verify the morphological changes of dermis (elastic fiber system and collagen) associated with aged skin we performed a light and electron microscopical study on irradiated, shaved albino mice ocurring after irradiation disruption (0,30,60 and 90 days). Experimental group consisted of 48 irradiated animals, which were divided into other three groups and submitted to different radiation doses (A-28800 J/m2, B-57600 J/m2, and C- 86400 J/m2). As controls, non irradiated shaved and non shaved animals were included. Since the day of disruption, and subsequently, elastic system and collagen network were progressively modified. Increase of collagen fibrils diameter was prominent in the 60 and 90 day groups ($p<0.05$), aspect verified in the ultrastructural observations. Elastogenesis figures were also seen in deep dermis. We found that collagen metabolism is profoundly altered after UVB irradiation and that it is progressive even after UVB disruption. Our comparative study of groups disclosed the clear relationship between dose and "elastotic changes" and also that chronological aging of mice skin apparently was intensified after UVB irradiation. Skin elastogenesis seems to be a major consequence of UVB irradiation, apart from elastolysis, and occurs not only in humans but also in hairless mice submitted to continuous, long-term UVB exposition.

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Variable pulsed light for the treatment of hypertrichosis: a pilot clinical study

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The aim of the present study was to evaluate the efficacy and safety of variable pulsed light (VPL, Elite Plus, Energist Ltd. U.K.), an alternative to intense pulsed light,in the treatment of hypertrichosis. Eighty-four(84)otherwise healthy volunteers(skin types II-VI) with previously treated or untreated hypertrichosis were included in this study.Treatment was performed with a variable pulsed 610nm light source with an off-delay time ranging from 1 to 20ms; the spot size was 5cm x 1cm. Clinical evaluation was done on an individual basis and exact photographic documentation was performed using the Teach-screen-monitoring system (Foto-finder-mediscope, Germany). Prior to every treatment procedure the area to be treated was covered with a refrigerator cooled (4 Deg. C)optical coupling gel(Energist Ltd., U.K.). Determination of fluence used was based on the individual's sensitivity to pain; this depended to a great extent on the specific skin type and coarseness. The average fluence used was 36.5 J/cm2(range :13.7-45 J/cm2). Eighty-two percent of the areas treated showed hair clearance of over 50%, thirty-five percent showed hair clearance of over 75% while fourty-seven percent had clearance between 50-75%. The side effects associated with the treated areas included transient erythema(43%) and leucotrichia(15%). Side effects were minimal and in no patient were cause for exclusion from the study. Transient pigmentary changes were not observed. The variable pulse light system (VPL) used in this trial presents itself as an effective and safe method for hair removal due to hypertrichosis. It is particularly effective in fair-skinned individuals with dark hair(skin type II-VI). Adverse effects are minimal and transient and in no patient sigificant enough to interrupt treatment.

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Transactivation of EGFR via HB-EGF shedding protects human keratinocytes from UV-irradiation-induced apoptosis

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We reported last year that UVB irradiation triggered EGF receptor (EGFR) phosphorylation by EGFR transactivation mechanism through HB-EGF shedding, the conversion of HB-EGF from a membrane-anchored form to a soluble form by shedding protease(s). Since EGF has an anti-apoptotic effect through EGFR phophorylation, we hypothesized that EGFR transactivation through HB-EGF shedding works as an anti-apoptotic intrinsic mechanism. Evaluation of apoptosis was performed by measurement of lactic dehydrogenase activity (LDH); we quantitated the apoptosis as the percentage of LDH activity of UVB-irradiated keratinocytes in the presence of various reagents to that of Tween 20-treated keratinocytes after deduction of LDH activity of non-irradiated keratinocytes from each activity. UVB irradiation at 50 mJ/cm2 induced 6.9% of apoptosis. Next, we examined the effect of reagents that blocks each step of EGFR transactivation through HB-EGF shedding: Addition of KB-R8301 (10 µM), a shedding inhibitor, increased the apoptosis to 16.9 % (2.8 fold). Anti-HB-EGF neutralizing antibody also increased the apoptosis to 15.3 % (2.5 fold). AG1478, an EGFR kinase inhibitor, enhanced the apoptosis to 18.0 % (3.0 fold). This indicates the blockage of EGFR transactivation through HB-EGF shedding results in enhancement of UVB-induced apoptosis. Furthermore, addition of exogenous soluble HB-EGF (100 ng/ml) rescued the apoptosis enhanced by KB-R8301 markedly, but not the apoptosis enhaced by AG1478 at all. This also confirms the involvement of EGFR transactivation through HB-EGF shedding mechanism in anti-apoptotic intrinsic mechanism. Taken together, this study reveals for the first time the new role of EGFR transactivation through HB-EGF shedding in normal human keratinocytes, namely the intrinsic protection mechanism for UVB-induced apoptosis.

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Enhancement of ultraviolet-induced apoptosis by NF-kB decoy oligonucleotides

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A decoy strategy utilizing oligonucleotides (ODN) containing the specific binding sequence of a certain transcription factor has been developed and is considered to be a potential new class of anti-gene therapy. However, the application of this new therapeutic modality to skin diseases has not been fully documented.The aim of this work was to examine the effects of the NF-kB decoy ODN on UV-elicited skin change.Mouse keratinocyte Pam 212 cells were transfected with NF-kB decoy ODN to examine the effects of the decoy ODN on UVB-induced apoptosis. Tape-stripped rat dorsal skin was treated with an ointment containing NF-kB decoy ODN for the examination of the in vivo impact of the decoy ODN on sunburned cell (SBC) formation and UVB erythema.NF-kB decoy ODN specifically induced apoptosis of Pam 212 cells, and SBC formation was significantly enhanced by topical NF-kB decoy ODN ointment, while UV-induced erythema was not affected.These data suggest that enhancement of UV-induced apoptosis by NF-kB decoy ODN may play a cancer-preventive role by further eliminating photo-damaged keratinocytes.

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Low dose ultraviolet-B radiation renders resistant melanoma cells sensitive to TRAIL-induced apoptosis

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Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is a potential anticancer drug since it has been initially described to induce apoptosis preferentially in malignant but not in normal cells. However, it has turned out that not all cancer cells are susceptible to TRAIL. Among eight melanoma cell lines tested, only three turned out to be sensitive towards treatment with TRAIL, whereas five cell lines remained resistant. Chemotherapeutic drugs and ionizing radiation have been found to be able to sensitize resistant tumor cells to TRAIL-induced cell death. Since ultraviolet B radiation (UVB) is a potent inducer of apoptosis but exhibits much less adverse effects than chemotherapeutics and ionizing radiation, we studied whether UVB may switch melanoma cells from a TRAIL-resistant into a TRAIL-susceptible state. Therefore, we analyzed the TRAIL-sensitive human melanoma cell line A-375 in comparison to the resistant cell line IGR-37. Differences in the response to TRAIL were not due to differences in the expression pattern of TRAIL receptors or of components of the death inducing signalling complex. Both cell lines showed expression of the long form of the antiapoptotic FLICE inhibitory protein (FLIPL), but none of the short splicing variant FLIPS. Only in TRAIL-sensitive A-375 cells most of the 55 kDa FLIPL had been cleaved preapoptotically into the 43 kDa form, indicating basal caspase activation even in unstimulated cells. Upon exposure to sublethal UVB-doses, TRAIL-treated IGR-37 cells underwent pronounced apoptosis, and TRAIL-sensitivity of A-375 cells was dramatically increased. In both cases UVB caused an inhibition of flip transcription. Taken together the study indicates that i) the expression level and the processing status of FLIP plays a crucial role in determining the sensitivity of melanoma cells towards TRAIL treatment and ii) expression of FLIP is regulated by UVB.

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Synthetic dipeptide (Cys-Gly): a new antioxidant active ingredient that can be of great use in anti-aging skin care products

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The skin is constantly exposed to environmental stress such as UV irradiation. Therefore, the skin is the primary target for oxidative stress and reactive oxygen species (ROS) formation. Moreover, free radical damage to proteins causes cross-linking, carbonyl formation and protein denaturation. In the skin, protection from oxidative stress is ensured by enzymatic (SOD, catalase, Glutathion reductase and peroxydase) and non-enzymatic antioxidants. Catalase has become increasingly recognized as being an enzyme centrally involved in the aging process of skin, and it has also been recognized that catalase plays an important role in protecting the skin from UV-oxidative stress. As recent data has demonstrated a significant decline of antioxidant enzyme activity in photoaged skin, we were interested in investigating the anti-oxidative stress effect of the synthetic dipeptide (Cys-Gly) at 1%, on cultured human fibroblasts. To accomplish this, we studied the expression of SOD and catalase after UVB stress (100 mJ/cm2), and evaluated the outcome on protein carbonylation and cell viability. Our studies showed that on SOD gels, the application of Cys-Gly dipeptide at 1% enhanced SOD expression in UV-stressed cells, compared to the controls. This result corroborates the findings of catalase assays that showed that dipeptide-treated cells exhibited a higher level of catalase activity in response to UV stress. Interestingly, the protective anti-oxidative stress effect of the Cys-Gly dipeptide was supported by other studies that showed a significant decrease in protein carbonylation, and an enhanced cell viability in the dipeptide-treated cells. Comet assay confirmed this protective effect and showed a remarkable decrease (73%) of DNA damage in dipeptide-treated cells. These results demonstrate the great anti-oxidative effect of the synthetic dipeptide, an active ingredient that can be of considerable application in skin care and anti-aging products.

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Bax activation and induction of apoptosis in human keratinocytes by the protein kinase C δ catalytic domain

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The induction of apoptosis by UV radiation protects keratinocytes from the carcinogenic effects of sunlight. Protein kinase C (PKC) δ is a required effector molecule for UV-induced apoptosis, and is activated by caspase-3 cleavage to generate an active catalytic domain fragment. The PKCδ catalytic domain induces apoptosis when ectopically expressed in keratinocytes, however the mechanism of apoptosis induction is unclear. We constructed a chimeric protein encoding the PKCδ catalytic domain fused to a mutant estrogen receptor ligand-binding domain in order to selectively activate the PKCδ catalytic domain. The enzymatic activity of the PKCδ catalytic domain/ER fusion protein was induced in human keratinocytes (HaCaT) treated with 4-hydroxytamoxifen, and its activation triggered loss of mitochondrial membrane potential and apoptosis. The apoptosis was associated with release of cytochrome c from the mitochondria and caspase activation, and was blocked by caspase inhibitors (59% inhibition) and the anti-apoptotic proteins Bcl-2, and Bcl-x_L (59-82% inhibition), suggesting a role for mitochondrial pore formation. Consistent with this, the active PKCδ catalytic domain disrupted mitochondrial architecture and triggered the redistribution and activation (oligomerization) of Bax, a Bcl-2 family protein that can directly induce cytochrome c release. Bax is activated by BH3 domain-only proteins that are normally sequestered by anti-apoptotic Bcl-2 family members such as Mcl-1. Activation of PKCδ catalytic domain reduced Mcl-1 levels, and the PKCδ catalytic domain directly phosphorylated Mcl-1, potentially at a PKCδ phosphorylation site in its BH3 domain. These results suggest that Mcl-1 may be the direct target of PKCδ responsible for Bax activation, release of cytochrome c, and induction of apoptosis. In summary, despite being an effector activated late in in the UV apoptotic cascade, PKCδ also activates upstream components of the UV death effector pathway to insure the demise of cells unable to repair damage induced by genotoxic doses of UV radiation.

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Release potency and photoprotection by tocopherol nanoemulsion against UV-mediated damage in HaCaT keratinocytes

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Alpha-Tocopherol (vitamin E) is a lipophilic vitamin that exhibits an antioxidative activity, antiaging and antiphotodamage. The study was to clarify the release feature of tocopherol in nanoemulsion vehicle (vit E NM) in HaCaT culture system and photoprotection effect of vit E NM on UVB-induced damage in HaCaT keratinocytes.Tocopherol nanoemulsion (vitE NM) was prepared by high pressure homogenization methods. HaCaT keratinocytes were incubated in the culture medium supplied with 1/200 and 1/400 of vit E NM before different dosages of UVB irradiation. Cell growth and cellular activity was detected. The vitamin E level left in the culture medium was measured by HPLC. The photoprotection effect of vit E NM on cultured HaCaT cells was detected by MTT assay. HaCaT KC could proliferated normally and no cytotoxicity effect of vitE NM on HaCaT KC was observed. With prolonging the culture time, the vitamin E amount remained in the culture medium decreased from 93.38 μg/ml at 0h to 5.36 μg/ml at 24h. Although photodamage to HaCaT KC was dependent on the irradiated dosages (25%~80% decrease of cellular activity), pre-incubation with Tocopherol NM may reduce such UV-induced injury. Anyway the photoprotection of tocopherol NM was reduced because of increasing UVB irradiation from 30 mJ/cm2 (1.38 at 4h) to 90 mJ/cm2 (0.68 at 4h), but the cellular activity still increased after prolonging culture time to 24 h (2.46 at 30 mJ/cm2 and 1.22 at 90 mJ/cm2).Compared with the control group, vitE NM is characterized by stable penetration and release potency which is of high importance for vitamin E to function on photodamage in cultured KC for the first step. Pre-exposure of HaCaT KC to alpha-tocopherol could improve the cellular activity decreased by UVB irradiation. The photoprotection efficiency is related to both the released level of vitamin E and to the UVB dosage within 24h.

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Photoprotective mechanisms of hydroxychloroqine and TCMs on ultraviolet B-induced human keratinocytes damage

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Hydroxychloroqine and the TCMs[EGCG(Epigallocatechin-3-gallate), baikal skullcap root and szechwan lovge rhizome] have photo-protective, anti-inflammatory and anti-oxidant effects on UV-irradiated keratinocytes. P53-p21 expression halt cell division so that DNA damage can be repaired or cause damaged cells to undergo apoptosis. C-fos could be a target gene for chemoprevention of UVB-induced skin cancer. In our study, we investigate the influence of the above drugs on UV-induced cytotoxicity, apoptosis and expression of p53,p21,c-fos genes to clarify the correlative photoprotective mechanisms. Human keratinocyte HaCaT cells were cultured in 1640 medium with 10% fetal bovine serum. The cells were treated/pre-treated and exposed to UVB(0, 30, 60 and 90 mJ/cm2). The apoptotic cells or debris were detected by flow cytometric analysis. The mRNA level of p53-p21 and c-fos were also detected through everse-transcriptase-polymerase chain reaction (RT-PCR). The apoptosis and debris rate of HaCaT cells was dependent on the irradiated dosages. When the UVB dosages changed from 0 to 90 mJ/cm2, the apoptotic cells increased from 0.21% to 71.18% and the debris from 1.57% to 73.01%. The intervention of the above drugs may reduced the apoptosis and debris rate, respectively 8% ~96.8% and 0%~86.5%.The level of each gene mRNA expression was also down-regulated with treation/pre-treation of the drugs. These results suggest that hydroxychloroqine and the TCMs may blocked the UVB-induced of apoptosis and cytotoxicity through reducing of p53-p2l and c-fos expression to inhibit the development of UVB-induced skin tumor.

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Narrow band UVB–induced oxidative DNA base damage in human keratinocytes

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Oxygen radicals are produced by ultraviolet B (UVB) irradiation as well as many other environmental carcinogens. They are also endogenously produced in cells by the oxygen metabolism. In 1984, Kasai reported the formation of 8–hydroxy–2'–deoxyguanosine (8–OH–dG) in DNA by oxygen radicals. Recently, narrow band UVB (311–313nm) has greatly improved the therapeutic efficacy and safety of phototherapy compared to the conventional broad band UVB (290–320nm). In order to evaluate the narrow band UVB–induced oxidative DNA damage, we digested cellular DNA with nuclease P1 and alkaline phosphatase, and analyzed the released 8–OH–dG using a high–performance liquid chromatography system equipped with an electrochemical detector (HPLC–ECD). By this method, non–irradiated human keratinocytes (HaCaT) contained 1.48 (±0.22) 8–OH–dG per 10^6 2'–deoxyguanosine (dG) residues in cellular DNA, which increased linearly to as high as 10.97 (±0.44) 8–OH–dG per 10^6 dG after broad band UVB irradiation at 1000mJ per cm^2. The lower level of 8–OH–dG (2.06±0.31 residues per 10^6 dG) was also detected in HaCaT cells after narrow band UVB irradiation at 1000mJ per cm^2. This lower level of oxidative DNA damage suggests that narrow band UVB phototherapy may be less potent in UVB–induced skin carcinogenesis.

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Elastin exon 26A-containing primary transcript and 26A protein are induced by UV irradiation and heat treatment in human skin in vivo

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Photoaged skin contains elastotic materials in the upper reticular dermis. This phenomenon is commonly known as solar elastosis. In our previous report, it was demonstrated that UV irradiation induced tropoelastin mRNA expression in the epidermis of human skin in vivo and also in the cultured keratinocytes in vitro. It was also known that the primary transcript of elastin undergoes extensive alternative splicing resulting in the translation of multiple heterogeneous protein isoforms. In this study, we found that UV irradiation and heat treatment increased the levels of elastin transcript containing exon 26A and its encoding elastin isoform (26A protein) in the epidermis of human skin in vivo and in cultured human keratinocytes in vitro by nested RT-PCR, Western blot analysis and immunohistochemical staining. It was also shown that there was increased elastin transcript containing exon 26A in the forearm (sun-exposed) skin of elderly persons, compared with upper-inner arm (sun-protected) skin of the same individuals. These data suggest that elastin containing exon 26A peptide induced by UV irradiation and heat treatment in human skin in vivo, plays some roles in the development of solar elastosis

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Adaptive response of human keratinocytes to UVB damage: effect of dose fractionation

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UVB induces DNA damage and the most important DNA lesions are cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts. The cell will be able to repair these lesions after a low UVB dose. When damage is too high (after a high dose) cells go into apoptosis. The aim of this project is to study the effect of repetitive UVB damage on survival (MTT assay), apoptosis (PARP cleavage) and DNA repair (detection of the induction and removal of CPDs by southwestern blot analysis). Preexposure to a low UVB dose (8 mJ/cm2) will significantly increase survival, when the second dose is apoptotic and time interval is 24 hrs. Increase in survival is paralleled with decrease in apoptosis as determined by PARP cleavage. Dose fractionation of 24 mJ/cm2 to 3x 8 mJ/cm2 increases survival and changes the response from apoptosis to repair, with a repair capacity comparable to cells only exposed once to 8 mJ/cm2. This adaptive response is only observed when time-interval between the fractionated doses is long enough (24 h). Dose fractionation (3x 8mJ/cm2) with shorter time intervals (30 min, 1 h, and 3 h), results in accumulation of damage. These cells fail to repair damage 24 hrs after the last exposure and lose gain in survival, observed with fractionated doses with long time intervals (24 h). We conclude that dose fractionation induces an adaptive response, but only when time-interval between the fractionated doses is long, allowing more time for DNA repair and thereby preventing accumulation of DNA damage.

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Further analysis of Infrared A (IRA) radiation-induced MMP-1 expression

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Human skin is increasingly exposed to IRA radiation, which is not only the major part of natural sunlight but also emitted by artificial irradiation devices used for reasons of lifestyle or therapeutic purposes. There is, however, evidence from animal as well as in-vitro studies that IRA radiation causes premature skin aging. Accordingly, we have previously shown that IR-A radiation leads to an induction of matrixmetalloproteinase-1 (MMP-1) expression in human dermal fibroblasts in-vitro via the ERK-MAPKinase pathway. We now report that physiologically relevant doses of IRA radiation cause MMP-1 expression in-vivo in the skin of human volunteers (n=3) as well. Realtime-PCR analysis revealed a 3 - 14 fold upregulation of MMP-1 mRNA levels in IRA-irradiated skin, which was associated with a concomitant increase in MMP-1 protein expression (immunohistochemistry). In additional in-vitro studies we have also obtained first evidence for the involvement of reactive oxygen species (ROS) in IRA radiation-induced signalling. This conclusion is based on the following observations which were made in cultured human dermal fibroblasts: (i) IRA irradiation affected intracellular ROS levels as assessed by means of oxidation-sensitive fluorescent dyes; (ii) IRA irradiation shifted the balance between oxidized and reduced cellular glutathione towards the oxidized form and, most importantly (iii) IRA radiation-induced MMP-1 expression could be partially prevented when glutathione levels were increased prior to irradiation by treatment of cells with N-acetylcystein. These studies demonstrate the in-vivo relevance of IRA radiation-induced MMP-1 expression and indicate that antioxidative strategies may be used to protect human skin against IRA-radiation-induced detrimental effects.

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Ultraviolet B radiation up-regulates expression of dectin-2 on epidermal Langerhans cells

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Since ultraviolet B (UVB) irradiation of mouse skin alters the antigen presenting cell (APC) function of epidermal Langerhans cells (LC), leading to suppressed Th1, but activated Th2 responses, and because dectin-2 is a pattern recognition receptor (PRR) on LC and dendritic cells (DC) shown to mediate suppression of contact hypersensitivity responses by UVB and is required for DC expression of Th2 cytokines, we hypothesized that UVB may upregulate dectin-2 expression on LC and DC. To address this hypothesis, we cultured mouse bone marrow cells with GM-CSF for 6 days to produce DC and then treated these cells with sham or UVB radiation (up to 300 J/m^2). A day after, we assayed dectin-2 mRNA by RT-PCR/Southern blotting, and found it to be 3.5-fold greater in UVB-irradiated (vs. sham-irradiated) DC. We also treated DC with various doses of IFNγ, PMA, and LPS, and found none of these factors (known to stimulate APC function) to augment dectin-2 mRNA expression. Next, we examined effects *in vivo* using a conventional protocol for inducing UVB-induced immunosuppression. BALB/c mouse right ears were treated with UVB (1 kJ/m^2/day) and left ears with sham irradiation for 4 consecutive days. A day after the last irradiation, we isolated epidermal cells from ear skin and used anti-Ia^+ magnetic beads to sort Ia^+ LC from Ia- cells. We confirmed dectin-2 mRNA expression to be restricted to Ia^+ LC. Moreover, dectin-2 mRNA was 3-fold greater in UVB-irradiated (vs. sham-irradiated) LC. By contrast, mRNA expression of other PRR (e.g., TLR-2, TLR-4, dectin-1) were unaffected. Finally, using Western blotting and anti-dectin-2 Ab, we found UVB to also upregulate dectin-2 protein expression. These findings lead us to conclude that UVB is uniquely capable of augmenting dectin-2 expression on LC and DC, and to hypothesize that such augmentation may be an important mechanism accounting for the immunosuppressive and Th2-polarizing properties of UVB radiation.

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Detecting reactive oxygen species in skin using two-photon fluorescence imaging microscopy

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Reactive oxygen species (ROS) contribute to skin photodamage including photoaging, immunomodulation, actinic keratosis and skin cancers. Because these highly-reactive derivatives of molecular oxygen are extremely short-lived and essentially non-emissive, they are difficult to detect directly. In addition, until recently with the realization of two-photon excited fluorescence (TPEF) imaging, the opaque and heterogeneous environment of the skin has inhibited detection and quantification of UV-induced ROS within the skin. We have developed a TPEF imaging method to detect the presence of ROS with 0.5 m spatial resolution and >100 m depth penetration. Dihydrorhodamine (DHR, 100 uL, 50 uM) is applied to the skin surface (Epiderm-200 (epidermis) and Epiderm-200FT (epidermis/dermis) (MatTek Corp.)) and incubated for 1 hr (37 oC, 5% CO2). DHR is non-fluorescent until it reacts with ROS and forms fluorescent rhodamine-123 (R123). Samples are imaged before and after UV irradiation (200-1600 J m2, 280-400 nm, solar simulator, Solar Light Co.). A detailed description of the instrument and experimental parameters will be presented. ROS are generated predominately in the lipid rich extracellular matrix of the corneocytes. With increasing depth, R123 fluorescence is detected primarily in the cytoplasm of the keratinocytes within each epidermal layer. In the dermis, fibrous regions of R123 fluorescence are detected. The data show that at commonly obtained UV doses, detectable ROS are generated within all layers of the epidermis and in the dermis. Both ex vivo and skin equivalent tissues yield similar results, with the advantage of the latter being reduced variability (scattering coefficients, pigmentation differences) between samples that ex vivo tissue affords. By coupling the TPEF microscopy method with skin equivalent tissue, the effects of topical applications like antioxidants and sunscreens upon UV-induced ROS levels can be studied.

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Heat shock-induced expression of matrix metalloproteinase-1 is mediated by activation of extracellular signal-regulated kinase and c-Jun N-terminal kinase and via an interleukin 6-dependent autocrine mechanism in human skin fibroblasts

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Repetitive exposure of human skin to sunlight has been suggested to be a major factor for premature skin aging. Infrared irradiation as well as ultraviolet irradiation of human skin cells was shown to induce matrix-metalloproteinases (MMPs), which degrade extracellular matrix. Infrared irradiation leads to the generation of heat. Heat shock is widely considered as one of environmental stresses. Here we have investigated the effect of heat shock on the expression of MMP-1 and MMP-2 in cultured human skin fibroblasts. Heat shock induced the expression of MMP-1, but not MMP-2, at the mRNA and protein levels in a dose-dependent manner. Heat shock caused the rapid activation of three types of mitogen-activated protein (MAP) kinase. The heat shock-induced MMP-1 expression was inhibited by treatment of skin fibroblasts with a MEK inhibitor, U0126 and a JNK inhibitor, SP600125, but not a p38 MAP kinase inhibitor, SB203580. Furthermore, heat shock increased the synthesis and release of interleukin-6 (IL-6) into the culture media. The induction of IL-6 mRNA by heat shock preceded the increase of MMP-1 mRNA expression. The specific inhibition of IL-6 using a monoclonal antibody against IL-6 greatly reduced the expression of MMP-1 induced by heat shock.

Taken together, our results suggest that ERK and JNK, but not p38 MAP kinase, play an important role in the induction of MMP-1 by heat shock and that the heat shock-induced expression of MMP-1 is mediated via an IL-6-dependent autocrine mechanism.

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Ultraviolet radiation and oxidative stress in *hairless* rats. Photoprotective effect of a *Polypodium leucotomos* extract

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Previous studies demonstrated the antioxidant and photoprotective properties of an extract of the fern *Polypodium leucotomos* (PL). In the present work we evaluated the photoprotective effects of PL on *hairless* rats exposed to ultraviolet radiation (UVR). Male *hairless* rats were divided into four groups (n=8 each) as follows: control non-irradiated; control non-irradiated and pretreated with PL; irradiated; irradiated and pretreated with PL. PL doses of 30 mg/kg p.o. were given once daily for seven days; last dose two hours before UVR. Two UVR schedules were applied (UVB/UVA lamps; B/A ratio = 0.9) on the dorsal skin: 1.25 MED for biochemical assays and 2.5 MED for morphological analysis. Samples were obtained 48 hours after. The following parameters were determined in plasma, erythrocytes and soluble extract of epidermis as described previously (Romeu et al., Life Sciences, 2002): GSH, GSSG, GST, CAT, SOD, GSH reductase (GR), GSH peroxidase, and TBARS. Langerhans cells (LC) were stained with a fluorescent anti-S-100 antibody and their morphology was blindly evaluated by five observers. The linear general multivariate model (SPSS, 11.0) revealed that PL had several protective effects. In erythrocytes of irradiated rats pretreated with PL the GSH/GSSG ratio was increased by 20% and GR activity decreased a 22%, in comparison with irradiated but not pretreated with PL rats. In epidermis, rats irradiated and pre-treated with PL showed a SOD activity 51% higher than those only irradiated.

Morphological analysis revealed strong disturbances in LC of irradiated animals without preteatment with PL. The goodness of the model was checked by a χ^2 analysis: χ^2=32.47; p<0.001; κ=0.8; global predictive value=90%. Using this model, the morphology of LC of rats pretreated with PL was indistinguishable from that of the control non-irradiated rats. These results support the beneficial photoprotective properties of PL after oral intake, probably due to its antioxidant and anti-radical activities.

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Wound healing response of a full thickness *in vitro* human skin equivalent (EpiDerm-FT 200) after solar UV-irradiation

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Normal human epidermal keratinocytes (KC) and dermal fibroblasts (FB) were cultured to produce full-thickness skin equivalents with FB-containing dermal and stratified epidermal components and fully developed basement membrane at the dermal/epidermal junction (EpiDerm-FT 200, EFT). The wound healing response of EFT after solar UV-irradiation was compared to excised human skin. H&E stained paraffin sections of EFT showed a dose-dependent increase in apoptotic sunburn cells at 24 h post-irradiation. After 48 h, high dose (61 J/cm2, metal halide lamp) samples showed extensive epidermal damage and some dermal damage. At 72 h, sunburn cells still persisted in mid dose samples (40 J/cm2), which were thinner than controls (indicating decreased KC proliferation) but still without major epidermal damage. Epidermal destruction of high dose samples was nearly complete at 72 h with loss of dermal matrix also evident. However viable basal cells remained in some areas, with signs of proliferation and epidermal regeneration. Between days 5-7, substantial regeneration of the epidermis had occurred. Active MMP-1 protein in the culture media was also evaluated by ELISA. A 50% increase in MMP-1 was observed in irradiated samples at 24 h. By 48 h, mid dose samples showed a 2-fold increase in active MMP-1 protein, while high dose samples showed a 3-fold increase. At 72 h, mid dose sample MMP-1 protein was comparable to controls, while high dose sample activity remained elevated by 3-fold. Similar experiments were conducted with excised human skin. The response was similar to EFT. The earliest response was increased sunburn cell formation, with tissue thinning at 40 J/cm2 and extensive damage at 61 J/cm2. A viable, regenerative basal epidermal cell layer was also observed in the excised human skin at 72 h in high dose samples. These results show that the EFT human skin equivalent behaves similarly to excised human skin in terms of solar UV induced damage and wound healing. The model may thus prove useful for additional applications in dermal/epidermal wound healing phenomena.

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Epidermal peroxisome proliferator-activated receptor gamma (PPARγ) as a target for UVB radiation

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Ultraviolet B radiation (UVB) is a pro-oxidative stressor with profound effects on skin in part through its ability to stimulate cytokine production and induce toxicity. Peroxisome proliferator-activated receptor gamma (PPARγ) has been shown to regulate inflammatory processes and cytokine release in several cell types; however, its effects on human epidermal cells have not been examined. The present studies demonstrate the presence of PPARγ mRNA and functional protein in human keratinocytes and epithelial cell lines HaCaT and KB. Moreover, treatment of epidermal cells with the PPARγ specific agonists ciglitazone and the natural oxidized lipid 1-hexadecyl-2-azelaoyl glycerophosphocholine (azPC) alone did not affect cyclooxygenase-2 (COX-2) levels, but augmented COX-2 expression and enzyme activity induced by phorbol 12-myristate-13-acetate (PMA) and IL-1. Since UVB has pro-oxidative effects, and oxidative stress has been shown to produce the potent PPARγ agonist azPC, we then assessed whether UVB treatment of epidermal cells resulted in a PPARγ activity. Lipid extracts from UVB-irradiated, but not control cells, contained a PPARγ activity identified by PPRE reporter studies and this activity upregulated COX-2 expression induced by PMA. Use of the PPARγ inhibitor GW9662 and cells expressing a dominant-negative PPARγ mutant confirmed that the lipid extracts from UVB-irradiated cells augmented COX-2 expression through their ability to act as a PPARγ agonist. Several separate lines of evidence suggest that UVB-generated PPARγ agonistic activity was due to free-radical mediated cleavage of endogenous glycerophosphocholines. First, pretreatment of epidermal cells with antioxidants ablated UVB-generated PPARγ activity. Second, subjecting purified 1-hexadecyl-2-arachidonoyl-glycerophosphocholine to UVB irradiation generated a PPARγ activity, confirming a non-enzymatic process. These studies suggest that epithelial cells contain a functional PPARγ system, and this system is a target for UVB through the production of novel oxidatively modified endogenous phospholipids.

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UVA and UVB activate the Fanconi anemia/BRCA DNA damage response pathway

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The identification of genes defective in the chromosome breakage disorder Fanconi anemia (FA) has recently facilitated the elucidation of a novel DNA damage signaling pathway. Central to this pathway is the ubiquitylation of the FANCD2 protein, which then forms nuclear foci with BRCA1, NBS1, MRE11, and Rad50, and activates DNA repair through recombination and DNA end-joining. So far, an activation of this pathway has only been described after exposure to DNA-crosslinking agents or ionizing radiation (IR), but not after exposure to UVA or UVB. Following exposure to IR, FANCD2 is also phosphorylated, which is part of a separate, ATM-dependent DNA damage response pathway. In order to elucidate whether these DNA damage response pathways are also activated by UV, we exposed primary human fibroblasts to various doses of UVA or UVB and assessed FANCD2-isoforms by immunoblotting at several time points. As early as two hours after irradiation, clear shifts to the activated, ubiquitylated L-isoforms were detected in a dose-dependent manner, with subsequent degradation of FANCD2 after 24 and 48 hours. This clearly indicates that the FA/BRCA DNA damage response pathway is activated by UVA and UVB. The same activation was also detectable in UVA- and UVB-irradiated xeroderma pigmentosum A cells, indicating that the nucleotide excision repair of DNA photoproducts is not the upstream signal for UV-activation of this pathway. Either UV-induced DNA damage itself, or stalled replication forks at sites of DNA damage might mediate the FA/BRCA pathway activation. Activation of p53, as detected by phosphorylation of p53 at serine-15, was seen as early as FANCD2-ubiquitylation, but persisted longer. Unlike IR, UV-irradiation did not result in band shifts or reactivity with a phospho-specific FANCD2-antibody, indicating that UV does not induce phosphorylation of FANCD2. Since the FA/BRCA pathway activates recombination repair and DNA strand break repair, we hypothesize that both UVA and UVB generate a biologically relevant amount of DNA strand breaks, e.g. through oxygen radicals, to require activation of this pathway.

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No major role for 8-oxoguanine in UVA-mutagenesis

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Oxidative DNA damage, in particular 8-oxoguanine (8-oxoG), has been suggested to mediate mutation formation and malignant transformation following exposure to UVA. It is processed primarily by the base excision repair pathway (BER). The initial step of BER is the removal of the damaged base by a damage-specific DNA-glycosylase, which is 8-oxoG DNA glycosylase (OGG1) for 8-oxoG. In order to study the contribution of 8-oxoG to UVA-mutagenesis, we compared UVA- and UVB-induced mutation frequencies in mouse embryonal fibroblasts from OGG1-knockout mice and their OGG1-intact littermates, using the ouabain-mutagenesis assay. Following irradiation with various doses of UVA or UVB, mutations in the Na,K-ATPase gene were detected by colony forming ability in a selective medium containing 3 mM ouabain and counted. In three independent experiments, $OGG1^{-/-}$ cells did not exhibit an increased frequency of UV-induced mutations, as compared to $OGG1^{+/+}$ cells, either with UVA, or UVB. This indicates that 8-oxoG, which is processed by OGG1, does not contribute significantly to either UVA- or UVB-mutagenesis. This is consistent with our database of UVA- and UVB-induced *hprt*-mutations in primary human fibroblasts, now much extended and based on sequencing in 2 directions of a total of 90 mutants. There we find that G to T transversions, which are considered signature mutations for 8-oxoG, constituted only 13 % of UVA-induced point mutations. This low frequency is similar to what we found in UVB-induced mutations (15 %). Our new data also shows that C to T transitions, including CC to TT tandem mutations, are the most common type of mutation not only with UVB (58 %), but also with UVA (48 %). These C to T transitions are considered signature mutations for pyrimidine dimers, which therefore appear to be major contributors not only to UVB-mutagenesis, as is well established, but also to UVA-mutagenesis. In addition, UVB- and UVA-induced C to T transitions were located at largely the same sites and hot spots, suggesting that UVA- and UVB-induced pyrimidine dimers are being formed through the same or similar mechanisms of DNA damage formation.

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Removal of oxidative DNA damage is imapired in xeroderma pigmentosum group A (XPA)

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Patients with XP have deficiency in repairing DNA damage caused by UV such as pyrimidine dimers and are susceptible to developing skin cancers on the sun-exposed area. However, the patients with XPA, B, D, G have neurological abnormalities, which cannot be explained by pyrmidine photoproducts caused by UV. We tested whether XP cells are impaired in removing oxidative stress. We assayed the kinetics of 8-hydroxydeoxyguanosine (8OHdG) produced by UVB using XPA cells because 8OHdG is recognized as a sensitive marker of oxidative stress. DNA was isolated anaerobically and 8OHdG was measured by HPLC using electrochemical detector. Preliminary study revealed that UVB most effectively produced 8OHdG among UVB, UVA and UVA+riboflavin and cellular survival 24 hr after 800 J/m^2 of UVB was 90 % by trepan blue staining, most studies have been done using UVB, mainly at the dose of 800 J/m^2. (1) 8OHdG increased dose dependently up to 3200 J/m^2 both in XPA and normal cells. Yield of 8OHdG was $3/10^9 dG/J/m^2$. (2) The removal of 8OHdG was statistically significantly impaired in XPA cells : 8OHdG remained twice the basal level 24 hr after irradiation in XPA cells, whereas in normal cells it returned to the basal level at 24 hrs. (3) 8OHdG in XPA cells 24 hr after UV irradiation reduced by additng SOD, catalase, deferoxamine into the medium. In *in vivo* study pcHA-*XPA*, which contains human *XPA* gnee and hemagultinin gene as a tag was introduced into XPA model mice skin. pcHA-*XPA* was treated 24 hr before 4 kJ/m^2 of UVB irradiation.(4) Recovery of removing/repairng UV induced 8OHdG by human *XPA* gene in XPA model mice *in vivo* was shown by staining the mice skin using monoclonal antibody against 8OHdG. These results demonstrated that *XPA* gene is involved in removing/repairing oxidative stress, which can be produced many environmental materials and by living cells. It might explain some of the reasons why XPA patients have neurological abnormalities.

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UVB substantially suppresses the expression of Interleukin-1 receptor antagonist in human dermal fibroblasts: a likely mechanism to enhance inflammatory effects of IL-α and IL-β in the dermis

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Cytokines are important immune modulators, and ultraviolet-B (UVB) is a major environmental regulator of their expression. Previously, we and others showed that tumor necrosis factor alpha (TNF-α), a pivotal proinflammatory cytokine, is upregulated in adult dermal fibroblasts (FBs) and neonatal keratinocytes (KCs) in response to UVB exposure, and that this upregulation is augmented by interleukin-1 (IL-1α). In the present study, we sought additional factors that may play a role in UVB effects on TNF-α production. Ribonuclease protection assays (RPA) for multiple cytokines on RNA samples from FBs and KCs after sham irradiation (control) or 30mJ/cm2 UVB indicated that UVB suppressed the IL-1 receptor antagonist (IL-1ra) mRNA to ~7% of the control value. In FBs treated with UVB plus recombinant IL-1α, IL-1ra mRNA levels were 15-30% of control. UVB irradiation of KCs produced small increases in IL-1ra mRNA (40-60% over control). Because the IL-1ra blocks both IL-1α and IL-1β, and both of these molecules are upregulated by UVB, we sought to compare their relative effects on TNFα production. UVB-induced upregulation of TNF-α mRNA was synergistically increased 30-40 fold, as shown by real time PCR, in the presence of IL-1β. ELISA data showed that UVB alone increased TNF-α protein levels to 3.5-fold over sham-irradiated control cells. There was an increase of 14 -fold in the presence of IL-1α??and 38-fold in the presence of IL-1β. When both cytokines IL-1α and IL-1β were administered together, no additive effects were found (32-fold as compare to 38 fold with IL-1β alone). Overall, our data indicate that UVB produces a large inhibition of IL-1ra mRNA levels in FBs, and that this inhibition is likely to enhance IL-1α and IL-1β mediated pro-inflammatory effects in the dermis.

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Optical coherence tomography (OCT) for noninvasive quantification of dermal ageing

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Purpose: To describe OCT for non-invasive imaging of aged skin Background: Current methods to measure aged skin rely on external assessment of visible signs of ageing, are difficult to quantify; skin biopsy is still the only method to quantitatively measure alteration in the dermis. Optical coherence tomography (OCT) is an optical biopsy method which produces high-resolution vertical images of skin in vivo in real time. One can reliably visualize 5mm x 1.5 mm portions of skin in under one second. OCT produces architectural information with clear demarcation of the epidermis, papillary dermis, and reticular dermis. A new modification, polarization-sensitive OCT (PS-OCT), quantitatively measures dermal collagen birefringence. We hypothesized that PS-OCT can quantitatively measure changes in chronologic ageing and photoageing, as a potential optical biopsy method to replace invasive tissue biopsy. Method: We designed and built a unique device with several OCT enhancements, including improved image quality, Doppler information, polarization sensitivity, and real-time imaging. We carried out a pilot study to examine features of chronologically aged and photoaged skin. Results: Imaging of non-aged skin revealed a strong dermal birefringence signal present in the upper dermis. In chronologically aged skin, there is a total decrease in dermal birefringence. In photoaged skin, there is a further decrease in dermal birefringence. Quantitative analysis allows one to measure these changes without tissue biopsy. Conclusion: OCT, when polarization-sensitivity is incorporated, has the potential to become a useful and practical tool for noninvasive quantitative measurement of dermal ageing. It may serve to guide laser or other skin rejuvenation interventions over time

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Eicosapentaenoic acid inhibits the UV-induced MMP-1 expression through JNK inhibition in human dermal fibroblasts

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UV radiation induces delayed UV-responsive genes, including matrix metalloproteinase (MMPs), which degrade extracellular matrix proteins (ECMs). UV-induced MMP-1 expression resulted in procollagen deficiency and wrinkle formation. UV radiation activates JNK and phosphorylates transcription factor c-Jun. Phosphorylated c-Jun formed activator protein-1 (AP-1) complex with constitutively expressed c-Fos. As the promoter of MMP-1 carries AP-1 site, UV-induced AP-1 activation increases MMP-1 expression. In this report, we investigated the mechanism of EPA to inhibit UV-induced MMP-1 expression. EPA is a dietary w-3 fatty acid and produces 3-series PGs (PGE3) by COX-2. EPA is used for modulation of inflammation and immunoresponsiveness. UV (75 mJ/cm2) radiation increased MMP-1 expression at 72 hr and pretreatment of EPA inhibited the UV-induced MMP-1 expression in a dose-dependent manner. EPA inhibited UV-induced MAPKs activation and c-Jun phosphorylation. EPA also inhibited UV-induced AP-1 activation. Inhibition of AP-1 activity resulted from decrease of c-Jun phosphorylation and c-Fos expression. Our results show that EPA inhibited UV-induced MMP-1 expression through inhibition of AP-1 activity.

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Ultraviolet radiation upregulates vascular endothelial growth factor production and downregulates thrombospondin production in human skin epidermis

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Vascular endothelial growth factor (VEGF) is a major skin angiogenic factor and thrombospondin (TSP)-1/2 are endogeneous inhibitors of skin angiogenesis. UV irradiation has been shown to be an inducer of VEGF in keratinocytes in vitro and mouse models in vivo. To determine the UV-induced cutaneous angiogenesis in human skin in vivo, we performed quantitative analysis of cutaneous blood vessels with immunostaining for the endothelial cell marker CD31, and determined the expression pattern of VEGF and TSP-1/2 with immunostaining and RT-PCR. We found that skin vascularization was greatly increased after UV exposure (2 MED) with an increase of both the number and the size of dermal blood vessels in human skin. After UV irradiation, the expression of VEGF increased and that of TSP-1/2 decreased in immunostaining. In RT-PCR, mRNA expression of VEGF was increased about 20% at 24 hr after UV irradiation compared with control skin. Maximal inhibition of TSP-1 and TSP-2 expression was about 60% and 40% at 24 hr, respectively, compared with control skin. The ratio of VEGF to TSP-1 expression was increased about 3 folds and might be an important factor in UVB-induced angiogenesis in vivo. We also confirmed the regulation of VEGF and TSP-1/2 in keratinocyte-derived cell line HaCaT. Irradiation with physiologic UV dose (40 mJ/cm2) substantially induced the expression of VEGF, whereas blocked the expression of TSP-1/2 in HaCaT. Our results demonstrate that VEGF production is upregulated and TSPs are downregulated in UV-induced angiogenesis in human skin, and the regulation of this balance may be a potential therapeutic approach for prevention of treatment of photoaging.

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Withdrawn

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Effects of different ultraviolet spectra on apoptosis pathways

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Ultraviolet (UV) radiation has profound effects on cellular function, which includes carcinogenic as well as immunomodulatory properties on the immune system. A primary effect of UV is to cause cell death by inducing apoptosis. However, the mechanism of different ultraviolet wavelengths on apoptosis pathway remains unclear. Ultraviolet radiation is separated into UVA(320-400 nm), UVB (280-320 nm) and UVC (200-280 nm). We studied the effects of different clinically relevant UV spectra to better understand how specific wavelengths of UV initiate apoptosis in primary T cells. We exposed peripheral blood mononuclear cells (PBMC) from normal volunteers to FS40 (61%UVB, 28.5%UVA and 0.5%UVC) and FS351 (80.1% UVA1, 18.5%UVA2 and 1.4%UVB) lamps. Both UV sources induced apoptosis in PBMC as determined by immunocytochemistry, flow cytometry, agarose gel electrophoresis for DNA laddering and Western blotting. The apoptotic processes are UV-dose dependent. For FS40, we demonstrated DNA laddering and detected cleaved caspase-3 at a total UV dose as low as 7.5 mJ/cm2, while the cleaved caspase-3 was detected at UV dose of 6 J/cm2 of using FS351. We also found that there was a progressive increase for cytochrome C, truncated BID (Bcl-2 Interacting Domain), cleaved caspase-3, cleaved caspase-8 and cleaved caspase-9 with increasing UV dose of FS40. For FS351 a progressive increase for cytochrome C and cleaved caspase-9 was detected, while truncated BID and cleaved caspase-8 was not detected in UV-treated PBMC for up to 8 J/cm2. Our data show that both UVA and UVB can induce apoptosis in PBMC and the process proceeds through caspases. UVB induced-PBMC apoptosis is mediated through members of the extrinsic and intrinsic pathway. The lack of truncated BID and cleaved caspase-8 detected, while the presence of cytochrome C and cleaved caspase-9 detected in FS351-treated PBMC suggested a difference in the mechanism of UVA and UVB in inducing PBMC apoptosis. This may lead to a better understanding of the therapeutic mechanism of UVA and UVB.

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Ultraviolet A -radiation destroys the architecture of plasma membrane -associated rafts in human keratinocytes

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We have shown that UVA-induced signaling in HKs is triggered by the release of ceramide from plasma membrane localized sphingomyelin (SM). Within the plasma membrane of mammalian cells, SM together with cholesterol (Chol) form microdomains (rafts) which differ according to structure and fluidity from the remaining plasma membrane and build up platforms, which are involved in signal transduction. They contain proteins including caveolin-1 (Cav1), which has been shown to be functionally involved in raft-mediated signaling. In this study, rafts were isolated from HKs which had been sham-irradiated or exposed to 30 J/cm2 of UVAR. This dose induces intracellular signaling and gene expression, but not apoptosis or necrosis. Analysis of the lipid composition of the isolated rafts revealed dramatic changes: the SM content decreased to a third of that observed in controls, corroborating previous observations that UVA-induced ceramide formation results from SM hydrolysis. In addition, the Chol of rafts from irradiated cells was decreased by more than 50%. This UVA-induced decrease in SM and Chol content in rafts was associated with a concomitant increase of those lipids in non-raft membrane fractions of the same cells. UVAR also markedly decreased Cav1 in rafts from irradiated cells, in comparison to untreated controls as shown in western blot. In order to follow the fate of Cav1 in irradiated HKs other cellular compartments were analyzed for Cav1 expression. Neither the cytosolic fraction of HKs which, in contrast to most other cell types, contain Cav1, nor mitochondria contained increased amounts of Cav1; in fact, in both cell compartments, Cav1 expression was decreased upon UVA treatment. In marked contrast, significantly increased amounts of Cav1 could be detected in the nuclear fraction of irradiated cells. Our studies indicate that UVA-induced signaling is associated with a profound disturbance in the architecture of rafts and the translocation of Cav1 from the plasma membrane into the nucleus.

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Inhibition of UV-induced MMP-1 expression by selective COX-2 inhibitor, celecoxib, in human dermal fibroblasts

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Exposure to UV radiation induces various responses in human skin. Among these responses, UV-induced MMP-1 expression resulted in procollagen deficiency and wrinkle formation. Also, UV radiation induced expression of cyclooxygenase-2 (COX-2) and secretion of prostagladins (PGs). In recent reposts, prostaglandin E2 (PGE2) has an ability to amplify its own production by inducing COX-2 expression. But, relationship of PGE2 and MMP-1 expression was not clear. In this report, we investigated whether selective COX-2 inhibitors inhibit UV-induced MMP-1 expression. UV radiation induced COX-2 expression and PGE2 production and decreased procollagen expression in a dose- and time-dependent manner. Pretreatment of celecoxib inhibited UVB-induced MMP-1 expression. In stable MMP-1 luciferase reporter plasmid-transfected NIH3T3 cells, celecoxib inhibited UVB-induced MMP-1 promoter activation. Celecoxib inhibited UV-induced AP-1 activation and c-Jun phosphorylation. Phosphorylation of c-Jun is required for formation of AP-1 complex and results from stress-activated protein kinases, especially JNK (c-Jun N-terminal kinase). Although celecoxib inhibited c-Jun phosphorylation, it did not inhibit UV-induced JNK activation. Our results demonstrate that celecoxib inhibited UV-induced AP-1 activation, thereby inhibited MMP-1 expression in a JNK-independent manner.

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Potentiation of ultraviolet B-induced apoptosis by novel phytosphingosine derivatives, tetraacetyl phytosphingosine in HaCaT cell line and mouse skin

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We previously demonstrated that the novel phytosphingosine derivatives, tetraacetyl phytosphingosine (TAPS) induced apoptosis in HaCaT. In this study, in order to reduce cumulative UV dose and to enhance apoptosis, we investigated to examine the effect of combination of the low dose irradiation of UVB and the phytosphingosine derivatives, TAPS on the induction of apoptosis. 10 mJ/cm2 UVB or 10 ?M TAPS alone showed a weak cytotoxicity, respectively, but combination of 10 mJ/cm2 UVB with 10 ?M TAPS dramatically enhanced cytotoxicity and apoptosis in HaCaT measured by MTT assay, TUNEL assay and FACS analysis. Western blot analysis showed that cells cotreated with UVB and TAPS showed higher cleaved levels of caspase-3, caspase-8 and caspase-9 than treatment with UVB or TAPS alone, suggesting that combination of UVB with TAPS synergistically induced apoptosis via extrinsic and mitochondrial apoptotic pathway. In the hairless mice, UVB irradiation alone caused to increase apoptotic cells in the epidermis and TAPS-treated mice skin showed the increased apoptotic cells in dermis as well as in epidermis. As in vitro assay, the level of cleaved caspase-3 was more effectively induced in the mice skin cotreated with UVB and TAPS than single treatment of UVB or TAPS. Then, we investigated the role of mitogen-activated protein kinases (MAPKs) in the enhancement of UVB-induced apoptosis by the TAPS. We found that UVB irradiation gradually activated phospho-ERK1/2, plateau at 6 h, and thereafter returned to control level. TAPS strongly induced ERK1/2 phosphorylation and lasted 24 hr later. However, combination of UVB with TAPS prevented TAPS induced- or UVB induced-ERK1/2 phosphorylation, suggesting enhanced apoptosis by the combination of UVB with TAPS may be due to reduction of ERK1/2 phosphorylation. These data showing the synergic apoptotic effect of the UVB and phytosphingosine derivatives shed a light on their potential use as a drug against hyperproliferative diseases like psoriasis.

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Cellular protein changes *in vitro* following recovery from mild/moderate oxidative stress and in *ex vivo* photodamaged skin tissue

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It is well known that oxidative stress contributes to human ageing and photodamage. However the long-term effects of oxidative stress remain to be fully evaluated on cell phenotype after recovery from acute stress. We treated normal human dermal fibroblasts (HDF) with 450µM hydrogen peroxide(H_2O_2) for 7 days followed by a 7-day recovery period. Cell extracts from neonatal HDF were studied by 2-D-gel/MALDI-TOF mass spectrometry and from adult HDF by western blot arrays (BD Bioscience, PowerBlot™). Protein expression changes along with other potential candidates, were examined by immunocytochemical analysis in H_2O_2 treated HDFs and in paired samples of sun-protected and photodamaged human skin sections. The latter sections provide a comparative ex vivo model for normal and in vivo recovered, oxidative stressed tissues. Proteome analysis revealed several proteins that changed in HDFs in response to H_2O_2 treatment. A number of these showed altered staining by immunocytochemistry in H_2O_2 treated cells: transgelin (Smooth Muscle (SM)22), ninein, vimentin, tropomyosin, DNA excision repair protein ERCC-1, fragile histidine triad (FHIT), and lens epithelium-derived growth factor (LEDGF/transcription factor p52/p75). With the exception of SM22 these changes have not previously been noted in other proteomic or geonomic studies. Of several candidate biomarkers selected following literature analysis, the fibronectin splice variant ED (extra domain)-A, osteonectin, thrombospondin, integrin, transglutaminase II and procollagen I also showed marked altered expression on treated cells. However, for only procollagen 1 was good discrimination observed between sun protected and photodamaged skin. The protein changes fall into two strong themes, those of cytoskeletal and myofibroblast change. In addition we confirm the importance of putative in vivo collagen synthesis in photodamage repair following UV linked oxidative stress.

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Protective role of extracellular superoxide dismutase on ultraviolet irradiation

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Superoxide dismutases (SOD) are believed to play a crucial role in protecting cells against oxygen toxicity. In human, there are three forms of SOD: cytosolic Cu, Zn-SOD, mitocondrial Mn-SOD and extracellular SOD (EC-SOD). EC SOD is primarily a tissue enzyme but the role of EC SOD in skin is unclear. In this study, we have investigated the distribution of EC SOD in the skin using immunohistochemistry and established EC SOD gene expression systems in MEF/3T3 cell and HaCaT cell lines to analyze the functional significance of EC SOD gene expression in skin. In addition, Human EC SOD was fused with a gene fragment encoding transduction domain of the HIV-1 Tat protein (RKKRRQRRR) for development of a noble material preventing from the UV-induced deterious effects of UV irradiaion. Immunohistochemical analysis showed the EC SOD was abundantly located in the epidermis as well as in the dermis. Intracellular ROS was decreased and cell death was diminished following UV irradiation in EC SOD-overexpressed cell lines. Also, the purified fusion proteins trasduced effectively into HaCaT cell in dose-dependent manner and had their activities when added exogenously in culture medium. This study demonstrate that EC SOD have the defensive roles against UV-induced injury of the skin and EC SOD fusion protein may provide a therapeutic strategy for treatment of ROS-induced skin disease or cosmetics for protection from chronological ageing and photoaging.

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Protective effect of IL-4 on UVB-induced apoptosis

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A crucial event following exposure of skin to UVB is the formation of sunburn cells within the epidermis, which are UV-damaged cells that undergo apoptosis. Induction of apoptosis following UVB exposure has been considered to be protective mechanism, getting rid off damaged cells that bear the risk of becoming malignant. IL-4, is a cytokine with various biological activities, improves the antigen presenting capacity on B cells by inducing the expression of MHC class II gene, induces the isotype switching, promotes growth and increases survival of B cells. Furthermore, IL-4 induces the proliferation and maturation of mast cells, macrophages and prolongs the survival of particular T cell clones. In this study, we generated transgenic mice that over-expressed IL-4 specifically in skin using keratin 14 promoter to investigate the effect of IL-4 in UVB-induced apoptosis. We irradiated UVB (2kJ/m2) and detected apoptosis using TUNEL assay. As the result, apoptosis in epidermis was decreased in transgenic mice compared with that in normal mice. In addition, the expression of caspase 3 was decreased in transgenic mice compared with that in normal mice after UVB irradiation. Next, to investigate whether IL-4 has the protective effect in keratinocyte directly or not, we prepared stable cell line which over-expressed IL-4 constitutively using mouse keratinocyte cell line, Pam212. The results of TUNEL assay and FACS analysis showed that apoptosis induced by UVB (200J/m2) was remarkably decreased in IL-4 over-expressed Pam212 compared with that in normal Pam212. In summary, the results in this study show that IL-4 has the protective effect on UVB-induced apoptosis and suggest that IL-4 may be an important regulator in skin-immune system against UVB.

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Antioxidative capacity, absorption and metabolism of aromatic components of an extract of Polypodium leucotomos

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An oral photoprotectant (Fernblock® from the fern Polypodium Leucotomos) has been shown to exhibit strong photoprotective properties. Recently, studies have been performed to define its chemical composition. 4-hydroxycinnamic acid (p-cumaric), 3 methoxy-4-hydroxycinnamic acid (ferulic), 3,4-dihydroxycinnamic acid (caffeic), 3-methoxy-4-hydroxybenzoic acid (vanillic) and 3-caffeoilquinic acid (chlorogenic) have been found among the major components. Despite their chemopreventive role no conclusive data exist on its antioxidative capacity in skin cells or its absorption and metabolism after an oral intake. Antioxidative capacity was evaluated by using luminol/H2O2 in vitro assay. Cytoskeleton protection in human fibroblasts was analyzed by indirect immunofluorescent assays. To study compound absorption an adenoma-derived cell (Caco-2) was used to resembled the intestinal barrier. Finally, compound metabolism was investigated by using cultured primary rat hepatocytes. As expected, antioxidative and photoprotective properties was observed in a dose dependent fashion, being caffeic acid the most powerful antioxidant molecule. Apparent permeability resulted in a value that correlate with a 70 to 100% oral human absorption for all tested substances. Cumaric, Ferulic and Vanillic acids were metabolized and conjugated to glucuronic acid and sulfate in a 35%, 50%, and 10%, respectively. Serum esterases did not participate in their metabolism. No binding to cell proteins was observed. In conclusion, we showed first evidences of antioxidant and photoprotective activity of some constituent molecules of Fernblock®, and their absorption and metabolism properties. These results may help to optimize the benefits of this photochemopreventive agent.

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Cockayne Syndrome A and B proteins and repair of oxidative damage to mitochondrial DNA: mechanism of import and functional relevance *in vivo*

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Cockayne Syndrome (CS), characterised by neurodegeneration, photosensitivity and premature aging, is caused by mutations in the CSA and CSB gene. We have shown that the CSA and CSB proteins are localised in the mitochondrion (mt) and involved in the repair of oxidative mtDNA deletions. However, their import into mt, exact type of repaired damage and functional relevance in vivo are unclear. For localisation, CSA and CSB miss a mt leader sequence but computer based k-NN sequence analysis predicted 52.2 % and 30.4 % mt localisation for CSA and CSB, respectively. Furthermore, co-immunoprecipitation showed requirement of CSA/CSB proteins in a heterodimeric state to allow mt import. Employing a reconstituted in vitro repair assay comprising fapy-glycosylase and endo III as reporter enzymes for oxidative damage in pyrimidines and purines, respectively, we could show that, after repetitive sublethal UVA irradiation, removal of thymine glycol from mtDNA was defective in fibroblasts from CS patients. However, stable transfection of the CSB gene reconstituted this defect. Semiquantitative PCR showed increased levels of mtDNA deletions in CSB knockout mice in vivo compared to wild-type littermates in the brain but not in muscle, cartilage spleen or testis, correlating with the neurodegenerative changes found in aging animals. In aggregate, these results strengthen the role of CS proteins in the repair of oxidative damage to mtDNA and indicate a heterodimeric composition of both proteins as requirement for mt import. Furthermore, the correlation of neurodegenerative symptoms and defective removal of oxidatively induced mtDNA deletions in CS knockout mice indicates a close relationship of oxidative stress, its repair by CSA and CSB and processes such as neurodegeneration and aging.

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Protective effect of commercially available sunscreens with respect to UVA-radiation

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The aim of this study was to establish the extent of protection against UVA-radiation provided by sunscreens available on the Norwegian market in 2002 and 2003. At this time, there are no defined standards for either declaration or control of UVA protection in Europe or the USA. The American Academy of Dermatology Association (AADA) has recently sent its recommendations to the U.S. Food and Drug Administration (FDA) for measurement methods and requirements for UVA protection. In this project we have used those recommended *in vitro* adapted methods. In addition, the products were tested in compliance with the Australian standard (AS/NZS 2604). A selection of samples was also tested for UVA protection at the Australian Photobiology Testing Facility, University of Sydney. The specification of UVA protection on the packaging/container was also checked and controlled. Of the sunscreens analysed (54) for UVA protection, 47 products (87 %) satisfied the requirements of the Australian standard. 40 products (74 %) satisfied the requirement for a critical wavelength ≥ 370 nm, which indicated that they give broad-spectrum protection. With reference to the results for all the UVA indicators and criteria used, we have evaluated seven sunscreens to give very weak or no protection against UVA radiation. All the products claimed to protect against UVA radiation. 25 products were labelled with a quantitative UVA protection indicator. Five of these are considered to be incorrectly labelled with respect to UVA factor. 29 products were labelled with a general UVA protection indicator. Our results show that seven of these do not give sufficient UVA protection and could be also regarded as incorrectly labelled. From our study, we can conclude that the recommended measurement criteria from AADA give a reasonably good evaluation of the protection afforded by sunscreens against UVA radiation. By using the requirement for broad-spectrum protection (critical wavelength ≥ 370 nm) we have shown that 26% of the products do not protect sufficiently against UVA.

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Regulation of UV-mediated activation of the transcription factors AP1 and NF-kB by an extract of Polypodium leucotomos

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We have previously described that an extract of Polypodium leucotomos (PL) (FERNBLOCK®), mainly composed by antioxidative phenolics, has beneficial effects in preventing acute sunburn in humans. PL has also been reported to inhibit the formation of reactive oxygen species induced by UV light and to possess anti-inflammatory properties. Skin exposure to UV light may promote the depletion of cutaneous antioxidants, leading to altered gene expression programme and finally to development of skin diseases. The redox status of the cell has been shown to be involved in the regulation of the activity and/or expression of transcription factors such as AP1 and NF-kB, whose inhibition has been demonstrated to suppress human keratinocytes transformation. The aim of the present study was to investigate its possible effect in UV-induced activation of AP1 and NF-kB in the HaCaT keratinocyte cell line. The activities of these transcription factors were analyzed by reporter gene and electrophoretic mobility shift assays. Our results showed that when PL was removed from the culture medium before irradiation, an increase of the UV-induced AP1 transcriptional activity was observed, whereas NF-kB activity remained unaffected. In contrast, both AP1- and NF-kB-dependent reporter gene transcription induced by UV radiation were significantly blocked by PL when cells were treated before and after UV light stimulation. Interestingly, when nuclear extracts of cells pre- and post-treated with PL were analyzed for AP1 by mobility shift assays, a cooperative effect of UV stimulation and PL treatment on AP1 DNA-binding activity was observed. These results suggest that PL modulates the redox state of the cells and may avoid keratinocyte transformation.

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The proactive effects of lipopolysaccharide and UV-induced cell damage by saccharomycopsis ferment filtrate in human 3D skin model

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The development of new skin care formulations requires precise assessment of their safety and efficacy. Today, legislation demands quality control combined with severe safety measures, as well as a limited use of animals for such testing. Consequently, safety assessment protocols are oriented towards in vivo tests on human volunteers and in vitro alternative methods to animal use, especially tissue engineered skin substitutes. Our previous studies demonstrate that yeast extract SFF are able to inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production in a concentration-dependent manner without notable cytotoxic effects in vitro. A decrease in NO production by saccharomycopsis ferment filtrate (SFF) was associated with an induction of heme oxygenase-1 (HO-1) protein expression and an inhibition in iNOS protein by Western blotting. There is an interesting correlation between HO-1 induction and NO inhibition. Besides, UV-induced cell damage was also prevented by SFF pretreatment. In order to further conform the protective effect of yeast extract SFF in vivo, the human 3D skin model were selected. The present study was performed to assess morphologically the effect of SFF on human bioengineered skin. Upon LPS and UV treatment, cells in human 3D model were obtained and detected by HE stain, electron microscopy and immunohistochemistry. Our data shows that SFF can prevent cells from UV and reactive oxygen species induced cell damage on human bioengineered skin. The proactive effects of SFF are conformed in vitro or in bioengineered skin models.

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Targeted inactivation of vascular endothelial growth factor sensitizes mouse skin to UVB-induced cutaneous photo-damage

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Exposure of skin to UVB results in erythema, dilation of dermal blood vessels and vascular hyperpermeability. Vascular Endothelial Growth Factor (VEGF) is one of several pro-angiogenic factors that are induced in skin after UVB irradiation, and epidermal keratinocytes (KC) are the major source of VEGF in skin.

Using the Cre/LoxP system under the control of the Keratin 5 promoter, we have generated mice in which VEGF has been inactivated in epidermal KC (VEGF-A-/-), and used these animals to study the contribution of KC-derived VEGF to acute and chronic UVB-induced photo-damage. We found that VEGF-A-/- mice developed burn-like lesions after a single UVB irradiation, whereas the control mice were unaffected. Microscopic examination of the irradiated skin showed photo-damage characterized by erythema, loss of the epidermis, increased inflammatory cell infiltration and impared vascularization in the upper dermis of VEGF-A-/-. Double immunofluorescent stains for CD31 and active Caspase 3 revealed increased numbers of apoptotic endothelial cells in UVB irradiated VEGF-A-/- mice and differential immunofluorescent stains for CD31 and Ki67 demostrated reduced numbers of proliferating endothelial cells in VEGF-A-/- mice. Quantitative analysis of cutaneous blood vessels of mice after 10 weeks of UVB irradiation showed that cutaneous vascularization was greatly diminished in mutant mice with a prominent effect on large-sized vessels and impaired endothelial cell proliferation. Several reports have implicated VEGF as a major survival factor for endothelial cells exposed to stress stimuli. Exposure of HDMVEC to UVB irradiation in the presence or absence of VEGF showed that this cytokine (100ng/ml) significantly protected the cells from death, suggesting that this may be a mechanism for maintaining the integrity of UVB irradiated skin.

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A novel role of the melanocortin 1 receptor in repair of UV-induced DNA damage and survival of human melanocytes: implications on melanoma susceptibility

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A new role for melanocortins and the melanocortin 1 receptor (MC1R) in human melanocyte survival and the repair of UV-induced photoproducts is hereby described. The MC1R gene is a major contributor to the diversity of human pigmentation. Specific allelic variants of the MC1R, namely Arg151Cys, Arg160Trp, and Asp294His, are associated with red hair phenotype, poor tanning ability and risk for melanoma. We have demonstrated that these variants represent loss of function mutations in the MC1R. We have shown that activation of the MC1R by its ligands α-melanocortin (α-MSH) or ACTH stimulates human melanocytes proliferation and melanogenesis, and enables melanocytes to overcome the UV-induced growth arrest. Recently, we found that α-MSH promotes melanocyte survival after UV irradiation by suppressing UV-induced apoptosis and activating the Akt/PKB pathway and the transcription factor Mitf. The survival effect of α-MSH is absent with loss of function of MC1R, and is independent of melanogenesis, since it is evident in tyrosinase-negative albino melanocytes. α-MSH enhances the repair of UV-induced cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts. These novel findings ascribe a new role for α-MSH as a survival factor that restores the genomic stability for human melanocytes. Our results explain why loss-of-function mutations in the MC1R increase the risk for melanoma.

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Inhibition of ultraviolet B-mediated activation of nuclear factor κB and phosphorylation of MAPKs in normal human epidermal keratinocytes by pomegranate fruit extract

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Solar ultraviolet (UV) radiation, particularly its UVB component causes many adverse effects that include hyperpigmentation, photoaging, immunosuppression and skin cancer. Pomegranate fruit extract (PFE) derived from the tree Punica granatum contains several polyphenols and anthocyanidins and possesses strong antioxidant and anti-inflammatory properties. Previously it has been shown that PFE possesses anti-tumor promoting effects in a mouse model of chemical carcinogenesis. In this study, we determined the effect of PFE on UVB-induced adverse effects in normal human epidermal keratinocytes (NHEK). Studies have shown that nuclear factor kappa B (NF-κB), plays an important role in inflammation, cell proliferation and UVB-induced effects on the skin. In dose- and time-dependent studies, we evaluated the effect of PFE on UVB-mediated modulation of the NF-κB pathway. Immunoblot analysis demonstrated that the treatment of NHEK with PFE (10-40 µg/ml) for 24 hrs before UVB (40 mJ/cm^2) exposure dose-dependently inhibited UVB-mediated degradation and phosphorylation of IκBα, activation of IKKα and NF-κB/p65. We also observed that PFE (10 µg/ml) inhibited UVB-mediated degradation and phosphorylation of IκBα, activation of IKKα, nuclear translocation and phosphorylation of NF-κB/p65 in a time-dependent manner. Because of the role of MAPK in inflammatory responses, cell growth and proliferation, we next assessed the effect PFE on UVB-mediated activation of MAPK pathway in NHEK. Employing western blot analysis, we found that PFE treatment of NHEK resulted in a dose- and time-dependent inhibition of UVB-induced phosphorylation of ERKl/2, JNKs and p38 protein. Taken together, the data shows that PFE protects against the adverse effects of UVB radiation by inhibiting UVB-induced modulation of NF-κB and MAPK pathways and provides a molecular basis for the photochemopreventive effects of PFE.

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COX-1 deletion enhances apoptosis but does not protect against ultraviolet light tumor induction

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Squamous cell cancer growth is influenced by cyclooxygenase (COX-2) activity in many mouse models: COX-2 deletion, overexpression, and COX-2 selective inhibitors. Concurrent changes in apoptosis and proliferation due to altered COX-2 activity are seen when both have been studied, suggesting each contribute to the chemoprotective effect of selective COX-2 inhibition. Recent work has shown that COX-1 deletion may be nearly as protective. In this study, we used SKH hairless mice with a selective deletion in COX-1 to examine its role in photocarcinogenesis. Wild-type, COX-1 +/- or COX-1 -/- mice were exposed to broad spectrum ultraviolet light (UV) acutely or chronically (15 weeks). For 15 weeks following UV exposure, tumor number and size were measured. 24h after cessation of chronic UV, or at the end of the experiment, proliferation (BrDU incorporation), apoptosis (TUNEL staining) and prostaglandin (PG) synthesis (ELISA) were also measured. After acute or chronic (UV) exposure, induction of PGE_2 and PGF_{2a} in epidermal extracts were ~40% less in COX1 -/- animals than in wild-type controls. A four-fold induction of keratinocyte apoptosis in COX1 -/- animals was also observed. However, only a minimal decrease in epidermal keratinocyte proliferation occurred in COX-1 -/- animals. When susceptibility to tumor formation was studied, tumor number and incidence in COX-1 -/- animals were identical to wild type controls, although average tumor size was decreased in COX-1 -/- mice. Thus, enhanced apoptosis did not protect COX-1 -/- animals against UV-induced skin cancer, suggesting other effects are most important in NSAID chemoprevention. In addition, these data contrast sharply with data obtained using the classic DMBA/TPA cancer model in which a prominent protective effect of COX-1 -/- is present. This indicates cancer mechanisms are distinct in UV- and tumor promotor-induced cancer models, and that chemoprevention strategies must specifically address cancer causes.

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Proteomic analysis of keratinocyte responses to single and combined chemical and UV exposures

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Environmental agents modify the skin through complex mechanisms. Some of these are reflected in mRNA expression, but transcriptional profiling does not capture many of the alterations that can occur in the proteome. In order to investigate the proteomic response of keratinocytes to environmental modification, we used DNBS (2, 4-Dintrobenzenesulfonic acid) and UVB radiation on the HaCaT cell line. HaCaT cells were treated with UV plus DNBS, UV, DNBS and control media. Confluent HaCaT cells were cultured in EpiLife medium (150x25 mm2 dishes) for 18h post UV irradiation with Kodacel-filtered FS40 sunlamps (20mj/cm2), with DNBS (final concentration 0.05%) for 2h. For combined exposure, DNBS was added 18h post UVB, for 2h. The cells were then washed and cultured in fresh medium for another six hours. The soluble/cytoplasmic proteins were extracted with Sigma ProteoPrep Universal Extraction Kit. Four hundred micrograms of protein were used to perform 2D gel assays. Coomassie and/or silver staining were prepared with BioRad kits. Quantity One (BioRad) and PD quest software are used to analyze protein spots on the 2D gels. Thirty eight protein spots that were well defined and approximately equal density on the gels from each of the four conditions were identified using matrix-assisted laser desporption/ionization time-of-flight mass spectrometry. The Mascot online database searching algorithm identified proteins with probability scores > 57 and which matched in each of the 4 gels for 38 of 108 spots. The density of these spots was within 15% of control cultures in 35/38 of the proteins, indicating robust consistency at both the analytic and quantitative levels. Although these spots were selected for homogeneity, glutathione transferase and the PDX6 antioxidant proteins appeared to be modestly induced by these environmental stimuli. These data validate the application of 2D gel analysis to select a full range of proteins for establishment of a functional proteomic profile.

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UV irradiation reduces type I procollagen production by transcriptional repression through a 90 base pair sequence in the promoter of TGF-β type II receptor in human skin fibroblasts

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Transforming growth factor-β (TGF-β) is a major regulator of collagen gene expression in human skin fibroblasts. Cellular responses to TGF-β are mediated primarily through its cell surface receptor complex, composed of type I (TβRI) and type II (TβRII) receptors. Ultraviolet (UV) irradiation reduces TβRII expression, thereby decreasing type I procollagen synthesis, in human skin fibroblasts. We have investigated the mechanisms by which UV irradiation decreases TβRII. UV irradiation did not alter either TβRII mRNA ($t_{1/2}$=4 hrs, n=3) or protein ($t_{1/2}$=2.5 hrs, n=3) stability, measured 2 to 8 hours after UV irradiation in the presence of transcription inhibitor actinomycin D, or protein synthesis inhibitor cycloheximide, respectively. These data indicate that UV reduction of TβRII expression likely results from transcriptional repression, rather than increased turnover of either TβRII mRNA or protein. Two transcription factors implicated in regulation of TGF-β signaling are early growth response-1 (Egr-1), and c-Jun. We utilized separate Egr-1 or c-Jun knockout cells to examine the roles of these two transcription factors in TβRII down regulation. UV irradiation strongly induced both EGR-1 and c-Jun in the respective wildtype cells. However, neither loss of EGR-1, nor c-Jun prevented UV reduction of TβRII in knockout cells. Using a series of TβRII promoter-luciferase 5'-deletion constructs (covering 2kb of the TβRII promoter) we identified a short sequence in the promoter region that was strongly regulated by UV irradiation. This promoter region exhibited high promoter activity that was inhibited 80% ($p<0.05$, n=6) by UV irradiation. Further 5'-deletion resulted in 90% (n=6, $p<0.05$) loss of UV responsiveness. Taken together, these data indicate that UV irradiation reduces TβRII expression by transcriptional repression. This repression is mediated by a 90bp region in TβRII promoter, in human skin fibroblasts.

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Oral administration of green tea polyphenols inhibits ultraviolet radiation-induced oxidative damage and expression of matrix metalloproteinases in mouse skin which may lead to prevention of photoaging

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Chronic exposure of solar ultraviolet (UV) radiation to skin results in premature aging of the skin or photoaging. UV-induced oxidative stress and induction of matrix metalloproteinases (MMP) have been implicated in this process. The use of dietary botanical supplements has received considerable interest to prevent solar UV radiation induced skin photodamage. Since polyphenols from green tea (GTP) prevent several UV-induced adverse effects in the skin, we determined whether multiple UV irradiation-induced oxidative stress and induction of MMP can be prevented in *in vivo* mouse skin by oral administration of GTP. GTP was administered in drinking water (0.2%, w/v) to SKH-1 hairless mice which were then exposed to multiple doses of UVB (90mJ/cm^2, for two months on alternate days) following *in vivo* skin photoaging protocol. Oral administration of GTP resulted in inhibition of UVB-induced hydrogen peroxide production (54-73%), and prevention of UVB-induced depletion of antioxidant defense enzymes like glutathione peroxidase (37%), catalase (41%) and endogenous antioxidant glutathione (51%) in the skin. Treatment of GTP inhibited UV-induced protein oxidation *in vivo*, a hallmark of photoaging, when analyzed biochemically, by Western blotting and immunohistochemistry. In *in vitro* treatment of GTP to human skin fibroblast HS68 cells also prevented UV-induced oxidation of proteins, thus further support the inhibition of photooxidative damage of the skin by GTP. Additionally, treatment of GTP resulted in inhibition of UVB-induced expression of matrix degrading MMPs, such as MMP-2 (67%), MMP-3 (63%), MMP-7 (62%) and MMP-9 (60%) which are critically involved in photoaging of the skin or wrinkling of the skin. These data suggest that GTP as a dietary supplement could be useful to attenuate solar UVB radiation-induced premature aging of the skin.

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Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photodamaged skin

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The present study provides evidence from a number of different approaches (i.e., comparison of cell shape in 1-µm sections of photodamaged versus healthy skin at the light microscopic level; comparison of cell shape and attachment to collagen fibrils in ultrathin sections of the same tissues examined by transmission electron microscopy; and confocal fluorescence analysis of cells stained for attachment-site and cytoskeletal proteins including β1 integrin, vinculin, focal adhesion kinase and actin) that dermal cells in healthy skin are attached to collagen fibrils over a large part of the cell border, have a flattened/spread (two-dimensional) appearance, have a large number of well-organized focal adhesion sites and have abundant actin in their cytoplasm. In contrast, cells in photodamaged skin are often in contact with fragmented collagen or amorphous debris rather than intact collagen, have a collapsed/elongated shape with few organized adhesion sites, and have a lower amount of actin. Collagen synthesis is reduced in severely photodamaged skin relative to collagen synthesis in corresponding sun-protected skin (New Eng. J. Med. 329:530, 1993). We hypothesize that fibroblasts in severely damaged skin have less interaction with intact collagen and as a result experience a reduction in mechanical tension. Decreased collagen synthesis is (presumed to be) the result.

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A novel class of chemopreventive agents for topical suppression of skin photodamage: quenchers of photoexcited states

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Based on our previous research on the role of photoexcited states of endogenous skin chromophores as sensitizers of photooxidative stress we have developed a novel class of chemopreventive agents for topical skin photoprotection: quenchers of photoexcited states (QPES). Photoexcited states of endogenous UVA-chromophores such as porphyrins, melanin precursors, and crosslink-fluorophores of skin collagen exert skin photodamage by direct reaction with substrate molecules (type I photosensitization) or molecular oxygen (type II photosensitization) leading to downstream formation of reactive oxygen species. QPES compounds antagonize the harmful excited state chemistry of endogenous sensitizers by physical quenching, facilitating the harmless return of the sensitizer excited state to the electronic ground state by energy dissipation. To develop QPES compounds for photoprotection, a primary screening assay was based on QPES-suppression of photosensitized plasmid cleavage under anaerobic conditions excluding compounds that are only antioxidants. A secondary screen tests non-sacrificial quenching of dye-sensitized singlet oxygen (1O2) formation using ESR detection of 2,2,6,6-tetramethyl-piperidine-1-oxyl [TEMPO], a 1O2-specific stable free radical. We identified a pyrrolidine-pharmacophore with pronounced QPES activity and non-cytotoxic derivatives were selected for efficacy studies in cellular models of sensitized photodamage. Protection against dye-sensitized apoptosis and suppression of proliferation was achieved in cultured human skin fibroblasts when cell exposure to light and sensitizer occurred in the presence of QPES compounds. In support of our hypothesis that direct molecular antagonism of photoexcited states by physical quenching is a unique mechanism of action for chemoprevention of skin photodamage we report here results demonstrating QPES-photoprotection of reconstructed full thickness human skin exposed to solar simulated light. Supported in part by grants from NIH (CA-43894) and Niadyne, Inc.

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Creatine enhances DNA repair in human keratinocytes and living skin equivalents

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The creatine/creatine kinase system is known to play an important role in mitochondrial function and is one of the cell's energy (ATP) storage systems. Recent work suggests that creatine induces cellular protection of neuronal cells from energy depleting agents known to cause neuronal apoptosis. Ultraviolet radiation induces ATP depleting processes, some which are consequences of oxidative, DNA, and mitochondrial damage in keratinocytes. Therefore, we hypothesized that by increasing cellular energy reserves, creatine supplementation will protect the skin from UV insults. Using different model systems, we observed that creatine treatments reduce sunburn cell formation in living skin equivalents, increase DNA repair capabilities, and increased UVB survival in human epidermal keratinocytes. Creatine reduced the number of UVB induced apoptotic bodies in Organogenesis living skin equivalents by 50%. This may be attributed to increased DNA repair as creatine was found to improve the ability of normal human epidermal keratinocytes to remove UVB induced TT dimers. In cells exposed to 24mJ/cm2 UVB, those treated with creatine showed approximately 50% of initial TT dimers to be removed, compared with no TT dimer removal in untreated cells. Creatine may enhance DNA repair processes by stabilizing or increasing the energy levels of the cell during or after environmental insults. As a result, creatine may be a valuable agent for topical use to protect the skin from UV damage because it increases the capacity of the tissue for cellular and DNA repair.

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Successful penetration of topically-applied silicon phthalocyanine photosensitizer Pc 4 and new Pc 4 salts into human skin

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Photodynamic therapy (PDT) combines a photosensitizing agent and visible light to elicit an oxidative stress mediated apoptotic response, and has been used to treat various solid organ malignancies. Phthalocyanine (Pc) 4 is the first organosilicon photosensitizing agent in Phase 1 trials. Pc 4 exhibits low toxicity and, upon I.V. infusion, is readily metabolized and cleared. To determine if Pc 4 and newly-synthesized Pc 4 salts penetrates epidermal skin, we analyzed Pc 4 fluorescence by confocal microscopy. Penetration of Pc 4 was detected using a 650nm long pass filter following laser activation at 633nm. Briefly, Pc 4 (vehicle of 70% EtOH and 30% propylene glycol) was applied epidermally to 0.4 mm thick keratomes obtained from normal human volunteers for 1, 2 and 4 hrs. Pc 4 was also prepared as Pc 4 Pyruvate and Pc 4 HCL and applied epidermally (same vehicle). Concentrations of 0.1, 0.05, and 0.01 mg/ml were tested per Pc 4 formulation. Confocal analysis of all formulations revealed Pc 4 fluorescence in membrane and/or peripheral cytoplasmic patterns at the stratum granulosum with cytoplasmic patterns in the basal layer. Maximum fluorescence was detected using a laser transmission output of 3% for the 0.1 mg/ml and 30% for the 0.01 mg/ml for Pc 4. Both Pc 4 pyruvate and Pc 4 HCl could be visualized with a laser transmission output of 2-3% for the 0.1 mg/ml and 7-9% for the 0.01 mg/ml concentrations. Because fluorescence was inversely proportional to the laser transmission at a constant laser gain, we conclude that all Pc 4 formulations successfully penetrated the skin to the basal layer in a dose dependent fashion and that the salts may have enhanced penetration capacity. Epidermal penetration is one important aspect for development of Pc 4 compounds as topical PDT drugs for epithelial cancers and inflammatory disorders.

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Effects of T4 endonuclease liposomes and RNA fragments on UV-induced DNA damage

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We have previously demonstrated that a single, suberythemogenic dose of 0.75 MED from simulated solar radiation (SSR) is sufficient to cause significant cutaneous immune suppression (65%) as measured by in vivo contact hypersensitivity (CHS) response to dinitrochlorobenzene (DNCB). RNA fragments and T4 endonuclease liposomes were significantly protective against such UV-induced suppression when applied as lotions to healthy volunteers. We next aimed to determine the effects of these topical formulations on cutaneous thymine dimer formation and oxidized guanine, following exposure to SSR using a Xenon arc lamp filtered to produce an output that is 95% UVA and 5% UVB. Data from eight subjects revealed that T4 endonuclease liposomes induced partial prevention of thymine dimer formation relative to untreated controls (28% decrease; p=0.004). This effect was not observed in vehicle treated skin. RNA fragments also provided protection against thymine dimmers induced by SSR, albeit to a lesser extent (14% decrease in thymine dimer formation as compared to untreated skin; p=0.04). The T4 endonuclease liposome and RNA fragment formulations were determined to have SPF's of 1, suggesting that their benefits can not be attributed to UV absorption. We conclude that T4 endonuclease liposomes, which are currently in clinical trials for enhancing DNA repair in patients with xeroderma pigmentosum, may be active in prevention of UV damage in skin of normal individuals as well. The mechanism of photoprotection by RNA fragments remains speculative.

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Eukarion 134 increases removal of damaged DNA in skin

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Eukarion 134, a SOD/catalase mimic, has been reported to have cytoprotective effects in several different model systems. We observed that pre-treatment with Euk-134 increases the removal of UVB induced TT dimers, and decreases UVB induced toxicity in human epidermal keratinocytes. Additionally, we further observed that pre-treatment with Euk-134 led to accelerated removal of UVB induced TT dimers and reduced levels of UVB induced sunburn cells in living skin equivalents. Classically, DNA repair pathways are not associated with cellular anti-oxidant defense pathways. However, these results suggest that Euk-134, a SOD/catalase mimic, may have a direct or indirect effect in upregulating cellular DNA repair mechanisms. These findings suggest that Euk-134 may possess properties beyond classical SOD or catalase analogues, further enhancing its ability as a cytoprotective compound.

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Chemoprevention of ultraviolet radiation-mediated skin cancer development by resveratrol: involvement of survivin

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Chemoprevention by naturally occurring plan-based agents is a newer dimension in the management of neoplasia, including skin cancer. Here, we evaluated the protective effects of *trans*-resveratrol (*trans*-3,4',5-trihydroxystilbene), a potent antioxidant found in grapes and red wine, against UVB radiation-mediated skin tumorigenesis in SKH-1 hairless mice. For our studies, we employed UVB initiation-promotion protocol where the control mice were subjected to chronic UVB exposure (180 mJ/cm^2, twice weekly, for 28 weeks). The experimental animals received either a pre-treatment (30 minutes prior to each UVB) or post-treatment (5 minutes after UVB) of resveratrol (25 and 50 µ mole/0.2 ml acetone/mouse). The mice were followed for skin tumorigenesis and were sacrificed at 24 h after the last UVB exposure, for further studies. Resveratrol treatment (both pre- and post-) resulted in a significant reduction in tumor incidence, tumor multiplicity and tumor volume/mouse. The onset of tumor was also significantly delayed by resveratrol. Interestingly, the post-treatment of resveratrol was found to impart equal, if not better, protection than the pre-treatment; suggesting that the effects of resveratrol are systemic rather than sunscreen effects. Because survivin is a critical regulator of survival/death of cells and its over-expression has been implicated in several cancers, we evaluated the involvement of survivin in chemoprevention of UVB-mediated skin carcinogenesis by resveratrol. The Western blot and Real Time-PCR analyses demonstrated a significant over-expression of survivin and phospho-survivin in skin tumors whereas treatment with resveratrol resulted in a significant inhibition in these critical molecules. The levels of Smac/DIABLO, a pro-apoptotic protein, were found to decrease in UVB-induced tumors and pre-treatment with resveratrol reversed the response. Our study demonstrated that resveratrol imparts strong chemopreventive effects against UVB exposure-mediated skin carcinogenesis (relevant to human skin cancers) via inhibiting survivin-pathway.

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T-oligo treatment enhances UV-induced DNA repair capacity in human skin explants

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UV irradiation causes DNA mutations that may lead to skin cancer. We have shown that DNA oligonucleotides substantially homologous to the telomere sequence (T-oligos) increase DNA repair capacity (DRC) in cultured human cells. To investigate the effect of T-oligos on DRC in intact skin, we treated human skin explants obtained from sun protected areas of 12 donors aged 52.5±17.2 yrs with T-oligos (pTT and pGAGTATGAG) or diluent alone. After 24 hr, paired explants from each donor were UVB- or sham irradiated and processed after 16, 24, 48 and 72 hr for histologic analysis (at least 600 linear µm skin surface/donor at 400x). There was no deterioration in sham irradiated T-oligo- or diluent-treated explants for up to 160 hr. TUNEL + cells indicating apoptosis were comparable in diluent- and T-oligo-treated UV-irradiated skin through 72 hr. Proliferating (Ki67+) cells were sparse in sham irradiated skin and absent at 16 and 24 hr after UV. Compared to diluent controls, at 48 and 72 hrs there was ~50% inhibition of UV-induced hyperproliferation in T-oligo-treated skin as measured by Ki67 + nuclei; with maximal difference at 48hr: 13±2 vs 24.7±3 (per 200 µm epidermal length/3 areas/treatment) ($p<0.03$, n=3 donors). Maximum and comparable cyclobutane pyrimidine dimers (CPD) were detected by antibody staining immediately after UV irradiation (time 0) in diluent- vs T-oligos-treated skin (n=10 donors), confirming no sun screen effect of T-oligos. CPD + cells were ~50% less in T-oligo- vs diluent-treated skin through 48 hr: 27±3% vs 51±9% at 24 hr ($p<0.001$) and 6±4% vs 12±2% at 48 hr ($p<0.05$) of all epidermal cells. At 72 hr there were 0 vs 7±2% CPD + cells ($p<0.001$). There was also increased immunostaining of epidermal nuclei for total p53 protein. Compared to controls, T-oligo-treated skin had 52% and 28% more total p53 + nuclei at 6 and 24 hr post-UV ($p<0.001$). We conclude that T-oligo treatment enhances DRC in intact human skin by prolonging UV-induced epidermal growth arrest and accelerating CPD removal.

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UV-B irradiation differentially affects VEGF production in human microvascular endothelial cells and keratinocytes

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An increased vascular response to noxious stimuli such as sunlight and emotional stress is a major characteristic of rosacea. UV-irradiation and neuropeptides, such as substance P (SP), vasoactive intestinal polypeptide (VIP) and somatostatin (SOM) have been implicated in the pathogenesis of rosacea in part by affecting vasodilatation and inflammation. Also, a possible role for vascular endothelial growth factor (VEGF) in the vascular response in rosacea has been suggested. Therefore, we aimed to define how neuropeptides alone or in combination with UV-irradiation affect VEGF production by human microvascular endothelial cells (HMEC) and keratinocytes. The HMEC-1 cell line and fresh human keratinocytes were plated at 2x10^6 and 0.5 or 0.25x10^6 cells/well, respectively, in 12 well plates and irradiated with UV-B doses from 5 to 30 mJ/cm^2. Cells were then cultured for 24 h and conditioned supernatants collected to assess VEGF production by ELISA. In HMEC-1, UV-B significantly upregulates VEGF-production at low doses (5-15 mJ/cm^2), while it downregulates at higher doses (25-30 mJ/cm^2). Although UV-B dose-dependently kills a small number of cells within 24 h, the effects on VEGF production remain the same when adjusted for the number of living cells. VIP and SP have no additional effect on VEGF production, while SOM may downregulate VEGF. In fresh keratinocytes we found a dose dependent downregulation of VEGF production in concentrations from 7.5 to 30 mJ/cm^2, even when adjusted for 24h cell death. In a preliminary experiment with cells plated at 0.5x10^6, SOM decreased VEGF production significantly in both irradiated and non-irradiated cells, while there was no significant effect at lower density. We conclude that UV-B irradiation differentially influences the production of VEGF in endothelial cells and keratinocytes, while the role of neuropeptides needs further clarification.

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IGF-1R influences the response of NF-kB in UVB-irradiated keratinocytes

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Activation of the IGF-1R is essential for keratinocyte survival and has been demonstrated to protect normal human keratinocytes from UVB-induced apoptosis. NF-kB consists of a family of inducible transcription factors involved in a variety of cellular signaling pathways, including survival following cytotoxic stress. In the epidermis, NF-kB regulation is important for normal keratinocyte proliferation and differentiation. In this study, we have investigated whether the survival function of the IGF-1R following UVB exposure involved NF-kB. Normal human keratinocytes containing activated or inactive IGF-1R were irradiated with increasing doses of UVB. At specific times following irradiation, the keratinocytes were harvested and cell lysates analyzed for NF-kB DNA-binding activity using electrophoretic gel mobility shift assays (EMSA). Specific NF-kB DNA-binding activity was induced in a time- and dose-dependent manner in both IGF-1R-active and IGF-1R inactive cells. However, the specific pattern of NF-kB binding was different in UVB-irradiated IGF-1R-activated cells compared to UVB-irradiated IGF-1R-inactive cells. We hypothesize that distinct NF-kB subunits bind to NF-kB consensus sequences following UVB-irradiation depending on the activation status of the IGF-1R.

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Transcriptional profiling of chronically sun-damaged human skin

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Intrinsic, environmental, and lifestyle factors all contribute to the process of skin aging. Photoaging, a remodeling of the dermis that arises as a result of repeated exposure of skin to UV-light has been identified as the predominant contributing factor to the prematurely aged appearance of sun-exposed skin.

To study the phenotypic changes in human skin associated with chronic sun exposure at the transcription level, we had previously undertaken a Serial Analysis of Gene Expression (SAGE) of sun-damaged and sun-protected skin from a single patient undergoing elective facial plasty. We found 34 genes with at least a 4-fold difference in mRNA levels between the two full-thickness skin samples. Prior to a detailed study of the functional relevance to photoaging of some of these genes and the proteins these genes encode, it was critical to determine the inter-individual differences in gene expression profiles. To undertake this study of inter-individual differences, we have used RNA preparations from skin obtained from 11 Caucasian patients with moderate sun-damage and analyzed the changes in mRNA levels encoded by more than 22,000 different genes represented on the Affymetrix U133A chip. We developed an algorithm that exploits dependencies between control and test chips by computing a paired t-statistic for each gene as a measure of differential expression and a false discovery rate for lists of genes called significantly changed based on permutation testing. Using this algorithm, we were able to identify 566 mRNAs with consistently altered steady-state levels as a consequence of chronic sun exposure.

In conclusion, in this study, we were not only able to expand the number of genes assed to more than 22,000, but also confirm our prior findings, indicating that many of transcript levels identified in the SAGE study are indeed altered as a consequence to chronic sun exposure.

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Effects of topical white tea and green tea in UV-induced erythema and immunosuppression in human skin

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There is growing interest in the cutaneous photoprotective effects of antioxidants. In a previous study, we found that topically applied white tea and green tea extracts partially prevent UV-induced Langerhans cell depletion and oxidative DNA damage, after irradiating with 2 MED of simulated solar radiation (SSR). We next aimed to determine whether these protective effects were secondary to direct UV absorption by these formulations. We performed a sun protection factor (SPF) analysis in five healthy volunteers with Fitzpatrick skin types I-III (MED range 20-50; mean 34). Data showed an SPF of 1.21 (range 0.75-1.57) for the white tea and and SPF of 1.05 (range 0.73-1.42) for green tea. These indicate that the photoprotective benefits observed from these formulations can not be attributed to sunscreen effects. Studies are underway to evaluate whether topical green tea and/or white tea would prevent UV-induced suppression of contact hypersensitivity responses to dinitrochlorobenzene (DNCB) in vivo.

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A topical antioxidant solution containing vitamin C, vitamin E, and ferulic acid prevents ultraviolet radiation-induced caspase-3 induction in skin

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New photoprotection methods are necessary to prevent ultraviolet injury to skin resulting in skin cancer and photoaging changes. We have previously reported the photoprotective effect of topical application of 15% L-ascorbic acid and 1% α-tocopherol (JAAD 48:866, 2003). The solution provided fourfold protection against ultraviolet irradiation and reduced cellular apoptosis measured as sunburn cells. The stability and photoprotection of 15% L-ascorbic acid and 1% α-tocopherol has been augmented by addition of 0.5% ferulic acid. This natural plant antioxidant increases the stability of L-ascorbic acid approximately threefold and doubles photoprotection of the solution against solar-simulated irradiation. In this study, we further explored the mechanism of antioxidant photoprotection by measuring caspase-3 induction. 15% L-ascorbic acid, 1% α-tocopherol, or 15% L-ascorbic acid, 1% α-tocopherol, 0.5% ferulic acid antioxidant solutions were applied daily for four days to pig skin, 0.5ml to a 7.5 x 10 cm patch. Antioxidant-treated and untreated skin was irradiated (4 minimal erythema doses) with a solar simulator fitted with a 295nm band pass filter. Twenty four hours later the irradiated skin was biopsied, frozen in liquid nitrogen and shattered. Cell protein was extracted with detergents in the presence of protease inhibitors and electrophoresed in 12% SDS-polyacrylamide gel, transferred to PVDF membranes and treated with rabbit anti-human cleaved caspase-3 antibody. Levels of activated caspase-3 in control or vehicle-treated skin were 78±42%, in skin treated with 15% L-ascorbic acid, 1% α-tocopherol were 22±16% and in skin treated with 15% L-ascorbic acid, 1% α-tocopherol, 0.5% ferulic acid were 3±2%. All values are significant when compared to each other group ($p \le 0.05$, t-test, 2-tailed). Ferulic acid stabilizes an antioxidant solution of 15% L-ascorbic acid, 1% α-tocopherol and provides added photoprotection resulting in reduced activation of caspase-3 and subsequent apoptosis.

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Ferulic acid stabilizes a topical solution containing vitamins C & E and doubles its photoprotection for skin

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Skin protects itself from UV injury with antioxidants Tissue fluids are predominantly protected by vitamin C;lipid structures by vitamin E. These vitamins are delivered to skin following absorption from the gastrointestinal tract, but controls of absorption, transport, tissue uptake and metabolism limit skin tissue levels. We have previously reported that L-ascorbic acid, protonated at pH ⟨3.5, is absorbed into skin and can augment skin's natural antioxidant protection (Derm Surg 27:137, 2001). We demonstrated that a solution of 15% L-ascorbic acid augmented with 1% α-tocopherol can provide a 4-fold increase in protection from photoinjury to skin caused by UV irradiation (JAAD 48:866,2003). The combination of antioxidants was synergistic and resulted in unexpected protection against thymine dimer formation. We have recently discovered that the stability of a solution of 15% L-ascorbic acid and 1% α-tocopherol could be increased threefold by 0.5% ferulic acid, a ubiquitous natural plant antioxidant. Thus, stability at 45 degrees C at 1 month (considered equivalent to room temperature for 1 year) was greater than 90% for L-ascorbic acid and 100% for α-tocopherol. We tested photoprotection of 15% L-ascorbic acid, 1% α-tocopherol, 0.5% ferulic acid against 15% L-ascorbic acid, 1% α-tocopherol. Solutions were applied 0.5 ml to a 10 x 7.5 cm area of shaved pig skin daily for four days. Control and antioxidant-treated skin were irradiated with a solar simulator with a 295 nm band pass filter. 24 hrs later, erythema was evaluated with a chromameter and sunburn cells were enumerated in H&E-stained sections of skin (n=3). After 4 MEDs of irradiation, erythema measured was 33.3±2.6, 26.1±0.4 and 10.8±2.7 and sunburn cells were 35±0, 24.7±8.6 and 5.0±4.6 for control, C+E, and C+E+ferulic acid respectively. Ferulic acid increased stability of a solution of vitamins C&E 3-fold and provided approximately 8-fold photoprotection against solar-simulated irradiation.

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UV-induced tolerance is thymus-dependent

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Application of haptens onto UV-exposed skin does not result in induction of contact hypersensitivity (CHS) but induces hapten specific tolerance which can be adoptively transferred. This is mediated by the generation of suppressor T cells belonging to the CD4+CD25+ sybtype of regulatory T cells (Tr). In general, Tr have been recognized to play an important role in preventing the development of various autoimmune disorders, including diabetes and thyroiditis. Thus, the primary function of Tr appears to be the inhibition of autoreactive T cells which have not been eliminated by negative selection in the thymus (central tolerance). Therefore, Tr are important components of peripheral tolerance. Although the involvement of Tr in UV-induced tolerance is undisputed, it is still unclear how and where UV-induced Tr are generated. To study whether the thymus is involved in this process, C3H/HeN mice were thymectomized at day 3 after birth. At the age of 8 to 10 weeks, CHS against dinitrofluorobenzene (DNFB) was induced by epicutaneous application. In comparison to sensitized control mice, thymectomized mice showed a more pronounced CHS response, implying that the generation of negative regulatory T cells but not T effector cells may be impaired by thymectomy. Application of haptens onto UV-exposed skin did not result in sensitization in control mice, while the CHS response was not suppressed in UV-exposed thymectomized mice. resensitization of the mice after 2 weeks with the same hapten did not cause sensitization in control mice, indicating that tolerance had developed. In contrast, thymectomized mice showed an unimpaired ear swelling response upon resensitization, suggesting that UV-induced tolerance does not develop in thymectomized mice. Finally mice which were thymectomized into kidney capsules at week 8. Upon thymus retransplantation to originally thymectomized mice regained the ability to develop UV-induced tolerance. Taken together, the data demonstrate that development of UV-induced tolerance and consequently the generation of UV-induced Tr is thymus dependent.

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Contribution of pheomelanin and eumelanin to ultraviolet-induced apoptosis

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Red- or blonde-haired individuals are typically sensitive to sunlight. We investigated the role of pheomelanin and eumelanin on skin responses to UV by using three strains of mice with the same genetic background. Black (C57BL/6J) and albino (C57BL/6-Tyrc-2J/Tyrc-2J) strains differ by one amino acid in the tyrosinase gene. The heterozygous lethal yellow strain (B6.Cg-Ay) has one normal allele and one containing a mutation in the A locus resulting in a high level of pheomelanin in the hair shafts. Fontana-Masson staining showed that eumelanin in black and yellow mice was concentrated in dermal papilla and hair shafts. No eumelanin was seen in the skin of the albino mouse or in the interfollicular epidermis of black or yellow mice. After UVB exposure (1250 J/m^2) of shaved back skin, antibody detection of thymine dimers revealed a similar depth of UVB penetration in all 3 genotypes. To assess genotypic differences in UV-induced cell death, we counted sunburn cells and performed TUNEL and caspase 3 assays. All 3 genotypes had a similar frequency of sunburn cells and caspase 3 positive cells after UVB irradiation. The TUNEL assay also corresponded to these frequencies in albino mice. Surprisingly, black and yellow mice had 3 fold more TUNEL-positive cells in the hair follicles and peri-follicular epidermis than did albino mice. Performing TUNEL assays on epidermal sheets confirmed the geographical distribution of dead cells adjacent to hair shafts. These results indicate that both eumelanin and pheomelanin promote cell death of epidermis resulting from UVB irradiation in murine skin. We conclude that one effect of melanin (eumelanin and pheomelanin) on the response of mouse dorsal skin to UV exposure could be to enhance UV damage.

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Peptide methionine sulfoxide reductase (MsrA), a protein oxidation repair enzyme, is expressed in human epidermis and upregulated by solar simulated UV irradiation

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Recently, we have reported that photoaging correlates well with the amount of protein oxidation accumulated in the upper dermis, while protein oxidation levels found in the viable epidermis are generally very low[1]. We hypothesized that this might be due to preferential epidermal expression of the peptide methionine sulfoxide reductase (MsrA), which is able to reverse methionine oxidation and thus to repair oxidized proteins. To test this, MsrA was investigated on the levels of mRNA (RT-PCR), enzyme activity (^{3}H N-acetyl methionine assay), and protein (Western blot and immunohistochemistry) in primary human keratinocytes and human skin. Indeed, high mRNA, protein and activity levels of MsrA were found in keratinocytes and human epidermal skin extracts and confirmed by immunohistochemical staining of skin biopsies (n=10). Furthermore, to investigate the impact of solar exposure on epidermal MsrA in vivo, gluteal skin of 10 human subjects was repetitively irradiated with suberythemogenic doses of solar simulated light (SSUV; n=10). Non-UV-treated contra-lateral sites served as internal controls. Remarkably, in contrast to the previously investigated enzymatic antioxidants catalase and superoxide dismutase, epidermal MsrA levels were strongly upregulated (3-fold), while dermal levels remained very low. In conclusion, this is the first report of MsrA expression in human skin and thus extends the cutaneous antioxidant network. In analogy to DNA repair systems, MsrA appears to be upregulated by acute solar UV stress and thus may play an important role in maintaining the structural and functional protein integrity of the epidermis. The lack of MsrA in dermal skin, however, might contribute to the accumulation of oxidized proteins and thus makes it a susceptible target for the aging process.

1. Sander, Chang, Salzmann, Muller, Ekanayake-Mudiyanselage, Elsner,and Thiele JJ. *J Invest Dermatol, 2002;118(4):618-25*

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Expression and activation of protease-activated receptor-2 correlate with skin color

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The protease-activated receptor (PAR-2), a seven transmembrane G-protein coupled receptor, is activated by proteolytic cleavage of its N-terminus. Activation of PAR-2 is involved in skin pigmentation by increasing melanosome transfer via phagocytosis of keratinocytes. Previous studies have shown that the pattern of melanosome distribution within the epidermis is skin color-dependent. In vitro, this distribution pattern is regulated by the ethnic origin of the keratinocytes, not the melanocytes. In vivo, kinetics of PAR-2 upregulation by UV radiation is also different in skin of color. Therefore, we hypothesized that PAR-2 may play a role in the modulation of pigmentation in a skin-type dependent manner. We examined the expression of PAR-2 and its activator, trypsin, in human skins with different pigmentary levels using Western blot analysis and immunohistochemistry staining. Here we show that PAR-2 and trypsin are expressed in higher levels, and are differentially localized in highly pigmented relative to lightly pigmented skins. Moreover, highly pigmented skins exhibit an increased protease cleavage ability specific to a peptide containing the PAR-2 cleavage site. Microsphere phagocytosis was found to be more efficient in keratinocytes from highly pigmented skins, and PAR-2 induced phagocytosis resulted in more profound microsphere ingestion and cap formation in dark skin-derived keratinocytes. These results demonstrate that expression and activation of PAR-2 correlate with skin color, suggesting the involvement of PAR-2 in ethnic skin color phenotypes.

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12-O-tetradecanoylphorbol-13-acetate suppresses melanogenesis by proteasomal degradation of tyrosinase via activation of phospholipase D2 in mouse B16 melanoma cells

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The involvement of phospholipase D (PLD) isoforms in regulating melanogenesis in mouse B16 melanoma cells was examined. Treatment of the cells with 12-O- tetradecanoylphorbol-13-acetate (TPA) induced the activation of PLD and reduced melanin content. 1-butanol, but not 2-butanol, completely blocked the TPA-induced inhibition of melanogenesis. Reverse transcription-polymerase chain reaction revealed the existence of two PLD isoforms, PLD1 and PLD2, in B16 cells. Using cells in which PLD1 or PLD2 had been introduced by adenovirus vectors, it was shown that both PLD1 and PLD2 are activated by TPA. When PLD1 or PLD2 was over-expressed following infection with the adenovirus vectors, the basal PLD activity was enhanced whilst the melanin content decreased in a virus dose-dependent manner. The effect of PLD2 on the melanin content was much greater than that of PLD1. Also, the PLD2-induced decrease in melanin content was accompanied by a decrease in the expression and activity of tyrosinase, the key enzyme in melanin synthesis. Northern blot analysis revealed that the mRNA level of tyrosinase was not altered by over-expression of PLD2. Treatment with the proteasome inhibitor MG132 completely blocked the PLD2-induced inhibition of melanogenesis. When B16 cells were infected with an adenovirus vector encoding the catalytically inactive mutant of PLD1 or PLD2, the melanin content was slightly increased in the cells containing PLD2 but not with those containing PLD1. Furthermore, the TPA-induced decrease in melanin content was not observed in cells expressing the catalytically inactive mutant of PLD2. These results indicate that activation of PLD1 or PLD2 reduces melanin synthesis but that these enzymes are independently involved in the regulation of melanogenesis. Further, our results suggest that TPA inhibits melanogenesis by activating PLD2 via acceleration of the proteolytic degradation of tyrosinase.

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Proteinase activated receptor-2 stimulates prostaglandin synthesis in keratinocytes: analysis of prostaglandin receptors on human melanocytes and effects of PGE2 and PGF2a on melanocyte dendricity

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Reports suggest that prostaglandins (PGs) mediate post-inflammatory & ultraviolet radiation (UVR)-induced pigmentation through effects on melanocyte (MC) dendricity & melanin synthesis. Proteinase activated-receptor 2 (PAR-2) is expressed by keratinocytes (KC) & we've shown that PAR-2 stimulates melanosome uptake in a Rho-dependent manner. We now show that PAR-2 activation stimulates the release of PGE2 & PGF2a in KC, HaCat, & in an epithelial cell line genetically engineered to express PAR-2, but not in vector expressing cells. We examined the effect of PGs & PG receptor expression in MC. Of the 4 PGE2 receptors (EP1-EP4) MC express EP1 & EP3, as well as the PGF2a receptor (FP) at the protein & mRNA level. PGE2 & PGF2a both significantly stimulated MC dendricity at physiologic doses & treatment of MC with EP1 & EP3 receptor agonists increased dendricity, indicating that both EP1 & EP3 receptor signaling contribute to PGE2 mediated dendricity. Western blotting for EP1-EP4 & FP receptors following UVR showed that the FP receptor is upregulated by UVR (2 J/cm2 from a solar simulator) in MC, which peaked at 18 hrs. Real-time PCR showed that regulation of FP receptor occurs at the transcriptional level. EP1 & EP3 levels were not changed by UVR & de novo expression of EP2 & EP4 was not observed. We examined the potential production of eicosinoids by MC & show that MC produce PGF2a in an UVR-dependent manner. MC do not produce PGE2, leukotriene C4 or 5-HETE. The cAMP/PKA pathway modulates MC dendricity however treatment of MC with PGF2a or PGE2 did not elevate cAMP, showing that PG-dependent dendricity is cAMP-independent. Our data suggest that PAR-2 mediates cutaneous pigmentation through increased uptake of melanosomes by KC, as well as by release of PGE2 & PGF2a that stimulate melanocyte dendricity through EP1, EP3 & FP receptors. Further, UVR-dependent regulation of FP receptors & PGF2a production in MC may mediate effects of UVR in MC dendricity.

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Mesenchymal-epithelial interactions in the skin: increased expression of dickkopf1 by palmoplantar fibroblasts inhibits melanocyte growth and differentiation

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We investigated whether the topographic regulation of melanocyte differentiation is determined by mesenchymal-epithelial interactions via fibroblast-derived factors. The melanocyte density in palmoplantar human skin (i.e. skin on the palms and the soles) is 5 times lower than that found in non-palmoplantar sites. Palmoplantar fibroblasts co-cultured with melanocytes significantly decreased their proliferation and differentiation when both types of cells were in close apposition, suggesting that a juxtacrine rather than a paracrine factor is involved. Using cDNA microarray analysis, fibroblasts derived from palmoplantar skin expressed high levels of dickkopf 1 while non-palmoplantar fibroblasts expressed higher levels of dickkopf 3. Transfection studies revealed that dickkopf 1 decreased melanocyte function, probably via the inactivation of microphthalmia-associated transcription factor (MITF). Thus, our results provide a basis to explain why skin on the palms and the soles is generally hypopigmented compared to other areas of the body, and might explain why melanocytes stop migrating in the palmoplantar area during human embryogenesis.

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Human leukocyte antigen DQA1 and DQB1 alleles are associated with genetic susceptibility to vitiligo in Chinese

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To identify susceptible and protective HLA alleles involved in vitiligo, we performed genotyping of HLA-DQA1 and -DQB1 genes in Chinese Han population. Polymerase chain reaction sequence-specific primer (PCR-SSP) method was used to analyze the distribution of HLA-DQA1 and -DQB1 alleles among 187 vitiligo patients and 273 healthy controls through Epi Info version 6 package. The frequencies of HLA-DQA1*0302 (OR=1.98, $Pc<0.01$), -DQB1*0303 (OR=3.14, $Pc<0.001$), -DQB1*0503 (OR=3.36, $Pc<0.05$) alleles were significantly increased in patients with vitiligo vs. the controls, and HLA-DQA1*0501 (OR=0.40, $Pc<0.01$) allele frequency was highly decreased. HLA-DQA1*0302 (OR=5.19, $Pc<0.001$), -DQA1*0601(OR=2.95, $Pc<0.05$), -DQB1*0303 (OR=4.50, $Pc<0.001$), -DQB1*0503 (OR=6.69, $Pc<0.001$) alleles were positively associated, whereas HLA-DQA1*0501 (OR=0.05, $Pc<0.001$) allele was negatively associated with childhood vitiligo patients, and HLA-DQB1*0303 (OR=2.76, $Pc<0.001$) allele was positively associated with adult vitiligo patients vs. the controls. The frequency of HLA-DQB1*0303 (OR=3.72, $Pc<0.001$) allele was significantly increased in localized vitiligo patients vs. the controls, whereas HLA-DQA1*0302 (OR=2.47, $Pc<0.01$), -DQB1*0303 (OR=2.67, $Pc<0.01$), -DQB1*0503 (OR=4.46, $Pc<0.01$) alleles frequencies were significantly increased and -DQA1*0501 (OR=0.27, $Pc<0.01$) allele frequency was highly decreased in generalized vitiligo patients. HLA-DQA1*0302, -DQA1*0601, -DQB1*0303 and -DQB1*0503 alleles could be susceptible alleles of vitiligo, while HLA-DQA1*0501 allele could be protective allele in Chinese Hans. There may be different genetic backgrounds between vitiligo patients of childhood and adult, localized and generalized.

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Association of HLA-A, B, C alleles with vitiligo in Chinese population

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Vitiligo is an acquired, sometimes familial depigmentary disorder of the skin and hair that results from selective destruction of melanocytes. Serological typing of HLA has shown discrepancies in HLA associated with vitiligo in different ethnic population. To identify HLA alleles that may be involved in the genetic susceptibility to vitiligo. We determined the distributions of HLA-A, -B and -C allelic frequencies by using polymerase chain reaction with sequence-specific primers (PCR-SSP) in 187 Chinese Han patients with vitiligo and 204 healthy controls. The distribution of HLA allelic frequencies was further analyzed according to the age of onset and types of vitiligo. The Results as follows: (1) The frequencies of HLA-A*2501 (14.4% vs. 3.5%, OR=4.95, $Pc<0.00001$), -A*30 (4.4% vs. 1.5%, OR=7.33, $Pc<0.001$), -B*13 (28.0% vs. 14.8%, OR=2.45, $Pc<0.01$), -B*27 (11.3% vs. 4.0%, OR=3.20, $Pc<0.01$) and -Cw*0602 (16.0% vs. 5.3%, OR=3.63, $Pc<0.0001$) were significantly increased in the patients, while the frequency of HLA-A*66 (1.9% vs. 9.8%, OR=0.17, $Pc<0.01$) was decreased, when compared with the controls. (2) HLA-A*2501, -A*30, -B*13, -B*27 and -Cw*0602 were strongly associated with the childhood vitiligo. (3) HLA-A*2501, -B*13, -B*27 and -Cw*0602 were strongly associated with the generalized vitiligo. This study demonstrated the positive associations of HLA class I alleles with vitiligo, and there may be a different genetic background among different phenotypes of vitiligo in Chinese Hans.

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The genetic epidemiology and the mode of inheritance of vitiligo

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Vitiligo occurs with a frequency of 0.1%-2% in various populations and is classified into several subtypes by its clinical presentation. Although genetic factors are thought to be involved in the etiology of vitiligo, the genetic models for different phenotypes of vitiligo are unknown. Our purpose was to explore potential genetic models for different phenotypes of vitiligo and analyze genetic epidemiological characteristics of vitiligo in Chinese people. Concerned information from 2247 patients and members in their families was collected using a uniform questionnaire. Patients" clinical characteristics and their family history were analyzed using programs concerned in SPSS 10.0 and Epi Info 6.0. A complex segregation analysis was conducted to propose potential genetic models for vitiligo. We found a consistent distribution of ages of vitiligo onset between males and females. However, different subtypes of vitiligo had different ages of disease onset. The mean onset age of segmental vitiligo was approximate 15 years old, which was significant earlier than those of the other types of vitiligo. In relatives of vitiligo patients, the risk of developing vitiligo increased with increasing blood relatedness to the vitiligo patients. According to the results of complex segregation analysis, the polygenic additive model was the best model for focal vitiligo, vitiligo vulgaris, acrofacial vitiligo and segmental vitiligo with approximately 50% heritability in each. For universal vitiligo, the best model was an environmental model. This study indicate that regardless of the genders, the onset ages of vitiligo might be associated with both the patients" clinical subtype of vitiligo and the family history of vitiligo. Different phenotypes of vitiligo might have different pathogeneses and genetic backgrounds. Onset of vitiligo is possibly affected by both genetic backgrounds and environmental factors.

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All-trans retinoic acid induces differentiation of amelanotic melanocytes of human hair follicle

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Retinoids has been shown to induce the differentiaton and inhibit the growth of melanoma cells in vitro.On the other hand,they can induce differentiation and apoptosis of murine melanocyte precursors. There is a group of amelanotic melanocytes(AMMC) which localize in the outer root sheath(ORS) of hair follicles. AMMC has been cultured successfully,and been demonstrated to be melanocyte precursors in vitro. In this study,we investigated the effects of all-trans retinoic acid (ATA) on the differention and proliferation of AMMC. AMMC were incubated with ATA of different concentrations ($0,10^{-4},10^{-6},10^{-8}$M). The morphological transformation of cells was observed by using invert microscope, and the proliferation was tested by accounting cells. Laser scanning microscopy(LSM) was employed to carry out semi-quantitive analysis of expression of tyrosinase (TYR), tyrosinase realated protein-1 (TRP-1) and tyrosinase realated protein-2 (TRP-2) after indirect immunofluorescence staining. The change of melanosomes was observed by transmission electron microscopy(TEM). Before the treatment with ATA, the AMMC were small and bipolar, and contained lots of stage I and II melanosomes. After treatment with ATA, Lots of AMMC showed dendrite formation, and became larger. All melanosomes in the treated AMMC were showed in stage III and IV . It has been demonstrated that ATA increased expression of TYR and TRP-1, but had no effect on that of TRP-2. ATA inhibited proliferation of AMMC in dose dependent manner. All results suggested ATA could induce differentiation and inhibit proliferation of AMMC. In the study about tumor cells, ATA can induce apoptosis. So we assumed that inhibition of proliferation is related to apoptosis.

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Cytokine and melanogenetic marker profiles in vitiligo vulgaris: therapeutic effects of narrow band UVB

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Vitiligo vulgaris is a major medical problem especially for pigmented races including Japanese that can result in severe difficulties in social adjustment. Therefore, it is important for dermatologists to be aware of the various options for controls of this disease. Narrow-band UVB 311 nm, new light source, which is not available widely, is fairly effective without the use of psoralens. Three representative case are 64 year-old male, 20 year-old male and 19 year-old female had been diagnosed as vitiligo vulgaris. Well-demarcated depigmented spots existed on their face, neck, back and the periphery of their fingers and toes. Histological examination revealed that melanin granules in the basal layer of the epidermis were decreased. Narrow-band UVB (0.3 J/cm2/day, 5 days/week, 8 weeks,total 12 J/cm2) and topical calcipotriol therapy was started, and after 2 weeks, dotted pigmentation appeared in the depigmented spots especially on their face. Melanin granules in the epidermis and S 100-positive melanocytes in the basal layer were increased after 8 weeks of the treatment. The relative expression of a number of key mediators of pigmentation including tyrosinase, endothelin-1, c-kit and basic fibroblast growth factor (bFGF) were analysed and quantified in immunohistochemically stained skin sections using semiquantitative image analysis. Melanocyet number was increased about threefold in the treated skin. Tyrosinase protein expression in melanocytes in each case was significantly elevated after the traeatment. Interestingly, the higher expression of bFGF, a natural mitogen for melanocytes, were found in vitiligo skin. After the treatment, bFGF protein decreased to the level of normal control. bFGF might be in involved in the pathogenic chain of events leading to vitiligo.

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Apoptotic cleavage of livin in melanoma cells

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The inhibitor of apoptosis (IAP) protein Livin is highly expressed in melanoma, and has been shown to function as a caspase inhibitor in vitro and an immunologic target in some patients. We have reported that UVB treatment of YUSAC2 melanoma cells results in Livin cleavage, generating a 30-kD fragment visualized by Western blotting (JID 120:48). To further characterize this cleavage event, we examined multiple apoptotic stimuli and cell lines and investigated potential proteolytic mechanisms. In addition to UVB, treatment with 4-TBP or cisplatin under apoptosis-inducing conditions resulted in Livin cleavage in multiple melanoma lines. Additional stimuli activating death receptor or endogenous (mitochondrial) apoptotic pathways were also tested. None of the melanoma lines were responsive to TRAIL, but forced Bax expression produced cleavage. A Livin cDNA tagged with a C-terminal V5 epitope was transiently transfected into HeLa cells, and the expressed protein was cleaved upon exposure to TRAIL as well as other stimuli. The 30-kD fragment was visualized with an anti-V5 antibody, localizing the cleavage site to the amino-terminal third of the molecule. Although this region contains a putative caspase recognition homology domain, neither mutation of this site nor addition of the pan-caspase inhibitor z-VAD-fmk blocked apoptotic cleavage in melanoma cells. By contrast XIAP, a related IAP protein, was cleaved in apoptotic melanoma cells in a caspase-dependent manner. Screening of other protease inhibitors demonstrated that Livin cleavage could be blocked by the serine protease inhibitor AEBSF. The serine protease HtrA2/Omi, known to be involved in many apoptotic responses, was detected in mitochondria and found translocated to cytosol in apoptotic melanoma cells with kinetics similar to that of Livin cleavage. These studies demonstrate that although multiple apoptotic stimuli can cause Livin cleavage, activation of the mitochondrial apoptotic pathway in melanoma cells is sufficient for this proteolytic event which appears distinct from XIAP and may be mediated by HtrA2/Omi.

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Connexins are differentially expressed in melanoma and adjacent epidermis

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Melanocyte growth is controlled by keratinocytes through paracrine growth factors and cell adhesion molecules. Disruption of this balance can alter the expression of cell adhesion and cell-cell communication molecules and lead to development of melanoma. Gap junctional intercellular communication (GJIC) plays an important role in maintaining tissue homeostasis and is a critical factor in the life and death balance of cells. In the skin GJIC is involved in the regulation of keratinocyte growth, differentiation and migration as well as in keratinocyte-melanocyte interactions. Changes in connexin (Cx) expression, in particular loss of Cx43, may result in a reduction or a loss of gap junctional activity, which contributes to tumor progression. Using microarrays and RT-PCR we show that Cx43 is not expressed in 14 of 28 melanoma cell lines and only weakly in the other 14, but is highly expressed in melanocytes, keratinocytes, fibroblasts and endothelial cells. Cx32 is expressed in 24/28 melanoma lines and, at a lower level, in all melanocytes, but not in keratinocytes, fibroblasts and endothelial cells. Cx26 is weakly expressed in 12/28 melanoma lines. In contrast, Cx30 is only weakly expressed in 3/28 melanoma lines, which were also positive for Cx43 and Cx26 and were derived from the same patient. Using immunofluorescence on human tissue sections, we did not detect Cx43, Cx26 and Cx30 in melanoma (n=8). However, we show an induction of Cx26 and Cx30 in the epidermis adjacent to melanoma, while both are not detectable in the epidermis adjacent to benign non-epithelial skin tumors (melanocytic nevi and angioma). As the expression patterns of Cx26 and Cx30 are not identical with CK6, a marker for hyperproliferation, and CK17, a marker of trauma, we suggest that the induction of these gap junctional proteins is not a reflection of reactive hyperproliferative or traumatized epidermis. We instead discuss the putative roles of these gap junctional proteins in tumor progression.

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25-Hydroxycholesterol acts in the Golgi compartment to induce degradation of tyrosinase

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In addition to its well-known effects on cholesterol trafficking, the androstenedione U18666A alters the trafficking of tyrosinase. Because oxysterols play a significant role in cholesterol homeostasis we examined the effects of 25-hydroxycholesterol on murine melanocytes. Within the family of oxysterols, 25-hydroxycholesterol in particular has been demonstrated to regulate cholesterol homeostasis via oxysterol-binding protein and oxysterol-related proteins, the sterol regulatory element binding protein, and the rate-limiting enzyme of cholesterol biosynthesis, hydroxymethylglutaryl coenzyme A reductase. Incubation of cultured murine melanocytes with 25-hydroxycholesterol induced a decrease in pigmentation with an IC_{50} of 0.34 µM and a significant diminution in levels of tyrosinase, the rate-limiting enzyme of melanin synthesis. Pulse chase studies of 25-hydroxycholesterol-treated cells demonstrated enhanced degradation of tyrosinase following ER and Golgi maturation. Protein levels of GS28, a member of an ER/cis-Golgi SNARE protein complex, were also diminished in 25HC-treated melanocytes, however levels of the ER chaperone calnexin and the cis-Golgi matrix protein GM130 were unaffected. Effects of 25-hydroxycholesterol on tyrosinase were completely reversed by 4α-allylcholestan-3α-ol, a sterol identified by its ability to reverse effects of 25-hydroxycholesterol on cholesterol homeostasis. Interestingly, incubation of cells in the absence of exogenous cholesterol altered tyrosinase processing in a manner similar to 25-hydroxycholesterol in the presence of exogenous cholesterol. We conclude that 25-hydroxycholesterol acts in the Golgi compartment to modulate pigmentation. These data further support a link between cholesterol homeostasis and pigmentation in murine melanocytes.

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Flotillin-2 transformation of a non-metastatic melanoma line to highly metastatic phenotype is associated with thrombin activated receptor PAR-1

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Flotillin-2 (flot-2) is a 41.7 kDa, highly conserved, caveolae/lipid raft associated protein of unknown function. We have previously demonstrated that more aggressive, metastatic melanoma cell lines and metastatic lesions have increased flot-2 expression. When flot-2 was transfected into a non-metastatic melanoma line (SB2), two transformed SB2 flot-2 clones with properties of vasculogenesis in Matrigel were highly tumorigenic and metastasic in the nude mouse xenograft model. To determine how over-expression of flot-2 is involved in progression of MM, we compared differential gene expression of flot-2 transfected SB2 cells by microarray analysis. When mRNA was hybridized to a cancer related DNA pathway array (CG-8) of 1100 unique sequenced 75-oligomers, the thrombin receptor (Protease Activated Receptor, PAR-1) was upregulated, and this was confirmed by quantitative RT-PCR and Western blotting. PAR-1 plays a key role in both invasion and angiogenesis leading to the metastatic melanoma phenotype. PAR-1 and flot-2 protein expression were increased in metastatic melanoma lines compared to non-metastatic lines and PAR-1 was co-immunoprecipitated with flot-2, suggesting they bind to each other. The rate of invasion of SB2 flot-2 cells through Matrigel was significantly increased (p= 0.002) if cells were pre-activated with thrombin. Small interfering flot-2 RNAs down-regulated both flot-2 and PAR-1 mRNA expression in SB2 flot-2 cells. In conclusion, flot-2 modulates expression of the thrombin receptor, PAR-1 that is implicated in progression to a metastatic phenotype, controlling invasion, angiogenesis, and actin cytoskeleton reorganization. Understanding the relationship of flot-2 and PAR-1 in melanoma progression may be of general importance for understanding tumor progression and may result in novel therapeutic intervention for the treatment of malignant melanoma.

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Proliferation, apoptosis and survivin expression in benign melanocytic neoplasms

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Apoptosis, as a counterbalance to proliferation, is important in the maintenance of tissue homeostasis and dysregulation of apoptosis likely occurs in the development of cutaneous neoplasms. We have shown previously that survivin, a member of the inhibitor of apoptosis (IAP) family, is absent in normal skin but expressed in melanoma, non-melanoma skin cancers, and benign keratinocytic lesions. We determined its pattern of expression in various types of melanocytic nevi, and attempted to correlate expression with apoptotic and proliferation markers. Six cases of each of the following nevus subtypes were retrieved from our dermatopathology archive: compound dysplastic/atypical nevus, compound nevus, intradermal nevus, neurotized intradermal nevus, and Spitz nevus. Survivin expression was evaluated by in situ hybridization using a full length biotinylated riboprobe. Proliferation and apoptotic indices were calculated by counting immunoreactive cells in TUNEL- and PCNA-immunostained sections, respectively. All nevi, regardless of histologic type, expressed survivin. Compound melanocytic lesions expressed survivin in both the epidermal and dermal compartments. Positive staining in neurotized melanocytes was seen in four of six cases (67%). The apoptotic rate was low with only rare positive cells identified in the epidermal and dermal compartments of dysplastic, compound, and Spitz nevi. Apoptotic cells were not identified in any of the neurotized nevi. The proliferative index was highest for Spitz nevi (16.6% epidermal component, 11.3% dermal component), while dysplastic/atypical, compound, intradermal, and neurotized nevi demonstrated only rare PCNA positive cells (<1% positively staining cells). This study demonstrates that survivin is consistently expressed in benign melanocytic lesions, while apoptotic cells are rarely identified suggesting dysregulation of the apoptotic pathway leads to accumulation of cells in these neoplasms.

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The effects of arsenic trioxide on mouse malignant melanoma growth *in vivo* and *vitro*

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Arsenic trioxide (As_2O_3) induces clinical remission of patients with acute promyelocytic leukemia, but its effects on melanoma are not clear. In this study, murine melanoma cell line, Cloundman S91 was examined as cellular model for As_2O_3 treatment, andAs_2O_3 induced cell death and inhibition of cell growth and colony formation were evaluated. Finally, As_2O_3 (1mg/kg/d) was administered to C57BL/6 mice inoculated with murine melanoma B16 cells to estimate in vivo efficacy, the inoculated skin tumors were identified macroscopically and were confirmed by histochemical analysis with hematoxylin and eosin staining. The S91, at high concentrations (2-16*10^{-6}M), As_2O_3 induced apoptosis and, at low concentrations (0.5-2*10^{-6}M), growth inhibition, which were enhanced by Acorbic acid (AA). The murine melanoma model showed *in vivo* tumor growth inhibition in implanted tumors with no signs of toxicity. It was found melanoma tissue necrosis increased significantly and tumor cells reduced apparently than control, the remains enlarging, cytoplasm loosening, nucleus dwindling or disappearing, presenting a typical partly differentiation shape. Our results suggest that As_2O_3 could induce mouse melanoma apoptosis, differentiation and growth inhibition, but in vivo tumor growth inhibition indicates the limit of monotherapy with As2O3 because it could not induce total cell death and combinated AA with As_2O_3 at clinically obtained concentrations may be a useful, novel therapy for melanoma.

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CP-31398 induced apoptosis in human melanoma cells is p53 mutation dependent

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p53, a tumour suppressor gene, has the ability to induce cell cycle arrest, DNA repair and apoptosis. It is also one of the most commonly mutated genes in human cancers. A small pharmacological compound, CP-31398, was recently shown to have the ability to promote proper folding and stabilize the active conformation of p53 protein, enabling it to activate transcription and slow tumour growth in mice. Here we examine the effect of CP-31398 in human melanoma cell lines with different p53 status. Five p53 wild type and three p53 mutant melanoma cell lines were screened initially for apoptotic response. All cell lines responded to CP-31398 at high concentration (≥30μg/mL), but only three were sensitive at lower concentrations (≤15μg/mL). We hypothesize that the ability of CP-31398 to induce apoptosis is p53 mutation dependent in melanoma cells. Using one p53 wild type (MMRU) and two p53 mutant (MEWO and SK-110) cell lines, we demonstrated by flow cytometry that at a moderate CP-31398 concentration (14μg/mL) only MMRU and MEWO cells undergo apoptosis but not SK-110 cells. Upon CP-31398 treatment, we showed that MMRU and MEWO cells exhibited apoptotic characteristics including DNA condensation and fragmentation but not in SK-110 cells. Immunofluorescence staining confirmed that CP-31398 enhanced p53 protein level in all three melanoma cell lines yet lacking the ability to induce apoptosis in mutant-p53 SK-110 cells. We also found that CP-31398 had the ability to alter mitochondrial membrane potential and induce the release of cytochrome c in both MMRU and MEWO cells but not in SK-110 cells. Furthermore, western blots showed that p21 upregulation after CP-31398 treatment in SK-110 is slower than CP-31398 responsive MMRU and MEWO cells, suggesting that CP-31398 may be involved in cell cycle arrest. Taken together, our results indicate that CP-31398-induced cell death is p53 mutation dependent. Elucidating the molecular mechanism by which CP-31398 induces cell death may lead to better utilization and application of this compound as an anit-cancer drug for various forms of cancer.

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Chloride induced inhibition of tyrosinase

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Tyrosinase inhibition by halide ions has been reported hitherto and the results implied that a different manner of inhibition mechanism on tyrosinase from various sources could exist. In this study, Cl^- induced inhibition of tyrosinase was kinetically analyzed to elucidate Cl^- as a potent regulatory factor in melanogenesis. Inhibition kinetic studies were carried out on mushroom tyrosinase which is commercially available as well as on human tyrosinase which is transiently over-expressed in HEK293. Chloride bound compounds such as NaCl, KCl, and NH_4Cl_2 were applied for Cl^- source. Kinetic analyses showed reversible complex inhibitions of tyrosinase by Cl^-: partial hyperbolic uncompetitive inhibition for mushroom tyrosinase and slope-parabolic competitive inhibition for human tyrosinase expressed in HEK293, respectively. In case of mushroom tyrosinase, Cl^- inhibited tyrosinase activity at ES complex state, indicating that Cl^- binding site was induced after substrate binding to enzyme. Intrinsic fluorescence study confirmed this phenomenon. For human tyrosinase, apparent Km values were significantly changed accompanying parabolic apparent Vmax changes, showing that Cl^- binding to Cu^{2+} at the active site induced competition with DOPA substrate as a mixed-type. Kinetic results indicated that physiological concentrations of Cl^- and its related channel might regulate the melanogenesis. It is worth notice that high content of Cl^- exists physiologically and tyrosinase reacts sensitively to Cl^- in the manner of complex interactions.

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Slow-binding inhibition of tyrosinase by Zn^{2+}

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Many physiological roles of Zn^{2+} in human tissues including skin have been well elucidated. In the present study, we uncovered that Zn^{2+} could act as a tyrosinase inhibitor. Zn^{2+} inhibited tyrosinase in the manner of slow-binding inhibition observed by continuous substrate reaction. Sequential studies showed that apparent rate constants were not changed by different concentrations of Zn^{2+}, however, inhibition rate constants were linearly increased by substrate with a dose-dependent manner, which is a rarely observed phenomenon in general inhibition mechanisms. In equilibrium state after slow binding, most of enzyme activity was completely inactivated by Zn^{2+} even under the low concentrations. Zn^{2+} may interact with His residues existed in the active-site pocket of tyrosinase and this ligand-binding affect the substrate accessibility to Cu^{2+} of the active site. Zn^{2+} directly bound with bovine serum albumin as well as gamma immunoglobulin observed by complete activity protection from Zn^{2+} inhibition. This result indicated that prior to applying on pigment cell, interferences of most abundant proteins in normal culture system should be considered. The general utility of Zn^{2+} as a whitening agent is currently under investigation.

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Reversible pigmentation of TXM 13 human melanoma cell line

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A suitable pigment production cell line is essential to screening and exploiting an effective whitening agent in respect of low cost, rapid growing, easy treatment, and maintaining pigmentation. In this regard, we found that TXM 13, a human melanoma cell line is a good candidate to fit these requirements. Recently, we isolated several pigmented cell clones from TXM 13 where non-pigmented cells mostly existed with pigmented cells by using limiting-dilution method. Repeated subcultures rapidly amplified non-pigmented cells compared to pigmented cells. Therefore, specific cloning of pigmented cell was needed to maintain pigmentation. Interestingly, long-term culture after confluency on plastic dish, even mostly non-pigmented cells could reversibly produce pigment conspicuously. This result indicated that the extent of confluency and incubation time were directly involved in repigmentation. From the kinetic analysis of tyrosinase activity, we found that melanogenesis was resumed by tyrosinase catalysis. We also tested other melanoma cell lines, such as TXM 1, TXM 18, TXM 40, A375SM and A375P, respectively. However, these cell lines did not produce pigment. Consequently, TXM 13 could be usefully applied to develop further whitening agents.

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Tyrosinase inhibition of active site-targeted Cu^{2+} chelators

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Tyrosinase is ubiquitously distributed in organisms and has the nature of multi-catalytic function since it has two Cu^{2+} connected individually with three histidines at the active site. Therefore, Cu^{2+} of the active site has been targeted to inhibit tyrosinase activity. DETC (diethyldithiocarbamate) was detected as a potent tyrosinase inhibitor among several Cu^{2+} chelators tested in this study. DETC significantly inhibited tyrosinase activity (IC_{50} = 53 mM) irreversibly, which was measured by the plot of v (ΔOD/min) vs. [E]. Subsequent kinetic analyses showed that parabolic competitive interaction between DETC and Cu^{2+} of the active site was observed. The secondary replot of $K_{i\,slope}$ vs. [DETC] evaluated parameters as K_i = 46.8 mM and α = 0.0662, respectively. These results indicated that DETC has a relative high affinity to Cu^{2+} at the active site of tyrosinase and effectively retard catalysis. Cell viability was tested by MTT assay in the presence of various concentrations of DETC. The result showed that viability was not affected in less than 25 mM of additive DETC. In summary, we found that DETC is a potent whitening agent to inhibit the tyrosinase active site and its kinetic behavior can be unique comparative to other well-known inhibitors.

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Effct of Aloesin on melanogenesis of human epidermal melanocytes

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Aloesin [2-acetonyl-8-b-D-glucopyranosyl-7-hydroxy-5-methylchromone], a compound isolated from the Aloe plant, has been used for centuries in cosmetics. The mechanism for bleaching of Aloesin is not clear as a compound of cosmitic. In this study, the different concentrations of Aloesin (0.01, 0.1, 1, 10mM) were incubated with human epidermal melanocyte. The cell proliferation rates, tyrosinase acitivity and melanin levels of melanocytes were determined after treatment of Aloesin, then the results of Aloesin groups were compared with the control group. Aloesin induced the death of melanocytes in high concentration (10mM), and had no effect on proliferation rates in low concentrations(0.01 mM, 0.1 mM, 1mM). We found Aloesin obviously inhibit tyrosinase activity and melanogenesis in 0.1 mM and 1mM concentrations. There was no difference of the tyrosinase activity and melanin levels between in 0.01 mM Aloesin group and the control. Our results suggest that Aloesin shows promise as a pigmentation altering agent for cosmetic or therapeutic applications.Its mechanism for depigmentation would be mainly inhibited tyrosinase acitivity.

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Assessment of 3 xeroderma pigmentosum group C gene polymorphisms and risk of cutaneous malignant melanoma: a case-control study

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Xeroderma pigmentosum (XP) patients exhibit a 1000-fold increased risk for developing skin cancers including malignant melanoma. Inherited polymorphisms of XP genes may, thus, contribute to subtle variations in DNA repair capacity and genetic susceptibility to melanoma. We investigated the role of three newly identified variant alleles of the DNA repair gene XPC in a hospital-based case-control study of 294 caucasian patients from Germany with malignant melanoma and 375 healthy control individuals from the same area matched by age and sex. We confirmed that the PAT+, Intron 11A, and the exon 15C polymorphisms are in linkage disequilibrium. The allele frequencies (cases : controls) were for PAT+ 41.7% : 36.9%, for Intron 11A 41.8% : 37.0%, and for exon 15C 41.3% : 37.3%. The observed genotype distributions matched the expected genotype distributions as predicted by the Hardy-Weinberg theory. The homozygous PAT, Intron 11, and exon 15 genotypes were associated with nonsignificantly increased risks of melanoma: OR 1.527 (95%-CI: 0.967-2.418), OR 1.526 (95%-CI: 0.966-2.414), and OR 1.425 (95%-CI: 0.906-2.246), respectively. Exploratory analyses of subgroups revealed that these genotypes might be associated with significantly increased risks for the development of multiple primary melanomas (n=28), melanomas in individuals with a low number of nevi (n=273), melanomas in individuals older than 60 years (n=100), and melanomas thicker than 1mm (n=126). Our case-control findings support the hypothesis that the PAT+, Intron 11A, exon 15C haplotype may contribute to the risk of developing and progression of malignant melanoma by increasing the rate of an alternatively spliced XPC mRNA isoform that skipped exon 12 and leads to a reduced DNA repair function.

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Brassicaceae extract exhibits lightening effect on human skin, and reduces UV-induced tanning in skin samples: *ex vivo* studies

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Skin researchers are very interested in modulating melanin synthesis in the skin given that increased skin pigmentation is found in many skin disorders, and also because hyperpigmentations have psychosocial and cosmetic effects, as well as cultural impacts, particularly in Asian countries. In order to respond to this current search for new lightening compounds for the skin, we developed an extract of fermented and hydrolyzed proteins of Brassicaceae (mustard family), and investigated its effect on melanin synthesis in ex vivo human skin. Ex vivo samples of different types of caucasian skin were submitted or not to UVB irradiatiation and to treatment with different concentrations of the extract, twice a day, for different periods of time (2-5 days). Melanin synthesis in the skin was then evaluated histologically after Fontana-Masson staining of the samples. The results of these samples showed that, compared to the untreated control skin, application of Brassicaceae extract on the skin reduced melanin level, and this effect was seen with different applied doses. Moreover, when skin samples were irradiated with 100 mJ/cm2 of UVB and then treated with Brassicaceae extract, twice a day, for 2-5 days, the results showed that application of the extract restrained UV-induced melanin synthesis, in contrast to what was observed in the UV control skin. Interestingly, this effect was more evident in skin samples that were treated for 24 hours (2 applications) with the Brassicaceae extract, prior to UV irradiation. These results were time-independent, and the effect of the extract on melanin synthesis was consistent in the different studied skin types. These studies highlight the interest of using Brassicaceae extract in skin care products and cosmetics designed for lightening purposes and for the treatment of pigmentation disorders, as well as for moderation of UV-induced tanning.

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Role of $p150^{Glued}$ and microtubules in perinuclear maintenance of phagocytosed melanosomes in human keratinocytes

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The intermediate chain of cytoplasmic dynein links the retrograde microtubular motor to the $p150^{Glued}$ subunit of the dynactin complex that associates with membrane-bound organelles. We have demonstrated that cytoplasmic dynein intermediate chain in human keratinocytes is involved in the perinuclear-directed aggregation of phagocytosed melanosomes. We hypothesize that the $p150^{Glued}$ subunit of the dynactin complex localizes to the melano-phagolysosome- and microtubule- rich perinuclear region and that disruption of microtubules releases melano-phagolysosomes from the perinuclear region. The $p150^{Glued}$ subunit was identified in extracts of isolated human keratinocytes. Double-label immunofluorescent confocal microscopy demonstrated $p150^{Glued}$ and tubulin colocalization in the melano-phagolysosome-rich perinuclear region. Time-lapse video microscopy in cultured keratinocytes were performed in controls or after 2, 10, or 20 minutes after 1 µM nocodazole and cold induced depolymerization of microtubules. Depolymerization of the microtubules by cold and nocodazole induced early dispersion of melano-phagolysosomes from the perinuclear region in well-spread cultured keratinocytes situated at the periphery of keratinocyte colonies. Taken together, these findings provide further evidence for the $p150^{Glued}$/dynactin complex linkage of melano-phagolysosomes and microtubules, and their role in maintaining perinuclear aggregation to protect the nucleus from UV-induced DNA damage.

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Gene expression profiling and tissue microarray analyses reveal osteopontin as a major invasive melanoma associated protein

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Existing therapies for metastatic melanomas remain unsatisfactory. Improved understanding of the abnormal genes expressed in metastatic melanomas may reveal new targets for therapeutic development. Previous studies using DNA microarray technology have led to discovery of critical genes involved in melanoma metastasis and invasion. However, more advanced microarray technology is available that can provide a more complete molecular portrait of melanoma that was not possible with older versions of the technology used previously. Therefore, we compared the transcriptome of metastatic melanoma nodules and normal intradermal nevi using oligo arrays with more than 20,000 gene probes. Selected genes were confirmed using tissue microarrays consisting of multiple cores of melanoma samples corresponding to different Breslow thickness. Over 160 genes were significantly over expressed in metastatic melanoma nodules compared with benign intradermal nevi (>2 fold, p<0.05). Most of the genes were not reported before to be melanoma associated. The most abundantly and specifically expressed gene is a secretary adhesion molecule, osteopontin (OPN). Using tissue microarray analysis, OPN was observed to be present in most melanoma biopsies, regardless of the thickness. In contrast, benign or dysplastic nevi or melanoma in situ did not express significant amount of OPN. Osteopontin has been implicated in various biological processes including cell adhesion, migration, and anti-apoptosis. Our data indicates that the aberrant OPN expression is associated with development of invasiveness of melanocytic lesions. Thus, abnormality in this gene occurs early in the progression of melanoma. It may serve as a target for future melanoma therapeutic development.

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Fate of melanocytes during murine hair follicle involution (catagen): leads and lessons from TRP2-LacZ transgenic mice

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Melanin synthesis in hair follicle (HF) melanocytes (MCs) is strictly coupled to the growth stage of the hair cycle (anagen) and is interrupted during follicle regression (catagen) and resting (telogen). Using TRP2-LacZ transgenic mice as a model, we investigated the MC fate during catagen. In anagen HF, melanogenically active MCs are located above the dermal papilla (DP), while amelanotic MCs are seen in the outer root sheath (ORS) and bulge. During catagen, melanogenic and amelanotic MCs show distinct patterns of migration, survival and apoptosis. MCs located in the ORS moved upward in the regressing HF, were TUNEL-negative and most likely represented surviving MCs. MC population located above the DP became divided into two distinct sub-populations heterogeneous in their fate. The MC sub-population closest to the hair shaft moved upward in the regressing HF and underwent apoptosis. These MCs also expressed Fas and lacked p55TNFR, p75NTR and p53 expressions suggesting the involvement of Fas in apoptosis of these cells. The second MC sub-population located close to the DP migrated downward through the basal lamina into the DP, which was also supported by electron microscopic studies. These cells also showed the expression of c-kit receptor, were TUNEL-negative and subsequently could be seen in the HF connective tissue sheath and/or neighboring dermis. During subsequent hair cycle, restoration of HF pigmentary unit occurs most likely from the amelanotic MCs that survive catagen and are located in the secondary hair germ of telogen HF, as well as from the MC stem cells located in the bulge. Taken together, these data provide a possible scenario and mechanisms of the remodeling of the follicular pigmentary during HF anagen-catagen-telogen transition and may be used for the establishing in vivo models for pharmacological modulation of MC apoptosis and survival during the hair cycle.

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Molecular control of hair pigmentation by BMP signaling: evidence for the cross-talk between the BMP and melanocortin receptor type 1 signaling pathways

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Hair pigmentation is controlled by tightly coordinated programs of melanin synthesis and transport from the follicular melanocytes to hair shaft keratinocytes. This process requires the involvement of signaling through the melanocortin type 1 receptor (MC-1R) that regulates the switch between pheo- and eu-melanogenesis. However, the involvement of other signaling systems, including the BMP pathway in the control of hair pigmentation remains to be elucidated. To assess the effects of BMP signaling on hair pigmentation, transgenic mice overexpressing the BMP antagonist noggin (promoter: keratin 5) were generated. Dorsal hairs of wild-type (WT) mice showed a yellow band near the tips followed by the brown-black pigmented central/proximal portions. In contrast to WT mice, K5-Noggin mice showed darkening of the dorsal hairs characterized by the absence of yellow band and by the predominance of brown-black pigment in the hair shaft. To assess the putative involvement of MC-1R signaling in these alterations of hair pigmentation, the expression levels of MC-1R, agouti signaling protein (ASP) and attractin were compared in the dorsal skin of K5-Noggin versus WT mice. By RT-PCR, MC-1R transcripts were upregulated in K5-Noggin skin, while attractin transcripts were unchanged, compared to WT skin. By Western blot analysis, K5-Noggin skin had reduced levels of ASP, compared to the WT skin. By in situ hybridization, decreased levels of ASP transcripts were observed in the matrix and outer root sheath of hair follicles of K5-Noggin mice, compared to WT hair follicles. This suggests the direct or indirect involvement of BMP signaling in controlling the expression of MC-1R and ASP in anagen hair follicle. Taken together, these data provide evidence for the cross-talk between BMP and MC-1R signaling pathways in controlling hair pigmentation and in the switch between pheo- and eu-melanogenesis during hair growth.

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Evaluation of genetic melanoma vaccines in cdk4 mutant mice

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Until recently, the development of melanoma vaccines in mice was performed in experimental models involving transplantable melanoma cells. In patients however, melanoma arises autochthonously in the skin and subsequently metastasizes to other organs. In order to more adequately model the expected clinical situation, we set out to evaluate the efficacy of a candidate vaccine approach in mice harboring an oncogenic mutation (R24C) in the germline sequence of the cyclin dependent kinase 4 (cdk4), a protein critically involved in cell cycle regulation. These mice are genetically prone to develop melanoma following carcinogen treatment of the skin. A genetic prime-boost strategy stimulated melanoma antigen-specific cellular immune responses in cdk4-mutant mice, which were associated with localized autoimmune destruction of melanocytes. However, we did not observe significant destruction of carcinogen-induced melanocytic neoplasms in the skin with this immunization strategy which was optimized for rejection of B16 melanoma cells. We conclude that experimental melanoma vaccines need to be evaluated in novel melanoma models against autochthonously developing melanomas in order to identify strategies, which eventually may be of clinical benefit for melanoma patients.

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Pep-1 as a novel probe for the detection of melanoma

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Many cutaneous melanomas express the glycosaminoglycan hyaluronan (HA) abundantly on their cell surfaces suggesting its potential to serve as a tumor marker. However, in order for HA to be a reliable marker, it is necessary to identify probes that provide contrast between melanoma cells and HA in the surrounding extracellular matrix. As a first step in identifying probes for melanoma, we compared the staining patterns of two currently available HA probes (HABP and Pep-1) using cryostat sections of normal human foreskin and normal murine ear skin. HABP (Hyaluronan Binding Protein) is a complex of the aggrecan domain and link protein while Pep-1 is a synthetic peptide (GAHWQFNALTVR) isolated from a phage display library. We observed that: 1) HABP uniformly stained both the epidermis and the dermis of human and mouse skin, and 2) Pep-1, but not a scrambled peptide control (WRHGFALTAVNQ), stained HA associated with keratinocytes in the epidermis but showed weak to no staining in the dermal matrix. Moreover, pretreatment with hyaluronidase abrogated Pep-1 staining showing HA specificity. Pep-1 staining profiles were similar in human and mouse skin. These findings may suggest that Pep-1 binds preferentially to cell associated HA. Furthermore, Pep-1 has the ideal properties for the in situ detection of HA expressed by melanoma (i.e., strong cellular staining and weak extracellular staining). To test Pep-1 as a melanoma probe, we assessed its binding to HA expressed on the surface of B16-F1 melanoma cells. Pep-1, but not a scrambled peptide control, bound B16-F1 cells. Binding of Pep-1 to B16-F1 melanoma cells was abrogated by pretreatment with hyaluronidase showing HA specificity. Next, we established cutaneous tumors in the ears of syngeneic mice by local inoculation of B16-F1 melanoma. Pep-1, but not a scrambled peptide control, detected HA associated with melanoma cells in the dermis of cryostat sections. Again, Pep-1 staining was abrogated by pretreatment with hyaluronidase showing HA specificity. These results suggest that Pep-1 may represent a novel probe for in situ detection of melanoma.

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Modulation of PKC-β expression by bone morphogenic protein-4

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Bone morphogenic protein-4 (BMP-4), a small secreted molecule belonging to the members of the transforming growth factor -β family, when added to cultured human melanocytes (hMcytes), was shown to decrease the level of melanin and the expression of tyrosinase, the key enzyme in melanogenesis. To further delineate the mechanism by which BMP-4 regulates melanogenesis, we investigated effects of BMP-4 on the expression of protein kinase C- β (PKC-β), the enzyme that activates (phosphorylates) tyrosinase. Paired cultures of primary hMcytes were treated with either vehicle or BMP-4 (25 ng/ml media) and cells were harvested after 24 and 48 hours. Immunoblot analysis using a monoclonal antibody specific for PKC- β revealed that BMP-4 reduced the level of PKC-β by 50-70% at both 24 and 48 hrs after the treatment. To determine the level at which BMP-4 regulates the expression of PKC-β, paired cultures of melanocytes were treated with vehicle or BMP-4 for 72 hrs and northern blot analysis was performed using specific cDNA against PKC-bβ. BMP-4 treatment reduced the mRNA level of PKC-β was by 50-70%. To determine whether BMP-4 could directly suppress the promoter activity of PKC-β, a PKC-β promoter-CAT reporter construct was transfected into LH human melanoma cells. A Renilla-luciferase promoter construct was co-transfected to assess relative transfection efficiency between the plates. 20-24 hours after the transfection, cells were treated with BMP-4 (25 ng/ml media) or vehicle alone and cells were harvested 48 hours after the treatment. CAT activity was normalized against luciferase activity. Compared to vehicle, BMP-4 significantly suppressed PKC-β promoter activity (2.96±2.6 vs. 3.69±2.6, p<.01), suggesting the BMP-4 may transcriptionally suppress PKC-β gene expression. These results further support the concept that PKC-β is regulated in parallel with the melanogenic genes and suggest that BMP-4 may be an attractive therapeutic target for disorders of pigmentation.

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Expression of multiple isoforms of MITF in cultured human pigment cells

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Microphthalmia-associated transcription factor (MITF), a basic-helix-loop-helix (bHLH) and bHLH-leucine zipper transcription factor, has been implicated as the master gene for survival of melanocytes and the key transcription factor for regulating the expression of tyrosinase, the rate limiting melanogenic protein. MITF was originally identified in pigment cells (MITF-M), but subsequent studies revealed that MITF is a family of multiple isoforms whose exact individual biological functions are yet to be elucidated. MITF-A, -B, -E and -H isoforms were shown to be expressed in various cell types such as heart tissues, mast cells, Hela and Cos-7 cells, and the expression of MITF-M was thought to be restricted to melanocytes. To determine if isoforms of MITF, other than the M isoform, are expressed in human melanocytes, primers specific for each of MITF-M, -A, -B, -E and -H were generated and RT-PCR was performed using RNA isolated from cultured human melanocytes. RNA isolated from Hela and Cos-7 cells were used as positive controls for the expression of MITF-A, -B, -E and -H isoforms. RT-PCR revealed that melanocytes readily expressed MITF-M, as expected, and also expressed MITF-A, -B and -E isoforms. Interestingly, MITF-H isoform was not detected. To examine whether melanoma cells express similar type of MITF isoforms, RT-PCR was performed using RNA isolated from non-pigmented human melanoma cells (NP-MM4) with the specific primers of each isoform. In NP-MM4 cells, MITF-M was readily detected and MITF-B, -E and -H isoform were detected as well. However, MITF-A, which was readily detected in normal melanocytes, was not detected. These results suggest that further understanding of function of each MITF isoform may be important in deciphering the biology of melanocytes. In particular, MITF isoforms may subserve critical functions that distinguish benign from malignant pigment cells.

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T-oligos induce MITF and tyrosinase in cultured human melanocytes

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Oligonucleotides homologous to the telomeric 3' overhang (T-oligos), repeats of TTAGGG, have been shown to induce a more differentiated phenotype in several cell types, including pigment cells. To investigate the mechanism by which T-oligos induce differentiation in human melanocytes, effects of T-oligos on the levels of tyrosinase, the key and the rate-limiting enzyme in melanogenesis, and microphthalmia-associated transcription factor (MITF), a key transcription factor for the tyrosinase gene, were examined. Paired cultures of subconfluent primary human melanocytes supplemented once with either the T-oligo pGTTAGGGTTAG (40 µM) or control (complementary) oligonucleotide pCAATCCCAATC (40 µM), then harvested at days 2,3,4 and 5 after treatment, and immunoblot analysis was performed using specific monoclonal antibody against tyrosinase. The level of tyrosinase protein in T-oligo treated samples remained unchanged at day 2, but were induced by 2-4 folds at days 3, 4, and 5, compared to the control. To assess if T-oligos could also induce the level of MITF, paired melanocyte cultures were treated once with T-oligos (40 µM) or control oligonucleotides (40 µM) and harvested on days 3 and 5. T-oligo similarly induced the protein level of MITF by 2-3 folds at days 3 and 5, compared to control samples. These data expand earlier studies demonstrating that thymidine dinucleotides (pTT, identical to one-third of the telomere repeat sequence), induce tanning (increased melanogenesis) by upregulating mRNA and protein levels of tyrosinase, acting in part through p53 activation. In combination, the data support the concept that tanning is largely a DNA damage response in which UV-induced DNA photoproducts lead to telomere loop disruption and consequent exposure of the 3' overhang sequence, a signal mimicked by providing T-oligos to cultured melanocytes.

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The Foxn1 transcription factor converts epithelial cells into targets for pigmentation

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Foxn1 (Whn, Hfh11) is a transcription factor containing a winged-helix DNA-binding domain and a negatively charged transactivation domain. In rodents, the loss of Foxn1 function causes the nude phenotype, which is characterized by the lack of visible hair, structural abnormalities in the epidermis, defects in lactation, and the absence of a thymus. To assess the effects of a gain of Foxn1 function, we generated transgenic mice that express *Foxn1* from the keratin 5 (Krt5) promoter. This promoter targets Foxn1 to basal epithelial cells of the epidermis and hair follicles, cell types generally lacking endogenous Foxn1. In three independent lines of mice, the *Krt5-Foxn1* transgene caused changes in skin pigmentation. In contrast to wild-type epidermis, transgenic epidermis contained melanin, and the basal layer exhibited numerous melanocytes. At the same time, the transgenics showed no abnormalities in coat color or density, suggesting that melanocytes entered the hair follicles in normal numbers. Consistent with its effects on pigmentation, the transgene induced keratinocytes to secrete at least one diffusible factor known to regulate melanocyte behavior. Thus, the transgene broadened the actions of the pigmentary system and made it more human-like, as the epidermis joined the hair as a target for melanization. In wild-type hair follicles, Foxn1 was most abundant in the differentiating precursors of the cortex, which receive pigment from melanocytes. To a lesser extent, Foxn1 was also detected in the differentiating medulla, the other melanized structure of the hair. Consistent with this distribution of Foxn1, nude mice exhibited a deficiency of pigment in the hair cortex. Thus, at specific sites in the skin, Foxn1 confers on epithelial cells the properties necessary for a functional association with melanocytes. In all, the results suggest a mechanism by which epithelial cells signal to melanocytes, identify themselves as pigment recipients, and attract the dendrites, thereby inducing their own pigmentation.

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Combined modality *in vivo* confocal laser scanning microscopy for imaging melanoma progression in murine skin

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This study was aimed to develop and assess a non-invasive imaging system for in vivo evaluation of the growth and progression of melanoma in murine skin. A system was designed and developed for simultaneous fluorescence and reflectance mode in vivo confocal imaging of murine skin utilizing a 488nm (fluorescence mode) and 820nm (reflectance mode) light source. B16 melanoma cell lines were inoculated intradermally into transgenic C57BL/6-TgN (ACTbEGFP) 10sb mice (Jax Laboratories). The inoculation sites were imaged sequentially over a two week period. Intravascular injection of fluorescein dextran was used to assess tumor associated changes in the microvasculature. The in-vivo confocal images were correlated with ex-vivo conventional and fluorescence microscopy. The combined modality system provided single cell resolution and adequate image registration. In fluorescence mode the melanoma appears as a negative image within the background of GFP expressing murine cells. A population of fluorescent dendritic cells was observed in close apposition to the tumoral cells. The dermal microvasculature was readily assessed. In conclusion, combined reflectance/fluorescence in vivo confocal laser scanning microscopy holds significant promise for studies of tumor progression in murine skin.

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Reflectance spectrophotometry: a rapid, standardized and objective measure of phototype

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The purpose of this study was to develop an objective measure of skin phototype by reflectance spectrophotometry and evaluate how well it correlates with subjective assessments. Male and female healthy (85) and familial melanoma subjects (85), ages 18-72 yrs from varied racial and ethnic backgrounds were evaluated for skin phototype by trained investigators according to Fitzpatrick skin types (FST) I-VI criteria vs. the area-under-the-reflected light intensity over the 450-615 nm spectrum interval curve (AUIC) measured by a reflectance spectrophotometer. AUIC was calculated for the upper inner arm (photo-protected) and the dorsal forearm (photo-exposed) in all subjects. Inner arm had higher AUIC than dorsal arm and correlated well (r=0.9) as a function of clinician-assessed FST and ethnicity. Dorsal arm AUIC values changed as a function of season over a 2 yr period, with highest values in winter and lowest values in summer and thus, did not provide a consistent temporal site for skin phototyping. In contrast, the upper inner arm produced discriminating AUIC values between known FST I-VI (highest AUIC values in FST I, lowest AUIC values in FST VI) with the lowest within-subject (3% RSD), between-site, same region (5% RSD), and temporal variability (8% RSD). Clinician-assessed FST vs. inner arm AUIC fit a quadratic equation (r2=0.81), in which clinician and back-calculated instrument skin type agree +/- 1 FST. Self-assessment FST agreed <25% with instrument-determined skin phototype in FST I-III, but agreed 50-100% in FST IV-VI. Skin typing 279 healthy subjects using the instrument method alone fit a quadratic equation (r2=0.94) and 90% confidence interval that statistically differentiated between skin types I-VI. Thus, the reflectance spectrophotometric measure of the upper inner arm provides a rapid, easily accessible, noninvasive, reproducible and accurate method to objectively determine skin phototype. Whether this objective method is predictive of skin cancer risk remains to be elucidated.

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The role of Apaf-1 expression in human melanoma progression

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Malignant melanoma is a life-threatening skin cancer due to its highly metastatic character and resistance to radio- and chemo-therapy. It is believed that the ability to avoid apoptosis is the key mechanism for the rapid growth of cancer cells. However, the exact mechanism for failure in apoptosis pathway in melanoma cells is unclear. P53, a tumor suppressor gene which is the most frequently mutated gene, is a key apoptosis inducer. However, p53 mutation is only found in 15-20% of melanoma biopsies. Recently, it was found that Apaf-1, a downstream target of p53, is inactivated in metastatic melanoma. Specifically, loss of heterozygosity (LOH) of the Apaf-1 gene was found in 40% of metastatic melanoma. To determine if loss of Apaf-1 expression is indeed involved in melanoma progression, we employed the tissue microarray technology and examined Apaf-1 expression in 71 human primary malignant melanoma biopsies by immunohistochemistry. Our data showed that Apaf-1 expression is significantly reduced in melanoma cells compared with normal epidermis (P<0.005). Moreover, 17 of 71 (24%) melanoma biopsies showed loss of Apaf-1 expression and this Apaf-1 loss is significantly associated with the locations of the tumors. Fifty percent of the tumors grown in sun-exposed sites, whereas only 18% of the tumors in sun-protected sites, show loss of Apaf-1(P<0.05). Our data also revealed that loss of Apaf-1 was not associated with the tumor thickness or subtype, patients gender, age, or 5-year survival. Taken together, our data indicate that Apaf-1 expression is significantly reduced in human melanoma and loss of Apaf-1 could be caused by sun exposure. Loss of Apaf-1 expression may play a role in the initiation rather than progression in melanoma development.

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ING2 enhancement of apoptosis in melanoma cells after UV irradiation

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ING2, a recently cloned gene, is a homologue of the novel tumor suppressor gene, ING1b, which has been shown to enhance UV-induced apoptosis synergistically with functional p53 in melanoma cells. Recently, it has been shown that the ability of ING2 to induce apoptosis requires an intact PHD finger. However, the molecular mechanisms by which ING2 mediates apoptosis are yet to be determined. We hypothesized that ING2 enhances apoptosis after UV irradiation in a p53-dependent manner in melanoma cells. We found that overexpression of ING2 enhanced UV-induced cell death in MMRU cells compared to the control by flow cytometry and trypan blue exclusion assay. Induction of cell death by UV was further confirmed by propidium iodide (PI) staining. Upon UV irradiation, MMRU cells clearly showed apoptotic characteristics such as nuclear condensation. The enhancement of UV-induced apoptosis was abolished when we co-transfected MMRU with ING2 and mutant p53. Furthermore, this enhancement was not observed in the mutant p53 cell line MEWO. These observations strongly suggested that ING2-mediated apoptosis after UV irradiation is dependent of p53 in melanoma cells. We also found that ING2 altered the mitochondrial membrane potential of the UV-irradiated cells. Moreover, overexpression of ING2 enhanced the expression of endogenous Bax. Upon UV irradiation, Bax translocated to mitochondria and cytochrome c was released into cytosol. However, cells overexpressing ING2 were shown to have a minimal effect in these processes. Similarly, cells overexpressing ING2 showed little increment of the cleavage of caspase 9 and 3 after UV irradiation. These observations suggested that ING2 enhanced UV-induced cell death via mitochondrial apoptotic pathway, although alternative pathway may be involved.

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XPA gene polymorphism is associated with risk of cutaneous melanoma

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Sun exposure is widely accepted as the major environmental risk factor associated with skin cancer, including cutaneous melanoma. UV rays from sunlight cause DNA damage, including the formation of pyrimidine dimers, i.e., photoadducts. Lesions of this type are repaired through the nucleotide excision repair (NER) pathway. Proteins encoded by the xeroderma pigmentosum (XP) gene family are critically involved in NER. Polymorphic variants of the XP genes may influence an individual's inherent DNA repair capacity (DRC) and thereby affect the level of UV-induced DNA damage accumulated in melanocytes. In an ongoing study, we are examining the relationship between eight single nucleotide polymorphisms (SNPs) in five of the XP genes and risk of developing cutaneous melanoma. Peripheral lymphocyte DNA has been obtained from ninety-six invasive cutaneous melanoma cases recruited from the Fox Chase Cancer Center (FCCC) Melanoma Family Risk Assessment Program. DNA from cancer-free controls, matched to cases for age, gender, and ethnicity, was obtained from the FCCC Biosample Repository. SNPs are being typed utilizing pyrosequencing or PCR-RFLP assays. A significantly increased risk for melanoma has been found to be associated with the XPA 23 G allele (odds ratio=1.66; 95% confidence interval 1.1-2.5). This risk is enhanced in females and individuals with green or blue eyes. No increased risk for cutaneous melanoma has been found to be associated with SNPs in the XPC, XPD, XPF or XPG genes. These results suggest that the XPA A23G polymorphism contributes to genetic susceptibility to melanoma and differences in DRC may influence individual melanoma risk.

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Nuclear reduction of X-linked inhibitor of apoptosis protein-associated factor 1 in cutaneous malignant melanoma

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Dysregulation of apoptotic processes is likely one of the key factors contributing to the malignant nature of melanoma marked by strong chemoresistance. X-linked inhibitor of apoptosis protein (XIAP) suppresses apoptosis through the inhibition of activities of various caspases. Recently, XIAP-associated factor 1 (XAF1) has been identified as a nuclear protein that antagonizes the anti-apoptotic activity of XIAP by nuclear translocation of XIAP. In this study, we want to determine whether XAF1 plays a role in melanoma progression. Immunohistochemistry were performed on tissue microarray representing 73 primary melanomas to evaluate the expression of XAF1. We show that greater than 70% of the tumor tissues have reduced expression of XAF1 in nucleus and cytoplasm when compared to adjacent normal tissue. Specifically, 7 of 73 (10%) tumor tissues show loss of XAF1 expression. The percentage of nucleus and cytoplasm stained positive for XAF1 is significantly less in tumor tissue. In addition, thicker tumors are associated with a decrease in XAF-positive nucleus ($P < 0.05$, chi-square test). No correlation is found between XAF1 expression with age, gender, histological subtype and location of tumors. Our results suggest that nuclear loss of XAF1 may contribute to melanoma progression. Further study is required to understand the molecular mechanisms governing the selective loss of XAF1 expression in the nucleus of tumor tissue.

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Contribution of melanocortin 1 receptor gene variants to cutaneous sporadic melanoma risk in an Italian population

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Melanocortin 1 receptor (*MC1R*) gene variants have been recently shown to be low-penetrance melanoma susceptibility alleles in fair-skinned Australian and Northern European populations. We analyzed the contribution of the *MC1R* genotype to melanoma risk in an Italian population of 100 patients with cutaneous sporadic melanoma and 100 control subjects. The entire coding sequence of the *MC1R* gene was investigated by DNA sequence analysis and logistic regression models were used to evaluate the association between allelic variants of the *MC1R* gene and risk of cutaneous sporadic melanoma. We detected a total of 30 *MC1R* variants, including six novel low-frequency alleles (R34R, A111V, V119V, R213W, C315R, 123_124insT), with the V60L variant being the most frequent allele in our population. When the different variants were analyzed separately, the R151C and V60L variants, in heterozygous form, were significantly associated with cutaneous melanoma with OR= 3.57 (95% CI 1.22-13.11) and 1.82 (95% CI 1.03-3.27), respectively. The D294H allele was detected exclusively among individuals with melanoma, occurring in 6 melanoma cases and in none of the control subjects. No relationship between the V92M, R142H and R160W variants and melanoma could be demonstrated. No differences in the ORs were found after adjustment for skin type, hair color and eye color. These results provide evidence for a predisposing role of the V60L, R151C and D294H *MC1R* variant alleles to cutaneous sporadic melanoma risk in an Italian population.

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Telomere-homologue oligonucleotides induce apoptosis in human melanoma cells; co-treatment with all-*trans* retinoic acid or 1,25-dihydroxy-vitamin D3 potentiates this effect

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Disruption of the telomere loop structure and exposure of the 3′telomere overhang TTAGGG tandem repeat sequence induces a senescent phenotype as well as DNA-damage responses in human cells. Single-stranded DNA-oligonucleotides, homologue to the 3′overhang of human telomeres (T-oligos), have been shown to mimic these responses in the absence of telomere disruption in normal human cells as well as Jurkat cells. Here, we investigate whether three highly metastatic human melanoma cell lines (MV3, G361 and UISO-Mel6) undergo apoptosis after treatment with an 11-base long T-oligo and compare this to treatment with all-trans retinoic acid (ATRA), 1,25 dihydroxy-Vitamin D3 (VitD3) as well as combination of these compounds. Cells were incubated with either 20μM T-oligo, 10nM VitD3 or 1μM ATRA alone or with a combination of T-oligo/VitD3 or T-oligo/ATRA with the same concentrations. After 48 hours, cells were collected and processed for TUNEL-assay, FACS analysis and fluorescence microscopy. While treatment with the T-oligo led to a 1.7-fold increase compared to diluent treated cells, VitD3 and ATRA resulted in a 1.3-fold increase in apoptosis, respectively. The combined treatment with T-oligo/VitD3 led to an 11.9-fold increase and for T-oligo/ATRA a 9.4-fold increase compared to diluent. Thus, combining these treatments potentiates the pro-apoptotic effects. Nuclear receptors and mechanisms by which ATRA and VitD3 regulate cellular differentiation and apoptosis have been characterized, however, little is known for T-oligos. So far, no specific receptor has been identified; yet, it has been shown that they localize to the nucleus and share common downstream regulators such as p21 and the E2F transcription factor. Further studies are needed to elucidate the spectrum of cellular interactions for T-oligos. Combining T-oligos with ATRA or VitD3 or with cytotoxic agents might enhance therapeutic options for malignant melanoma.

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PIGMENT: a community-based trial of a new triple-combination agent for the treatment of facial melasma

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An 8-week, multicenter, open-label, community-based study evaluated a newer therapeutic approach to the treatment of recalcitrant melasma. The study drug was a combination of tretinoin 0.05%, hydroquinone 4.0%, and fluocinolone acetonide 0.01% in a hydrophilic cream formulation (Tri-Luma cream). The trial enrolled 1290 patients of diverse races and ethnicities as well as the full range of Fitzpatrick skin types (I through VI). The cream was applied to the affected areas of the face once daily for 8 weeks. Evaluation of melasma was performed at baseline, week 4, and week 8, using the Melasma Area and Severity Index (MASI) and Investigator Global Assessment of Improvement. Patients also completed a quality of life (QOL) questionnaire at baseline and endpoint to determine the impact of melasma and its treatment. The mean Melasma Area and Severity Index (MASI) decreased significantly ($P<.0001$) at both weeks 4 and 8 compared to baseline in the overall study population and across all Fitzpatrick skin types and races/ethnicities. The mean MASI darkness and homogeneity scores likewise fell significantly ($P<.0001$) at weeks 4 and 8 in all facial regions of interest and all Fitzpatrick skin types. By week 8, investigators global evaluations showed that 75% of patients had Moderate or Marked Improvement or were Almost Clear or Clear. The QOL parameters generally improved: for example, 67% of patients indicated they were less embarrassed or self-conscious and 58% were less likely to spend time hiding hyperpigmentation. The most common adverse events were skin irritation (6%) and erythema (4%). No serious adverse events were reported and discontinuation due to an adverse event was rare (3%). Tri-Luma cream was found to be safe and well tolerated. The study results demonstrate that this new triple formulation produces rapid, significant improvement of melasma across the range of patients seen in daily practice.

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A novel role for prohibitin in melanogenesis discovered using small-molecule probes

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Pigmentation is a complex cellular process involving multiple regulatory elements. Many of the genes involved in this biological pathway remain to be identified. An attractive gene-identification approach known as "chemical genetics" involves the screening, based on observable phenotypic properties, of small molecules in cells. This approach mimics the logic of classical genetic screens; however, instead of creating a mutation in a known protein, small molecules bound to affinity matrices are employed to isolate the target protein responsible for the cellular phenotype without prior knowledge of what that target would be. Here we describe the use of a chemical genetic approach to dissect the mammalian pigmentation pathway and identify novel protein participants. Using this approach we have screened a library of 1,200 triazine-based small-molecules and discovered a new class of potent pigment enhancing chemicals. From this class of promoters we have characterized the small-molecule *melanogenin*. *Melanogenin* enhances pigmentation in cultured wild-type melanocytes up to 4-fold at noncytotoxic concentrations (2.5-5μM). Using *melanogenin* bound to an affinity matrix and microsequencing, we have identified its specific intracellular binding target as the mitochondrial protein prohibitin. Subsequent studies employing siRNA have demonstrated that prohibitin is required for *melanogenin* to exert its pro-pigmentary cellular effects and reveal an unsuspected functional role for this protein in mammalian melanin induction. This represents a novel mechanism by which pro-pigmentary signals are transduced. *Melanogenin* itself may also serve as a new structural basis for designing future therapeutics to treat hypopigmentation. In conclusion, this work provides strong proof-of-concept for the "chemical genetic" approach to the dissection of mammalian melanogenesis, the identification of a novel role for prohibitin in the regulation of pigmentation and the discovery of a new class of research tools and potential therapeutic molecules.

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PC cell-derived growth factor in melanocytic tumors: a potential role in melanoma and other melanocytic neoplasms

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PC cell derived growth factor (PCDGF), also known as progranulin, is the precursor of granulin, a novel growth factor. PCDGF has been shown to stimulate cellular proliferation, confer epithelial tumorigenesis, and promote tumor invasion. Previous work has focused on PCDGF in epithelial tumors. To our knowledge, this is the first study on the expression of PCDGF in melanocytic tumors. Melanocytic tumors including 13 common nevi, 12 Spitz nevi, 10 dysplastic nevi, 9 superficial spreading melanomas, 5 desmoplastic melanomas, and 10 in-transit metastatic melanomas were examined using a monoclonal anti-PCDGF. Immunoreactivity was scored based on the number of tumor cells positive (0+ <5%, 1+ 5-25%, 2+ 26-50%, 3+ >50%). Strong PCDGF expression (2-3+) was seen in the majority of dysplastic nevi, Spitz nevi, superficial spreading melanoma, desmoplastic melanoma, and metastatic melanoma. The strongest expression was seen in Spitz nevi and the melanoma groups. Common nevi were essentially negative with only 1/12 showing 1+ immunoreactivity. There were some qualitative differences in the staining patterns. In some cases of Spitz and dysplastic nevi, the immunoreactivity was concentrated in the junctional component; PCDGF expression was more uniform in the melanoma groups. PCDGF is strongly expressed in melanoma, dysplastic nevi and Spitz nevi. Although it is only rarely expressed in common nevi, the expression of PCDGF may be a common growth factor involved in tumorigenesis of other melanocytic neoplasms. Interestingly, the strongest expression was seen in Spitz nevi and melanoma. Expression of PCDGF may provide new insights in the molecular events leading to the development of melanoma and other melanocytic tumors. Theoretically, PCDGF could provide a molecular target for treatment of melanoma as more is learned about the role of PCDGF in melanocytic neoplasia.

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Inhibition of NF κ B, ERK, and p38 pathways affects the pigmentation processes in three-dimensional epidermal models containing melanocytes, possibly through inhibition of melanogenic factors produced from keratinocytes

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Melanocytes of the in vivo epidermis are under the influences from surrounding epidermal kerastinocytes. Keratinocytes produce various melano-genic factors including stem cell factor (SCF), basic fibroblast growth factor (bFGF), endothelin-1 (ET-1), and prostaglandin E2 (PGE2). The purpose of this study is to test the effect of inhibitors of signaling molecules on pigmentation and production of these factors produced by keratinocytes. We treated three dimensional epidermal models containing melanocytes, and cultured human keratinocyte cell line, HaCaT, with PD98059, PD153035, parthenolide, Bay 11-7085, and SB202190, the inhibitors for ERK, EGF receptor, NF κ B, I κ B kinase, and p38 MAP kinase, respectively. Melanocytes in the three dimensional epidermal models were observed and photographed with digital camera from above, and the extent of pigmentation was assessed with digital image analysis software. Concentrations of SCF, bFGF, ET-1, and PGE2 in the supernatant of HaCaT keratinocytes were measured with ELISA. All of these inhibitors significantly suppressed pigmentation. Dopa reaction revealed that the numbers of melanocytes did not change by the treatment with these inhibitors. MTT assay revealed that none of these inhibitors affects cell viability in three dimensional epidermal models. Parthenolide, and Bay 11-7085 inhibited the production of ET-1 from HaCaT keratinocytes induced by TNFα and IFNγ, but did not inhibit bFGF, PGE2 or SCF production. SB202190 and PD98059 inhibited the production of PGE2 from HaCaT keratinocytes while they did not inhibit ET-1, bFGF, or SCF production. PD153035 did not inhibit either of the factors tested. These results indicate that inhibition of NF κ B, ERK, and p38 can suppress the melanization processes, which could be partially explained by the inhibition of kerainocyte-derived melano-genic factors.

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Cytogenetic characterization of mucosa-associated malignant melanomas

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Mucosa-associated malignant melanomas (MM) are extremely rare tumors. While cutaneous malignant melanomas are common UV-light induced neoplasms which metastasize to lymph nodes, mucosa melanomas arise independently of UV-light exposure and are characterized by a highly aggressive clinical course with early hematogenous metastases. Little information is available about these tumors regarding their etiology and pathophysiology. For sinonasal MM, comparative genomic hybridization studies showed complex cytogenetic aberrations involving chromosomes 1, 6 and 8. For vulvar MM, detailed cytogenetic analyses are lacking. Design: Three cases of vulvar MM and two cases of sinunasal MM were cultured and characterized cytogenetically. Molecular cytogenetic analysis using multicolor in situ-hybridization (M-FISH) was performed for detailed characterisation. Results: The results of combined analyses of G-banded metaphases and M-FISH staining showed complex chromosomal aberrations involving chromosomes 1, 6, 8, 10, 12, 13, 17, and 21. When drawn into an aberration map for efficient comparison of breakpoints gains and losses, a high grade of similarity of breakpoints gains and losses was observed. The hotspots were identified on chromosomes 1, 6, 8, but also on chromosomes 10, 12, 13, 17, and 21. Particularily the aberrations on chromosome 1q, 6 and 8 are similar to those described for sinonasal MM. The pattern of chromosomal abnormalities in vulvar and sinonasal MM is distinct from the common variants of cutaneous melanoma and is similar to the described gains and losses of chromosomes 1, 6 and 8 in sinonasal MM. Our preliminary cytogenetic analyses suggest that the chromosomal changes in mucosal malignant melanomas are similar, independent of the anatomic site in which they arise.

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Lack of BRAF mutations in Spitz nevi

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RAF proteins are serine/threonine kinases that mediate cellular responses to growth signals by activating the mitogen-activated protein kinase pathway. A high frequency of activating mutations in the BRAF gene has been identified in melanoma (59-80%) and common melanocytic nevi (73-82%), including junctional, compound, intradermal, congenital and dysplastic nevi. More than 90% of mutations in melanoma and nevi involve codon V599 in exon 15 of BRAF. Spitz nevi are benign melanocytic neoplasms that can histologically resemble melanoma. There is good documentation in the literature of metastatic melanomas that had been originally misdiagnosed as Spitz nevi. In this study, we have investigated the frequency of BRAF mutations in Spitz nevi and melanoma, using laser capture microdissection, PCR amplification and direct sequencing. None of the 30 Spitz nevi showed sequence alterations in exon 15 of the BRAF gene. In contrast, mutations affecting exon 15 were found in 13 of 23 (57%) melanomas. Although the mutational profile of BRAF in melanoma with Spitz-like features needs to be established, these data suggest that in some cases mutations in BRAF may be used to differentiate Spitz nevus from melanoma.

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Potential role of stem cells in melanoma development

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Melanoma progression is thought to occur through a stepwise "dedifferentiation" process from an epidermal melanocyte. Recent developments in stem-cell biology led us to re-evaluate these concepts. We evaluated melanoma cultures to discern whether cells with stem-cell-like features were present. A subpopulation of small-ovoid cells were identified and clonally purified from metastatic melanoma. These clonally purified cells had the capacity to self-propagate and differentiate into a secondary cell population with an increased proliferative rate, increased Tyrosinase-Related-Protein-1 expression, and increased numbers and maturation of melanosomes. The behavior of these small-ovoid melanoma cells is more consistent with that expected of a stem-cell based biologic process than that expected of a "dedifferentiation" process. Antigenic markers preferentially identifying the small-ovoid melanoma cells were found to identify a subpopulation of neoplastic melanocytic cells in tissue sections, but not normal appearing epidermal melanocytes. These findings suggest that events leading to melanoma (and nevus development) may first occur in a stem-cell compartment, not through a process of "de-differentiation", and challenge us to reconsider some of our tightly held beliefs regarding the development of melanocytic neoplasias.

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Immunophenotypic expression patterns of cutaneous in situ melanomas and conjunctival primary acquired melanosis with atypia

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Our prior immunophenotypic comparisons of melanomas, which had revealed significant differences between those of the skin and the eye, were extended to include in situ melanomas. The results were intended for use in our intraoperative imaging system for the evaluation of surgical margins. We compared cases of cutaneous melanoma in situ with their conjunctival counterparts, primary acquired melanosis with atypia (PAM with atypia). The immunophenotypic expression patterns of tissue from 18 patients with cutaneous melanoma in situ (including lentigo maligna melanoma in situ and superficial spreading melanomas) and two cases of PAM with atypia were quantitated. Controls consisted of cases of benign melanocytic hyperplasia and primary acquired melanosis without atypia. All cases were immunolabeled with a panel of antibodies that included S100, tyrosinase, melan-A, HMB-45 and HMB-50 combination, microphthalmia transcription factor, p75 neurotrophin receptor, and MIB-1. The results were statistically tabulated by intensity and pervasiveness of labeling. S100, tyrosinase, melan-A, HMB-45 and HMB-50 combination, and microphthalmia transcription factor immunolabeled all of the melanocytic lesions, with tyrosinase and melan-A yielding the highest levels. P75 neurotrophin receptor was expressed at very low levels, if at all. Cutaneous melanomas in situ were similar in immunophenotype to PAM with atypia.

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Post-transcriptional control of melanocyte differentiation markers

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Post-transcriptional mechanisms are emerging as effectors of gene expression that allow mammalian cells to implement adaptive modifications. These regulatory processes involve the interactions of RNA-binding proteins with *cis* AU-rich elements (AREs) of untranslated regions (UTR) in mRNAs. HuA (HuR), a member of the ELAV family of RNA-binding proteins, influences gene expression following cell exposure to UV light and stress, and because of the presence of AREs in the 3′UTRs of Tyrosinase, dopachrome tautomerase (TRP-2) and Tyrosinase related protein-1 (TRP-1) genes, we examine if HuA modulates the expression of these genes. We first demonstrate the *in vivo* presence of these mRNAs in immunoprecipitated HuA mRNP complexes and then modulate the expression level of HuA using both siRNA mediated knockdown in the B16 melanoma cell line and adenovirus mediated overexpression in human normal melanocytes. We show that tyrosinase and TRP-2 protein level are decreased upon HuA knockdown and TRP-2 is significantly upregulated upon HuA overexpression. Interestingly none of the effects are associated with variation in the mRNAs steady-state level as assessed by quantitative real-time RT-PCR. We then use sucrose density gradients cell fractionation to show that upon HuA overexpression, TRP-2 mRNA is relative to Tyrosinase and TRP-1 mRNAs, more efficiently recruited to heavy polysomes where the most actively translated mRNAs are located. This indicates an increase in translational initiation of TRP-2 mRNA. Finally we show the general effect of HuA as a differentiating factor, a consistent delay in pigment accumulation into the medium of the B16 melanoma cell line is observed upon HuA knockdown and HuA overexpression induces an increase in the number of large dendritic melanocytes. These findings demonstrate that post-transcriptional regulation of gene expression and variation in translational efficiency as mediated by HuA, known to redistribute between the nucleus and cytoplasm of cells submitted to stress or UV light, affect the expression of genes involved in melanin synthesis and melanocyte differentiation.

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What is the maximum tolerated dose of NB-UVB for patients with vitiligo?

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There is limited data on the maximum amount of ultraviolet light that narrow-band UVB (NB-UVB) treated vitiligo patients can tolerate. In addition, the effect of repigmentation on tolerance to NB-UVB light has yet to be defined. The objective of this study was to determine whether or not there is a difference in the maximum dose of NB-UVB irradiation between patients with vitiligo who respond and those who do not respond to treatment. This is a single-center retrospective study on patients with vitiligo who were treated at least 20 times with NB-UVB between April 2001 to July 2003. Patients were treated 2-3 times per week, on non-consecutive days. Patients initial dose of light was anywhere from 35 mJ/cm2 to 750 mJ/cm2 with 10% dose increments until patients developed phototoxicity such as erythema or pruritus. Twenty-three patients were able to be evaluated for this study. Their ages ranged from 6 to 91 years (mean=38 years; SD=25). Duration of disease ranged from 3 months to 27 years (mean=7 years; SD=7.2). Of the 23 patients, 15 responded with repigmentation and 8 did NOT respond with repigmentation and/or acquired new depigmented lesions. Maximum tolerated dose for the "responders" was 7479 mJ/cm2 (mean=2142 mJ/cm2; SD=2166.1). Maximum dose for the "non-responders" was 6011 mJ/cm2 (mean=2240 mJ/cm2; SD= 2191.9). The difference in the maximum tolerated light dose between the two groups was not significant. (p=0.9, Mann-Whitney test) Vitiligo patients are able to tolerate significantly higher doses of irradiation with NB-UVB light than the literature suggests. Repigmentation does not appear to influence the maximum amount of NB-UVB irradiation tolerated.

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A natural new active whitening molecule

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Tyrosinase has long been commonly considered as the sole enzyme involved in melanogenesis and has served as target to develop whitening agents. But, over the past decade, it has become well accepted that melanins pathways involve many other enzymes such as Nitric Oxide Synthase (NOS) and/or peroxidase-H2O2 as well as many integrative phenomena such as the cross-talk between keratinocytes and melanocytes including the paracrine factor Nitric Oxide Radical. The aim of this study was to search for natural active molecules with a complete depigmenting approach. LMD Pharmacognosie investigations supported by ethnobotanical data showed that a product extracted from a Brassicaceae plant was able to prevent vegetable browning. Phytochemical screening and chemical synthesis were performed to determine the active molecules. Their potential inhibition of the melanins synthesis enzymatic pathways and their depigmenting effect on melanoma cell culture and skin equivalent tissue model were studied. One molecule from the plant extract was highlighted for its significant ability to inhibit pigmentation. The results show that this molecule is an inhibitor of NOS, peroxidase and tyrosinase. Regarding its action on cell culture, the inhibitory effect can reach 6% versus 4% for the ascorbic acid control and, on skin equivalent, 30% with no cytotoxic effects compared with kojic acid and ascorbic acid that both show a significant cytotoxic effect. Moreover, we showed that this molecule protects keratinocytes against UV-mutagenic effects. All data argue that depigmenting effects are explained in part by inhibition of the major enzymes involved in melanogenesis and especially, inhibition of the NO synthesis with respect to its central role in the regulation of the melanogenesis. This new molecule represents a safe and efficient alternative to reduce skin pigmentation.

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Clinical and *in vitro* investigation of the effects of ferulic acid on human skin pigmentation

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Our clinical studies have implicated ferulic acid (FA), an antioxidant and free radical scavenger, as a potential modulator of skin pigmentation. Using the murine B16 melanoma cell line, we investigated the effects of FA on tyrosinase and other proteins involved in melanogenesis. Western blot analyses demonstrated biphasic responses in tyrosinase protein levels to FA treatment. Lower doses reduced tyrosinase protein levels to 80% of untreated controls, resulting in reduced pigmentation, whereas at higher doses the protein levels were observed to be 20% greater than untreated controls. Real Time PCR analysis confirmed this modulatory effect of FA on endogenous tyrosinase gene expression. In addition, FA had no effect on the glycosylation pattern of tyrosinase. The data suggested that FA may modulate pigmentation through an indirect action on tyrosinase expression. Indeed, microphthalmia-associated transcription factor, a major regulator of tyrosinase, was reduced 50% upon FA treatment. Utilizing an acute UVR pigmentation clinical model, biopsies from human subjects revealed that FA functions through down-regulation of factors related to melanin production, including tyrosinase and inducible nitric oxide synthase, suggesting NO as a critical component for the induction of *in vivo* pigmentation.

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Epigallocatechin-3-gallate (EGCG) causes cell cycle arrest and apoptosis in human melanoma cells via modulation of the cyclin kinase inhibitor (cki)-cyclin dependent kinase (cdk) network and Bcl2 family proteins

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The antioxidant polyphenolic constituent of green tea, EGCG, has been shown to prevent several cancers including skin cancer. Earlier, we demonstrated that EGCG inhibits the growth and proliferation of human melanoma cells via induction of apoptosis. However, the normal human melanocytes were not affected at similar concentration of EGCG. Here, we demonstrate that EGCG, via modulation of cki-cyclin-cdk machinery, results in cell cycle arrest followed by apoptotic death of human melanoma cells. EGCG treatment (1-80 microgram/ml for 24-48 hours) of human melanoma cells (A-375, Hs-294T and G-361) resulted in a dose-dependent i) inhibition of cell growth as shown by MTT assay, ii) cell cycle arrest (G0-G1 phase arrest in A-375 cells and S-phase arrest in Hs-294T cells) as shown by DNA cell cycle analysis, and iii) induction of apoptosis as assessed by flow cytomety. Immunoblot analysis revealed that EGCG treatment caused a significant dose- dependent i) decrease in PCNA and Ki-67 (markers of proliferation), ii) induction of p16, WAF1/p21 and KIP1/p27, iii) decrease in cyclin D1 and cdk2 protein expression. EGCG treatment of melanoma cells also resulted in a down-modulation of anti-apoptotic protein Bcl2 and an up-regulation of pro-apoptotic proteins Bax and Bak. EGCG treatment resulted in significant activation of caspase-3,-7, and -9. Taken together, our study suggests that EGCG treatment of melanoma cells results in cell cycle blockade and induction of apoptosis via modulation of the cki-cyclin-cdk network and Bcl2 family proteins. To our knowledge, this is the first study showing the involvement of each component of the cki-cyclin-cdk machinery and Bcl2 family of proteins during cell cycle arrest and apoptosis of human melanoma cells by a chemopreventive agent. We suggest that EGCG could be developed for the management of melanoma as an alternative approach or in conjunction with current therapies.

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Invasive human melanocytic neoplasia generated using defined genetic elements

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Genetic evidence links constitutive activation of the Ras cascade (Ras-Raf-MEK-ERK) and hypofunction of the Rb (p16INK4a/CDK4/Rb) and p53 (p14ARF/HDM2/p53) pathways to human melanoma pathogenesis. The Ras cascade may be activated via mutation of RAS or, most frequently, RAF isoform genes. The Rb and p53 tumor suppressor pathways are often inactivated via mutations or deletions at the CDKN2A locus, which encodes both p16INK4a and p14ARF. Other genetic alterations, such as telomerase activation and PTEN inactivation, have also been implicated in melanoma progression. To investigate directly the contributions of specific genes to human melanoma development, we engineered primary human melanocytes to express one or more genes of interest, specifically, different combinations of oncogenic Ras, CDK4R24C (a constitutively active mutant found in a subset of melanoma-prone families), dominant-negative p53R248W, and hTERT (the catalytic subunit of human telomerase), and combined them with normal human keratinocytes to regenerate human skin on immune-deficient mice. In the context of hTERT, co-expression of CDK4R24C and p53R248W produced normal skin, while Ras expression alone induced junctional melanocytic hyperplasia resembling a dysplastic nevus. Most interestingly, expression of oncogenic Ras together with inactivation of either Rb or p53 pathways led to invasive melanocytic neoplasia displaying the cardinal features of malignant melanoma, including cytologic atypia, pagetoid scatter, deep dermal invasion and ulceration. Ras-driven melanocytic tumors were polyclonal and expressed a number of melanocyte and melanoma markers, including Melan-A, S100 and HMB-45, as well as the genes originally introduced into the melanocytes. Further experiments showed that hTERT expression was dispensable for early-stage melanocytic hyperplasia but greatly accelerated progression to invasive melanocytic neoplasia. Current studies address the sufficiency of Ras effectors to induce melanocytic neoplasia and the identification of potential therapeutic targets using this human cancer model.

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Center-based approach for computerized analysis of non-uniform growth patterns in melanocytic lesions

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The diagnosis of early melanoma is a challenging problem for dermatologists and pathologists alike. Large numbers of benign lesions are biopsied every year in the pursuit of malignant melanoma, contributing to health care costs and patient morbidity. Numerous efforts are underway to use computer-based algorithms to increase the accuracy of melanoma detection. These approaches vary from neural networks to genetic algorithms, and emphasize feature detection similar to that utilized by the ABCD and 7 point checklist methods. We hypothesize that all melanocytic lesions originate from a single mutated cell and therefore have a biologically determined center. Furthermore, we believe that malignant melanocytic lesions acquire subsequent mutations which result in greater pigment asymmetry and nonuniform distribution than that seen in benign lesions. Thus the definition of center may be useful to assess a lesion's uniformity as one moves radially out from the defined center. There are many ways to calculate center, including geometrical mean, median of the coordinates of the lesion, etc. We found that two methods of defining center - the centroid of the lesion (calculated as an average or median) and the center of the lesion's convex hull - most closely matched the centers identified clinically. Furthermore, the centroid and convex hull centers had optimal symmetry of the lesion border, computed as a radial symmetry measurement. We also compared a pigmented-weighted center with the centers described. Our sigmoidal pigment-weighting algorithm is a two-step approach that emphasizes the darkest and lightest regions of an image. When this weighting was applied to nonuniform lesions, the pigment-weighted center had greater deviation from the centroid/convex hull center. Thus an algorithm that incorporates definition of center with deviation of a pigment-weighted center distinguishes uniform from nonuniform melanocytic lesions, and thus has potential diagnostic value in melanoma detection.

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Overcoming resistance of melanoma cells to TRAIL-induced apoptosis by modulating interferon signaling pathways

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Malignant melanoma (MM) cells are notoriously resistant to apoptosis. As TRAIL and interferon (IFN) represent therapeutic agents in oncology, we sought to identify molecular cross-talk between TRAIL and IFN pathways to facilitate more effective treatment protocols for MM. Compared to cultured normal human melanocytes (n=6), which constitutively express low or absent levels of IFN inducible protein-16 (IFI-16), numerous MM lines (n=6) express high nuclear levels by immunostaining and Western blotting. Elevated constitutive IFI-16 levels were not further augmented in MM cells by exogenous IFN-α (10^3 U/ml) or IFN-γ (10^3 U/ml) treatment (24 hrs). Treating MM cells with siRNA targeting IFI-16 revealed significant (>70%) reduction in IFI-16 levels compared to scrambled control (SC) siRNA. While exposure of SC treated MM cells to TRAIL (100 ng/ml; 24 hrs) produced less than 20% apoptosis (PI staining with sub-G_0 DNA content; FACS analysis), MM cells with reduced IFI-16 levels contained 2-3 fold greater apoptotic responses in 2 different MM cell lines (p<0.05). Apoptotic machinery activated included cleavage of caspases 8, 3, and 9. Enhanced apoptotic susceptibility in IFI-16 siRNA treated MM cells was not related to alterations in TRAIL death or decoy receptor expression, or changes in levels of survival proteins such as c-FLIP, Bcl-2, or XIAP. By contrast, when IFN-α (10^3 U/ml) or IFN-γ (10^3 U/ml) was simultaneously combined with TRAIL, synergistic induction of apoptosis was observed (greater than 50%) in MM cells. We conclude that at least two distinct IFN signaling pathways are operative in MM cells. The IFI-16 related pathway mediates survival signaling providing resistance to TRAIL-induced apoptosis; whereas the second pathway is dependent on IFN-α/IFN-γ and produces enhanced apoptotic responses to TRAIL. Taken together, these results reflect molecular cross-talk between IFN signaling pathways and TRAIL. Further studies targeting IFI-16 are indicated to devise new and more effective combination therapeutic strategies to combat this deadly neoplasm.

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Human melanoma development in reconstructed skin models

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Human skin reconstructs are three dimensional in vitro models consisting of a co-culture of epidermal keratinocytes and melanocytes plated onto a dermal substrate. In contrast to cells in monolayer culture, tissue-engineered skin equivalents contain well-developed epidermal layers with normal melanocytes being distributed at the basement membrane that separates the epidermis from the dermis. The incidence of cutaneous melanoma (CM) has increased dramatically in the last few decades. Development of specific CM stage models (e.g. radial or vertical growth phases) with their specific microenvironments and cell-matrix and cell-cell communication are crucial for understanding changes leading to the metastatic CM phenotype and for the development of CM therapies. A serum-free (SF) culture system, in which the precise effects of growth factors and chemo-therapeutic compounds can be tested without interference from the multitude of undetermined components in serum, is presented. Single cell suspensions of normal human epidermal keratinocytes and melanoma cells from different stages of melanoma progression are seeded on acellular or fibroblast-contracted collagen gels and induced to differentiate in SF medium. Incorporation of the metastatic melanoma cell lines, A375 and SK-MEL-28, into the epidermal/acellular dermal cultures results in progressively growing clusters of melanoma cells in the basal/spinous layers. Similarly, when A375 cells are added to MatTek's full thickness skin model (FTSM), nodes of melanoma cells develop in the epidermis by day 10. These clusters begin to invade the dermis by day 14. Incorporation of SK-MEL-28 into FTSM shows progressive disruption of the basement membrane. Finally, the Wistar metastatic cells (WM852) yielded a tumor invading the dermis on day 16. The development of three dimensional, serum-free tissue culture models, containing melanocytes at different stages of CM malignancy, will provide researchers with valuable tools to study, understand, and develop preventative and therapeutic treatments for this most serious cutaneous malignancy.

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Ribozyme-mediated inhibition of telomerase activity suppresses *in vitro* & *in vivo* growth and metastatic potential of murine melanoma cells

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While normal human somatic cells have diminished telomerase activity, telomerase activity is commonly high in human cancer cells. Abnormal expression of this ribonucleoprotein complex confers an unlimited potential for growth and division to transformed cells, and has therefore been recognized to be an important molecular target in cancer therapy. Recent studies in our laboratory showed that systemic administration of cationic liposome:DNA complexes encoding anti-telomerase RNA (TER) ribozymes reduced the metastatic progression of B16-F10 murine melanoma *in vivo*. In this study, we developed stable transformant B16-F10 melanoma clones expressing ribozymes targeting TER at position 180 (TER 180 Rz) under the regulation of a U6 promoter. Analysis of TER expression levels by TaqMan revealed TER levels to be down-regulated by > 70% in the TER 180 Rz-expressing clones, as compared to vector-only controls. Analysis of telomerase functional activity by TRAP revealed 60-90% lower telomerase activity in TER 180 Rz clones, as compared to vector-only controls. *In vivo* experiments involving injection of B16-F10 clones by tail vein revealed 85% reduction in number of metastatic melanoma lung tumors in the TER 180 Rz group as compared to vector-only controls. To elucidate the mechanism by which down-regulated telomerase activity results in reduced metastatic potential, several functional assays were performed. FACS-based TUNEL assays revealed increased apoptosis in the TER 180 Rz group as compared to vector-only controls. *In vitro* observations of the lower growth rate of TER 180 Rz transformants were confirmed by FACS-based BrdU incorporation assays that revealed up to 50% reduction in the percentage of TER 180 Rz cells in S phase as compared to vector-only controls. These results assign a pro-metastatic function to telomerase, and demonstrate the therapeutic utility of anti-TER ribozymes given their anti-metastatic activity.

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Activating mutations of BRAF in melanoma and benign nevi

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We examined the frequency of V599E BRAF mutations in paraffin-embedded samples of melanoma and melanocytic nevi. Previous studies have reported widely varying frequencies of V599E mutation in melanomas and nevi; hence, the significance of this mutation in melanoma development is unclear. Our study included 73 melanoma samples. In addition, we analyzed 52 dermal nevi, 9 compound nevi and 15 junctional nevi, which are considered precursor lesions for melanoma. To assess the specificity of our assay for the presence V599E mutations, we included 18 normal blood samples and 20 normal, paraffin embedded colon tissue samples. Paraffin blocks were sectioned, areas containing lesions of interest were isolated and DNA extracted. Mutation detection was performed by allele-specific polymerase chain reaction (AS-PCR) using oligonucleotides with 3' ends complementary to mutant BRAF sequence. The analytical sensitivity of this assay for the detection of cells with a BRAF mutation was 1 in 10,000. As a control, PCR of a larger segment of exon 15 of BRAF or PCR of a GAPDH gene segment was performed. All AS-PCR results were confirmed by sequencing. Our study revealed V599E BRAF mutations in 13/72 (18%) malignant melanomas, 48/52 (92%) dermal nevi and 9/9 (100%) compound nevi but none of 15 junctional nevi. All control samples (18 blood samples and 20 colon) were negative for the mutation. Our results suggest that V599E activating mutations might play a role in the genesis of benign melanocytic nevi with a significant dermal component but not in those without a significant dermal component (i.e. junctional nevi). In addition, a lower frequency of V599E BRAF mutations in melanomas in our study suggests that this genetic alteration might not play as predominant a role in development of sporadic malignant melanoma as initially speculated.

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Puma expression is significantly reduced in human cutaneous melanomas

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Cutaneous malignant melanoma is an aggressive form of skin cancer, characterized by strong chemoresistance and poor patient prognosis. The molecular mechanisms underlying its resistance to chemotherapy remain unclear but are speculated to involve the dysregulation of apoptotic pathways. In this study, we aim to determine whether expression of PUMA (p53 upregulated modulator of apoptosis) plays a role in human melanoma formation, tumor progression, and survival. We used tissue microarray technology to examine PUMA expression in 60 human melanoma biopsies. We show that the majority (>80%) of melanoma tumor tissues exhibit reduced PUMA expression compared to adjacent normal tissue. However, reduced PUMA expression is not correlated with tumor thickness, suggesting PUMA reduction is an early event in melanomagenesis. Our results also demonstrate that PUMA loss is more prevalent in the tumors of older patients (age of diagnosis >60 years) (P=0.036), despite a lack of correlation between age and PUMA expression in normal tissue. In addition, we found that patients with negative or reduced PUMA expression in their tumor tissue tend to have a poorer prognosis (P=0.1). Finally, we show that age of diagnosis is a strong determinant of both overall (P<0.01) and disease-specific (P<0.05) 5-year survival, and that this is not the result of delayed diagnosis or differences in stage of tumor progression. Therefore, in patients diagnosed after age 60, both PUMA expression and survival are significantly compromised.

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Improved detection of the V599E Braf mutation in clinical specimens by allele specific PCR

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Recent studies have found a high frequency of acquired B-raf gene mutations in melanoma. B-raf encodes a serine/threonine kinase in the MAPK pathway, which is important in melanocytic proliferation. A V599E missense mutation accounts for 92% of the Braf mutations detected in melanoma, and the mutated gene product has a 10 fold greater basal kinase activity relative to wild type B-raf. The significance of this mutation in predicting clinical outcomes and therapeutic response is currently a subject of interest. To determine B-raf mutation status in thin primary melanomas an assay must be able to detect mutated melanoma cells in the presence of excess epidermal keratinocytes containing wild type B-raf. We developed a specific and sensitive assay to detect V599E B-raf mutations in the presence of excess wild type B-raf by allele specific competitive PCR. To determine the sensitivity of this assay, we extracted genomic DNA from cell cultures homozygous for mutant B-raf and from wild type cells. The genomic DNA containing mutant B-raf was serially diluted with the wild type DNA. These samples were then analyzed by allele specific, competitive PCR. One primer set amplified a housekeeping gene. A second competing primer set amplifed mutant BRAF but was unable to amplify the wild type gene because of a substitution of two bases at the 5 prime end. This protocol for the allele specific PCR was found to have the capacity to pick up V599E mutant cells at a frequency of 0.01% of total cells. We are currently using this assay to screen large numbers of clinical specimens in order to determine the predictive value of the V599E mutation.

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Human keratinocytes secrete a potent pro-motility factor for human melanomas: implications in melanoma development

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Development of solid tumors is a combination of gene mutations and altered interactions with the adjacent normal cells in their immediate microenvironment. Melanocytes and the early-stage melanomas are surrounded by keratinocytes and a fibroblast-rich stroma. Little is known about the role of melanoma interactions with its surrounding cells during the process of melanoma development. While human melanoma cells, M21 and M24, have constitutively activated Ras and ERK1/2, under serum-free conditions, these cells are unable to migrate on ECM-coated substratum. Therefore, the genetic mutations alone in melanomas may be necessary but are clearly not sufficient for initiating cell migration. We then focused on the possible contributions of secreted factors from surrounding keratinocytes or fibroblasts. We found that serum-free conditioned medium (CM) from HKs induced dramatic migration of the melanoma cells on various ECMs. The CM pro-motility activity was 2-4 fold greater than published pro-motility factors for melanoma cells such as serum, EGF, VEGF, HGF and serum-enriched CM from NIH3T3 cells. In contrast, similarly prepared CM from cultures of HDFs, melanocytes, M21 or M24 cells showed minimal pro-motility activity. To study whether the secretion by HKs would be increased by the co-presence of melanoma cells, we found that serum-free CM from M21/HK or M24/HK co-culture did not show significantly higher pro-motility activity than the origicnal HK CM. Interestingly, GFP-labeled melanoma cells, co-cultured on a HK monolayer, show marked cellular proliferation compared with co-culture experiments using non-HK cell monolayers. Human Cytokine Array analyses (to detect 75 known growth factors/cytokines, RayBiotech, Inc) suggested that the HK-secreted factor(s) is not among the growth factors/cytokines tested. Thus, molecular identification of this potentially novel motility and invasion-promoting factor(s) from HKs may gain new insights into the ontogenesis of human melanomas.

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MEK inhibition kills melanoma cells with constitutively elevated ERK activity

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A large fraction of metastatic melanomas carry oncogenic mutations in the ras-raf-MEK-ERK kinase cascade with most of these mutations in B-raf. The MEK kinase is an attractive therapeutic target for melanoma because this molecule lies immediately downstream of B-raf. Moreover, we have shown that CI 1040, a well-tolerated, orally available MEK inhibitor, causes rapid regression of human melanoma lung metastases in a mouse xenograft model [Collisson, 2003]. However, the mechanism of cell death due to CI 1040 remains unknown. We tested the efficacy of CI 1040 in several melanoma cell lines. We found that cell lines carrying the most common B-raf mutation (V599E) showed constitutive ERK activation in the absence of serum stimulation. We also demonstrated that ERK phosphorylation of these cell lines was equally down-regulated by CI 1040 regardless of serum presence. One cell line was homozygous for the V599E B-raf mutation and underwent CI 1040 mediated cell death within 24 hours. Cell lines heterozygous for the V599E B-raf mutation underwent CI 1040 mediated cell death within four days. Of the cell lines tested, those without B-raf mutations did not show constitutive ERK activation and did not undergo cell death in response to CI 1040. All the CI 1040 sensitive cell lines underwent cell death more rapidly under low serum conditions. These results suggest that B-raf mutation status may indicate melanoma sensitivity to therapeutic MEK inhibitors.

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Frequent alteration of the p16INK4A tumor suppressor gene by deletion, promotoer methylation, and point mutation in human melanoma metastases

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Studies of the p16INK4A tumor suppressor gene in human melanoma have shown significantly higher rates of inactivation in cell lines than in tissue specimens. One explanation is that deletion, the predominant mechanism of inactivation in cell lines, is difficult to detect in tissue specimens using PCR-based techniques. This study compares the rates of p16INK4A inactivation by mutation, methylation and gene deletion in a cohort of metastatic melanoma tissue specimens. We used a combination of sequencing, methylation-specific PCR, microsatellite analysis, and a novel fluorescence-based semi-quantitative comparative multiplex PCR to determine the frequency and patterns of gene inactivation in a cohort of 60 metastatic melanoma specimens from 58 patients with Stage III and IV disease. The INK4A locus was altered by deletion in 15/58 (26%) unique tumors, promoter methylation in 14/58 (24%), and mutation in 6/58 (10%). Deletions involving exon 2 were sustained in 13/15 deleted cases, including 8/15 cases in which both exons 1 & 2 were deleted. Two mutations were observed in exon 1, including a 4-base pair microdeletion (43/46del4) previously described in pancreatic and esophageal carcinomas (1 case each). Four mutations were detected in exon 2, including a novel substitution Pro81His, which would also produce an Arg96Ser change in p14ARF (which shares exon 2 with p16INK4A). Two cases demonstrated INK4A alteration by two mechanisms, namely deletion and methylation or mutation. Interestingly, INK4A alterations were more frequent among patients with visceral metastases (15/22, 68%) in comparison to patients with lymph node and subcutaneous metastases (17/38, 45%). In conclusion, multiple genetic and epigenetic mechanisms altered the p16INK4A tumor suppressor locus in 33/58 (57%) melanoma metastases. This frequency is substantially higher than previous reports analyzing alteration of this gene in human melanoma metastatic tissues.

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Identification of cells of presumed melanocyte lineage in the dermis of melanoma in situ using multiple melanocytic markers: a retrospective study

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In order to determine the frequency of inconspicuous dermal cells of melanocytic lineage found in tumors previously diagnosed as melanoma in situ (MIS), we re-examined 30 cases of MIS using immunohistochemical staining on sequential sections with four melanocyte markers: S100, HMB45, anti-Melan-A and Microphthalmia transcription factor (Mitf). Lichenoid keratoses, large cell acanthomas and normal skin served as negative controls. 7% (n = 2) of MIS examined showed dermal cells positive for HMB45, 27% (n = 8) for MelanA and 30% (n = 9) for Mitf. S100 stained numerous non-melanocytic dermal cells; findings deemed non-specific. In certain cases, nests of positive cells in the dermis adjacent to areas of adnexal structures were found to be contiguous with adnexa on serial sectioning. Only one MIS had a positive dermal tumor cell nest (Melan-A, HMB-45 and Mitf) thought to be significant for melanoma in the dermis. Interestingly, this tumor had a robust dermal inflammatory infiltrate. Four cases (13%) had cells in the dermis positive for two markers (Melan-A/Mitf [n=3] or Melan-A/HMB45 [n=1]). Control cases of lichenoid keratosis also revealed Melan-A and Mitf positive cells within the dermis, similar in appearance to those in cases of MIS, while large cell acanthoma and normal skin did not. We conclude that since solitary cells staining positively for Melan-A and Mitf are seen within the dermis of benign lesions (lichenoid keratosis), similarly staining cells in MIS are not necessarily indicative of dermal melanoma. Our data support using caution in interpreting lesions as melanoma with a dermal component when additional staining is performed, particularly when evaluated by a single melanocytic marker. We suggest that, when performed, multiple markers should be used; and that dermal cells staining positively for 2 markers (i.e.,Melan-A/Mitf) may have greater specificity and greater prognostic significance when evaluated in conjunction with cell morphology.

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Molecular imaging of murine melanoma using αv $\beta 3$-directed contrast

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It is well established that tumor survival, growth and metastasis is dependent upon the establishment of new vasculature, or tumor angiogenesis.|alpha*|v $\beta 3$-integrin is a biomarker of angiogenesis expressed on the surface of both endothelial cells involved in active angiogenesis as well as invasive tumor cells. In certain malignancies, αv $\beta 3$-integrin expression correlates with tumor grade. $\alpha v \beta 3$-targeted nanoparticles have been developed to specifically and sensitively detect nascent tumors with clinically relevant 1.5 T MRI scanner. However, the ability of this molecular imaging agent to phenotypically differentiate aggressive from indolent tumor grades is unknown. In the present study, a variety of human melanoma lines were screened and variably aggressive murine melanoma tumor models were developed. Murine melanoma models were created in nude mice by subcutaneous injection of the human cell lines C32, MeWo, A2058 and A375 (ATCC) characterized for MAPKK, phospho-MAPKK, MAPK and phospho-MAPK protein. Tumors were harvested at days 7, 14, 21 and 28. By Western analysis, A2058 and A375 cell lysates demonstrated increased expression of phospho-MAPK, MAPK, phospho MAPKK relative to MeWo. Tumor growth (A2058 and A375 tumors (0.054g, 018g day 7; 0.18g, 0.06g day14, respectively) and MeWo (0.005g day 7, 0.02g day 14) correlated with phospho-MAPK expression and earlier formation of new vessels ($\beta 3$ expression by immunohistochemistry). In sum, we have shown that tumor growth is correlated with MAPK expression and angiogenesis in this murine model. These models will challenge the sensitivity of MR αv $\beta 3$-integrin imaging to phenotypically characterize and distinguish related tumors with different development patterns. We propose that these biochemicall characterized models will advance the development of MR molecular imaging agents and techniques required to diagnose, characterize, grade and stage primary and metastatic tumors, including melanoma, in clinical practice.

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Preclinical studies of contrast-enhanced magnetic resonance imaging for sentinel lymph node analysis in melanoma

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Staging of invasive melanoma increasingly relies on surgical sentinel lymph node biopsy (SLNB), which is an improvement over complete lymphadenectomy, but still has a significant morbidity rate of 5-10%. Contrast-enhanced magnetic resonance imaging (MRI) has shown promise in the non-invasive imaging of lymph node metastases in several cancers, and melanoma is a particularly attractive application for this for two reasons: (1) contrast can be injected in the tumor site to achieve high concentrations in the sentinel lymph node, and (2) the draining lymph node basins are generally superficial, allowing high resolution imaging with phased array surface coils. In this work, we tested three contrast agents for suitability in this application: gadolinium (Gd-DTPA), ultrasmall superparamagnetic iron oxide nanoparticles (USPIO), and perfluoro-15-crown-5-ether (PFCE), a perfluorocarbon similar to artificial blood substitutes and imaged using F-19 MRI. These were tested with B16/F1 melanoma metastasizing to inguinal nodes of C57/Bl6 mice and imaged at 4.7 Tesla. Gd-DTPA was found to flow through nodes too rapidly to allow sufficient accumulation for tumor contrast. USPIO and PFCE provide contrast by accumulation in macrophages of normal node tissue but not tumor, and we achieved high level accumulation of these agents as demonstrated by histology. Imaging with these two agents provided comparable results, with 0.1x0.1x0.5 mm voxel resolution each. Extrapolating this to the anticipated resolution achievable in human clinical MRI, and using the known size distribution of human SLN metastases, we calculate that contrast-enhanced MRI can identify metastases with a false negative rate only 6% worse than surgical SLNB. Given the small but significant morbidity of SLNB and the fact that sentinel node analysis provides important prognostic information but little therapeutic benefit, these results suggest that contrast enhanced MRI may prove to be an important alternative to surgical SLNB and that further clinical studies are warranted.

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Topical formulation of coenzyme Q10 inhibits the growth of melanoma tumors

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Treatment for skin cancer may require surgery, chemotherapy and/or radiation, all of which cause severe discomfort to the patient. These modalities usually result in some damage to normal, healthy cells. As evidenced by our *in vitro* data, Coenzyme Q10 (Q10) selectively inhibits the proliferation of cancer cells. Our laboratory has developed a liposome-encapsulated Q10 cream that facilitates the topical delivery of Q10 to underlying tissues. Moreover, oral administration is one of the least effective modes of delivery due to uncertainties of absorption and inactivation in the gastrointestinal system. In contrast, percutaneous application is more pharmacologically advantageous due to a more specific and direct delivery to the point of interest, resulting in a higher absorption/dosage ratio of Q10. In animal studies, melanoma tumors were induced in nude, athymic mice by subcutaneous injection of SKMEL28 cells. Each mouse was inoculated with two tumors ($7x10^6$ cells/tumor) on the superior dorsal region. A liposome-encapsulated formulation of Q10 (10%) was applied to the treatment group daily for 30 days. Following treatment, the mice were sacrificed and the tumors excised and weighed prior to histological analysis. The mean mass for all tumors in each group was determined and the results showed a 52.3% decrease in tumor mass in the treatment group as compared to the control. These results were replicated using a slightly higher concentration of Q10 (15%), yielding a 54.0% decrease in tumor mass. Prior *in vitro* data suggest that a dysfunction in the apoptotic pathway is involved in the pathogenesis of melanoma, possibly playing a crucial role in tumorigenesis. In conclusion, Q10 may induce apoptosis in malignant cells leading to an inhibition of tumor growth. Furthermore, it is highly probable that individuals diagnosed with melanoma or other skin tumors could use our Q10 delivery system to selectively treat and inhibit the growth of their malignancies without the side effects of current modes of treatment.

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BRAF point mutations in primary malignant melanoma show different prevalences by subtypes

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Recently, frequent somatic mutations of BRAF were reported in malignant melanoma (MM). So, at first, we performed a mutation analysis using 43 cell lines established from tumors that had developed in several kinds of human organs, to elucidate the biological significance of activating mutations of the BRAF gene in human malignant tumors. Interestingly, in spite of the fact that the same V599E point mutation was observed in three of six MM cell lines, no such mutations were observed in other types of cancers. Next, to examine the biological significance of V599E point mutation in MM, we added mutation analyses of NRAS, KRAS, b-catenin and p16/p14ARF genes in these 6 MM cell lines, and found three p16/p14ARF mutation, two of which were overlapped with BRAF mutation. We further searched for mutations of BRAF in 35 primary sporadic malignant melanomas from 35 Japanese patients by direct sequencing and allele specific oligonucleotide hybidization and detected the V599E BRAF point mutation in only nine (26%) of them. Significant differences in mutation frequency were observed among four histological subtypes; four (50%) of eight superficially spreading melanoma (SSM) and five (33%) of 15 acral lentiginous melanoma (ALM) had the mutation, whereas none of twelve other types (six nodular melanoma, five lentigo malignant melanoma, and one mucosal melanoma) had it. The BRAF mutation was observed frequently even in thin lesions, indicating that activation of this gene may be one of the early events in the pathogenesis of some melanomas.

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Coenzyme Q10 induces apoptosis in human melanoma cells

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Coenzyme Q10 (Q10) is a naturally occurring component of the inner mitochondrial membrane, which plays a crucial role in the intrinsic pathway of apoptosis. Our *in vitro* studies showed that Q10 exerts an inhibitory effect on melanoma cells, however no adverse effects were observed on normal cells. It was hypothesized that the decreased proliferation in melanoma cells was either directly or indirectly related to the function of Q10 in the mechanism of apoptosis. Studies have demonstrated that the intrinsic pathway of apoptosis is often altered in melanoma due to mitochondrial abnormalities. We therefore investigated the involvement of Q10 with respect to apoptosis in melanoma cells. Both melanoma SKMEL28 cells and human neonatal fibroblasts (nFIB) were incubated in the presence and absence of 50µM Q10 for 48hrs. The cells were trypsinized and subjected to annexin V-PE staining for indication of apoptosis. 7-AAD was used to determine the level of necrosis given that annexin may also stain necrotic cells. Subsequently, the cells were analyzed using flow cytometry. Incubation with Q10 elicited a significant ($p<0.01$) reduction of apoptosis in nFIB, 58.5% decrease as compared to the control. On the contrary, the SKMEL 28 cells treated with Q10 showed a 77.5% increase in apoptosis. Current studies are ongoing to elucidate the mechanism by which Q10 exerts the aforementioned effect on cells. Concordantly, Q10 selectively restores the apoptotic potential in malignant melanoma while rendering slight support to normal fibroblasts. These data suggest a potential use for Q10 either alone or as an adjuvant therapy for the treatment of melanoma.

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A gene locus responsible for non-syndromic multiple lentigines maps to chromosome 6(q24-25)

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Multiple Lentigines syndrome (MLS) is a rare autosomal dominant disorder, characterized by diffuse cutaneous lentigines. Significant clinical overlap exists between Noonan syndrome and ML, and it is not surprising some identified cases of LEOPARD syndrome are a subtype of the Noonan syndrome, caused by mutations in the PTPN11 gene. Other groups suggest that distinct mutations affecting the PTPN11 phosphotyrosine phosphatase domain explain the cutaneous anomalies seen in ML/LEOPARD syndrome, where the majority of mutations are found in the amino N-SH2 domains. While this could account for a fraction of phenotypic variation seen in NS and overlap with the LEOPARD syndrome, our findings indicate this does not necessarily extend to ML in general. Our research suggests that the MLS manifesting with diffuse cutaneous lentigines without non-cutaneous manifestations, is not a subtype of either Noonan or LEOPARD syndrome and should be classified as non-syndromic ML. This work extends previous studies in the same family in which we excluded PTPN11 as well as the genes for other clinically overlapping disorders, including von Recklinghausen neurofibromatosis (NF1), Carney syndrome types I and II (CNC1 and CNC2), Peutz-Jeghers syndrome (PJS), and Cowden disease (CD). Linkage informativeness is definitive in positioning the MLS locus in the 5-cM region between markers D6S1703 and D6S411. Know genes involved in pigmentation, such as RAB32, are being studied with wave analysis and sequencing to determine potential mutations. Further work on identification of the gene for non-syndrome ML will allow further exploration of the genetics of pigmentation.

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Phenolic stress induced autoimmune reactivity to melanocytes

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Exposure to 4-tertiary butyl phenol (4-TBP) can cause depigmentation in humans handling phenols. We hypothesized that skin bleaching is mediated in part by a stress response causing autoimmune reactivity to melanocytes. 4-TBP induced expression of stress protein HSP70 was evaluated by ELISA and Western blotting, and the cytoprotective effect of adenoviral HSP70 overexpression against 4-TBP exposure was measured. The effect of 4-TBP exposure and HSP70 overexpression on immune related markers was assessed by FACS analysis, and the cytotoxic responses of T cells and dendritic cells to stressed melanocytes were quantified. Immunohistology was performed to investigate the in vivo relevance. It was observed that PIG3V vitiligo melanocytes generate increased amounts of HSP70 in reponse to 4-TBP as compared to normal melanocytes or fibroblasts. Cells overexpressing HSP70 were not protected from 4-TBP induced cell death, and a >30% decrease in viability was noted at 62.5 mM 4-TBP for PIG3V melanocytes versus 125 mM for PIG1 control melanocytes. MHC class I molecule expression was elevated by 25% in response to adenoviral HSP70 overexpression, accompanied by a 20% increase in HLA-matched T cell mediated killing of 4-TBP exposed melanocytes. Interestingly, 4-TBP exposed melanocytes were sensitized 5-fold to dendritic cell effector functions and DC effector functions were enhanced by exposure to HSPs. Preliminary data suggest that DC cytotoxicity may be mediated by surface expression of TNF Related Apoptosis Inducing Ligand (TRAIL), accompanied by elevated TRAIL receptor expression by melanocytes following 4-TBP exposure. TRAIL and CD91 stress protein receptor expressing DCs were observed in depigmenting vitiligo skin. In combination with our reports of aberrant stress protein expression in vitiligo skin, the data support that 4-TBP induced expression of HSP70 can evoke an autoimmune response to melanocytes.