Genomic approaches to the investigation of wound microbiology and pathogenesis

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The purpose of the study is to develop a systematic metagenomic approach to understanding wound pathophysiology. Until recently, the complexity of wound microflora was underappreciated because standard culture techniques did not appreciate the full inventory of bacteria or their organization and interactions within the wound biofilm. We utilized high-throughput pyrosequencing of the 16S rRNA gene to identify the diversity of microbial species contained in the wound and to describe their relative abundance. Bioinformatic analysis of the 16S datasets demonstrated the presence of an average of 17 bacterial genera in our samples compared to an average of only 3 species identified by standard quantitative culture. This bacterial diversity included many anaerobes, such as Peptoniphilus, Anaerococcus, and Fusobacterium, which are difficult to culture. In addition, an overwhelming predominance of Staphylococcus (>90%) was correlated with decreased diversity in other organisms. The metagenomic data then informed our use of confocal microscopy, fluorescent *in-situ* hybridization (FISH), and quorum sensing analysis to further understand these microorganisms within the wound. Confocal microscopy localized these bacteria within highly organized biofilms and FISH visualized the spatial relationship between Staphyloccoccus and Pseudomonas. We discovered elevated levels of autoinducer-2 (AI-2), a quorum sensing molecule involved in density-dependent inter- and intra-species signaling. These quorum sensing molecules are linked to shifts in types and concentrations of microbial populations. This study provides an algorithm on how to investigate the complex microbial systems in chronic wounds and rationally approach the use of antibiotics in wound therapy.

TGF-beta 1-treated conditioned media from adipose tissue-derived stem cells induces increased expressions of type I collagen and MMP-1 in human skin fibroblasts, thereby promoting wound healing process *in vivo*

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To investigate the effects of TGF-beta 1-treated conditioned media (CM) from adipose-derived stem cells (ADSCs) on proliferation, migration, type I collagen and MMP-1 expressions. Furthermore we investigated whether TGF-beta 1-treated CM promote wound healing process *in vivo*. In this study, ADSCs were cultured with TGF-beta 1 and then CM was harvested. The TGF-beta 1-treated CM were added into fibroblasts and the proliferation, migration, type I collagen and MMP-1 expression were studied by proliferation, migration assay, Western blot, and RT-PCR analysis. In addition we injected TGF-beta 1-treated CM into wound model of hairless mouse, which was made by 3mm punch. Our data showed the proliferation and migration rates of fibroblasts were increased by TGF-beta 1-treated CM. Expression of MMP-1 and type I collagen of fibroblasts were increased by TGF-beta 1-treated CM. The wound healing process was accelerated by injection of TGF-beta 1-treated CM in wound model of hairless mouse. Collectively, these data suggest that TGF-beta 1-treated CM from ADSCs promotes fibroblasts proliferation, migration, type I collagen and MMP-1 expressions, thereby promoting wound healing. Our data indicates the TGF-beta 1-treated CM from ADSCs will be promising agent for wound healing.

003

Novel topical foam shows promise in healing of superficial skin wounds when compared to two current treatments

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The study compared Neosalus™ (water-lipid based topical hydrating foam) to 2 standard wound care regimens-Biafine® (trolamine/sodium alginate topical emulsion) and Release® (sterile, non-adherent absorbable dressing) - using 4 female Domestic Yorkshire crossbred pigs. Six split thickness skin wounds,(2.5cm x 2.5cm x 0.5cm), 3 on either side of the dorsal midline, were created on each animal using a fixed dermatome. Each animal had 2 wounds randomly treated with 1 of 3 treatments. Evaluation for infection, wound closure, and inflammation were conducted by a qualified veterinarian on Days 4, 7, 10 and 14. At study termination, histology was performed for markers of wound healing. None of the wounds showed signs of infection during the study. Application of Neosalus™ was associated with accelerated healing and better control of inflammation when compared to the other 2 treatments. Wound closure was minimal for all wounds on Day 4. On Day 7, wound closure was similar for Neosalus™ and Biafine®, but substantially lower for Release®. By Day 10, all (8 of 8) wounds treated with Neosalus™ had completely closed, whereas only 4 of 8 wounds treated with Release® and only 5 of 8 wounds treated with Biafine® showed complete closure. By Day 14, all wounds in the 3 treatment groups showed complete closure. Lower incidence of erythema was noted for Neosalus™-treated wounds than for Biafine®- and Release®-treated wounds (total erythema score of 6 vs.13 and 11, respectively). Exudate was eliminated from all Neosalus™-treated wounds by Day 7, in contrast to Day 10 for wounds treated with Biafine® and Release®. These findings were supported by histology. Lesser degrees of subacute inflammation and fibrosis, and slightly higher degrees of neovascularization and granulomatous inflammation were observed in Neosalus™-treated wound tissues compared to those treated with the other 2 treatments. The study shows that Neosalus™ can be an effective wound care agent which accelerates closure of superficial skin wounds while controlling inflammation.

004

Pericyte-derived MFG-E8 regulates Angiogenesis

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MFG-E8 is a secreted glycoprotein that is comprised of 2 N-terminal EGF-like domains and 2 C-terminal discoidion domains. One EGF-like domain contains an RGD sequence that binds to αvβ3/5 integrins. Previous studies indicated that MFG-E8 enhances VEGF-dependent angiogenesis, presumably acting via αv-integrins on endothelial cells (EC). Major sources of MFG-E8 *in vivo*, and precise mechanisms of MFG-E8 action remain to be determined. Immunoflorescence of B16 tumors revealed that MFG-E8 localized around blood vessels, and co-localized with PDGFRβ- and NG2-expressing pericytes (PC) rather than EC. RT-PCR analysis of flow-sorted tumor cells, leukocytes, EC, and PC showed that MFG-E8 mRNA expression by PC and EC was significantly higher than expression by tumor cells and leukocytes, and MFG-E8 mRNA levels were >2-fold higher in PC than EC. We characterized tumor vessels and growth in MFG-E8 KO mice to assess the significance of these findings. B16 tumor growth was delayed in MFG-E8 KO mice, and the vessel numbers in melanomas in MFG-E8 KO mice were reduced. PC coverage of vessels was also reduced in tumors in MFG-E8 KO mice and vascular permeability was increased. In mouse retinas, MFG-E8 also co-localized with PC. Pathological angiogenesis was markedly inhibited in MFG-E8 KO mice in a retinopathy of prematurity (ROP) model. Consistent with this result, retinal whole-mounts from ROP mice showed that vascular tufts were ensheathed with PC and MFG-E8. To determine if MFG-E8 regulated PC as well as EC, we studied the PC-like cell line 10T1/2. 10T1/2 cells produced large amounts of MFG-E8 mRNA and protein. Depletion of MFG-E8 from 10T1/2 cells by RNAi markedly inhibited basal and PDGF-induced migration. We conclude that PC are important sources of MFG-E8 *in vivo*, that PC-derived MFG-E8 promotes angiogenesis and vessel stability and that MFG-E8 enhances angiogenesis via actions on PC as well as EC. Our results also suggest that MFG-E8 is a relevant therapeutic target in several important disease states characterized by unwanted angiogenesis.

005

Calpain activity is essential for cell recruitment, myofibroblast differentiation and angiogenesis in early stages of skin wound

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Wound healing is a complex well-orchestrated phenomenon that implicates wound contraction, inflammation, angiogenesis and epidermisation. Calpains are ubiquitous calcium-dependant intracellular cysteine proteases that play an important role in cytoskeleton and adhesion molecule complex rearrangements with implications in cell motility and therefore inflammation, angiogenesis and fibrosis. Using a transgenic mouse overexpressing the natural calpain inhibitor, calpastatin, we asked whether calpains contribute to skin wound healing. Surgical skin wounds were performed on wild-type (WT) and Calpastatin transgenic mice (CPST-TG). Wound closure was delayed in the CPST-TG mice on days 3, 7 and 10. A marked delay was observed on day 3 (Residual wound day 3: 114% in CPST-TG v/s 41 % in WT, p<0,001), suggesting impairment of wound contraction. Immunolabeling revealed a reduction in α-SMA positive cells in the granulation tissue of CPST-TG wounds on day 3 (7% v/s 15%, p<0,05), and a 71% decrease in α-SMA RNA expression. We also found less CD45+ inflammatory cells in CPST-TG wounds (19% v/s 34%, p<0,05). On day 7 after wounding, cell proliferation in the epidermis and the granulation tissue as well as the blood vessel density (106 v/s 152 vessel/mm2, p<0,05) were decreased in CPST-TG animals. This was accompanied by a 28% decrease in VEGFa RNA (NS). Epidermisation was also impaired based on keratin 14 staining at each time point (p<0,05). Our results show a prominent role for calpain in the formation of the granular tissue and the recruitment or the differentiation of myofibroblasts resulting in delayed angiogenesis, proliferation and epidermisation.

006

Transgenic activation of lymphatic vessel function specifically reduces edema formation during acute skin inflammation

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Acute skin inflammation induced by UVB irradiation or delayed-type hypersensitivity (DTH) reactions results in pronounced dilation of cutaneous blood and lymphatic vessels. Whereas there is increasing evidence that blocking the angiogenic cascade through VEGF receptor inhibition prevents acute skin inflammation, the implications of the lymphatic endothelium are unclear. Lymphatic vessels contribute to the regulation of immune responses through their role in antigen transport and leukocyte migration to draining lymph nodes, and they drain edematous tissue fluid and inflammatory cells from inflamed skin. We investigated the contribution of the lymphatic endothelium, its specific receptor VEGFR-3 and its ligands VEGF-C and mouse VEGF-D in two models of acute skin inflammation. To this end, we induced DTH reactions using the cell-mediated hypersensitivity inducing sensitizer oxazolone or applied UVB irradiation in/to keratin 14 (K14)-VEGF-C and K14-mVEGF-D transgenic mice and their wild-type littermates. Our results indicate that genetic overexpression of VEGF-C or the VEGFR3-specific ligand mVEGF-D, leads to increased lymphatic flow, as evaluated by Evans Blue drainage from the inflamed ear and significantly reduces edema formation. Unexpectedly, none of the investigated parameters of acute inflammation, including expression of inflammation markers (such as CCL2, CXCL2, IFN-gamma, IL-6 and TNF-alpha), immigration of inflammatory cells (such as CD11b positive cells) were reduced in VEGF-C or mVEGF-D transgenic mice as compared to wild-type littermates in both models. These findings indicate that activation of lymphatic vessels leads to reduced inflammatory tissue swelling. It might be possible to dissect the process of edema formation from inflammation in general since these two processes are not necessarily interconnected.

007

Wilms Tumor 1 (WT-1) overexpression causes malignant transformation of endothelial cells

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WT-1 was initially described as a tumor suppressor gene lost in Wilms tumor of childhood. However, it is overexpressed in certain tumors, including leukemia. WT-1 is a complex gene, with at four isoforms, two of which are transcription factors and have nuclear localization, while the other two are cytoplasmic and regulate RNA stability. Recently, we observed that WT-1 was expressed in proliferative hemangiomas, but not in vascular malformations. In order to determine the role of WT-1 in endothelial lesions, we overexpressed all four isoforms of WT-1 in MS1 endothelial cells, which do not form proliferative tumors *in vivo*. Overexpression of two isoforms of WT-1 with cytoplasmic localization, resulted in transformation to angiosarcoma. Expression of the nuclear isoforms did not lead to transformation *in vivo*. Expression of angiogenic molecules, such as vascular endothelial growth factor (VEGF), angiopoietin-2, and hypoxia inducible factor-2 was increased by cytoplasmic WT-1 expresssion. These findings indicate that WT-1 is not only a marker of proliferative endothelium, but also a functional contributor to tumorigenesis. Targeting WT-1 may be of benefit in inhibiting angiogenesis and tumorigenesis.

008

Increased expression of secreted frizzled-related protein 2 in patient with Keloid: Crucial role in the tathogenesis of tissue fibrosis

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Recent study have implicated secreted Frizzled-related protein 2 (sFRP2) in the regulation of procollagen. However their effects on dermal fibrosis are unknown. Given the crucial role of collagen metabolism in the pathogenesis of tissue fibrosis, we sought to determine whether sFRP2 is also involved in the pathogenesis of tissue fibrosis in keloid. sFRP2 expression in tissues was analyzed by immunofluorescence and confocal microscopy. The extent of tissue fibrosis in sFRP2-knockout mice and keloid fibroblast was assessed by histologic/histochemical analyses, real-time polymerase chain reaction, Western blot and quantified by hydroxyproline assays. Western blot analysis measured extracellular matrix (ECM) production and phosphorylated signaling molecules in keloid. sFRP2 was markedly increased in the affected skin of keloid patients. Dermal fibrosis and increased collagen content were found in sFRP2-knockout mice. Upregulation of sFRP2 was maintained in cultured keloid fibroblasts, and sFRP2 induced both a dose- and time- dependent increase in collagen type I and fibronectin production. SFRP2 triggered the activation of both p38 mitogen-activated protein kinase (MAPK) kinase and NF kB kinase signaling cascades, the inhibition of which diminished sFRP2-induced ECM production. Our study demonstrates increased local sFRP2 expression in keloid-associated tissue fibrosis both *in vitro* and *in vivo* as well as sFRP2-induced ECM production through both p38 MAPK kinase and NF kB kinase signaling dependent pathways. Our results provide novel insights into the role of sFRP2 in the pathogenesis of tissue fibrosis.

009

Hypoxia and UV light modulate the level, localisation and activity of Hif1α/Hif1β proteins in epidermal keratinocytes.

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Epidermal keratinocytes are assumed to possess complex mechanisms to cope with environmental stressors such as oxygen, UV light and toxicants. Many adaptive mechanisms centre on ARNT (HIF1β) and other PAS transcription factors like HIF1α or AhR. Yet, the intricacy of PAS protein interactions in the epidermis and their potential role in managing with environmental challenge remain unclear. ARNT is thought of as a constitutively expressed protein. However, in epidermal keratinocytes (primary mouse, human N-TERT and SCC-derived) 1% hypoxia (48h) significantly reduced or even abolished ARNT expression. SCC-13 cells showed this effect even at 5% O_2. UV treatment (300 J/m^2) of PMK also results in downregulation of ARNT. Hif1α is understood to stabilise under hypoxia only, yet we detected Hif1α in both hypoxic and normoxic keratinocytes suggesting its putative hypoxia-independent function. Surprisingly, under hypoxia, the level of HIF1α decreased in all tested cell lines. The subcellular localisation of HIF1α was differentially modulated by Ca^{++}. In line with these expressional results, at low Ca^{++} overall HIF1 activity (TransAm HIF1 assay) showed a steady decline proportional to the time in hypoxia. At high Ca^{++} HIF1 transactivation shortly increased (for 3h of hypoxia) and then goes down. In contrast, epithelial cells of non-skin origin (Hct116) showed conventional pattern of increasing HIF1α/ARNT activity under hypoxia. Thus, keratinocytes respond to hypoxia differently than other cell types but in accordance with their differentiation state. Previously, we showed that ARNT deficiency results in severe alterations of mouse epidermal development, differentiation and barrier formation (Geng *et al*., 2006). Together with our current results this provides a mechanistic link between environmental stress and epidermal dysfunctions and suggests that modulation of Hif1α and ARNT may serve as a basic mechanism employed by epidermis to cope with environmental challenges.

010

Epidermal growth factor receptor-mediated reepithelialization in an *in vitro* diabetes model

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Diabetes is the sixth leading cause of death in the United States and a major cause of morbidity. Non-healing wounds represent a troubling complication of diabetes. Hyperglycemia and accumulation of advanced glycation end products (AGEs) and their precursors are believed to contribute to poor wound repair. AGEs are known to alter receptor and protein functions, and the epidermal growth factor receptor (EGFR) is a key regulator of reepithelialization. Therefore, the aim of this study was to investigate the role of AGEs on EGFR activity in an *in vitro* model of diabetes. The human keratinocyte line SCC12F was maintained in media containing physiological glucose levels (1 g/L). Cells were treated with 0.5 mM glyoxal, a well-studied AGE precursor, over a 48 hr period, and EGFR-regulated responses were studied. Increased levels of the AGE carboxymethyl lysine were detected as a result of glyoxal exposure indicating that glyoxal may lead to altered protein functions in keratinocytes. Glyoxal treatment of cells inhibited EGF-stimulated activation of the EGFR as detected by decreased protein tyrosine phosphorylation observed as early as 4 hrs of treatment. Reepithelialization occurs through keratinocyte proliferation and migration, so we examined the impact of glyoxal on these EGF-stimulated responses. Glyoxal exposure inhibited EGF-dependent cell proliferation as measured by bromodeoxyuridine (BrdU) detection. Similarly, EGF-stimulated migration measured using the Boyden chamber assay was decreased in glyoxal-treated cells. The transcription factor Snai2/Slug is a downstream effector of EGF-stimulated reepithelialization, and EGF-dependent induction of Slug protein was decreased following glyoxal treatment. This was evident within 24 hrs of treatment and became more pronounced with extended exposure. These studies indicate that the AGE precursor glyoxal interferes with EGF receptor signaling and downstream effects in keratinocytes. These findings suggest that AGEs may disrupt pathways required for optimal reepithelialization in diabetes.

011

Characterization of biofilm infected wounds in diabetic mice

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A reproducible and consistent animal model is critical in studying the mechanism and management of chronic wound healing. Our previous work demonstrated that *Pseudomonas aeruginosa* (PAO-1) biofilm infection on wounds in diabetic (db/db) mice significantly delayed wound healing at 4 weeks post-wounding. In the present study, we determined time-to-closure of 6 mm db/db mouse wounds infected with biofilm. PAO-1 bacteria were cultured for 72h to form biofilms and were transferred onto 2 day wounds (6 mm) created on the dorsal surface of the experimental groups in db/db mice. Both experimental and control wounds were covered with an occlusive dressing for 12 days. Wounds were harvested at 4, 6 and 8 weeks post-wounding. At 4 weeks, five of 6 (83%) control uninfected wounds healed; zero of 5 (0%) biofilm-infected wounds healed; by 6 weeks, four of 5 (80%) of the biofilm-infected wounds healed; and by 8 weeks, four of 4 (100%) of the biofilm-infected wounds healed. Colony forming units (CFU) on biofilm infected wounds only contained 10^3-10^4 *P. aeruginosa* after 4 weeks. In contrast, scabs from the same wounds had bacterial load of 10^7 CFU. Further studies on scabs showed that 5 μg/ml ofloxacin and 2 μg/ml gentamicin treatments for 4 hours had no effect on bacterial survival. These same conditions showed 100% kill of 10^7 planktonic PAO-1. Our results indicate that pseudomonas biofilm-infected wounds generally heal by 6 weeks, approximately 2 weeks longer than control non-infected wounds in db/db mice and that bacteria in the scabs were very resistant to antibiotic treatment. The presence of antibiotic resistant biofilms may be a factor in chronic wound healing, and this db/db mouse biofilm wound model will be a useful test bed for exploring new strategies to control biofilm *in vivo*.

012

Effect of superoxide dismutase (SOD) /catalase mimetic EUK-207 on the radiation-induced skin injury

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The skin is one of the first and most critical organs affected by radiation exposure, but there are currently no approved drugs to mitigate radiation-induced skin injury. Progression of radiation-induced injury may be driven by chronic oxidative stress. To determine if an synthetic SOD/catalase mimetic, EUK-207, ameliorates cutaneous radiation injury, we tested this compound in a rat model of combined skin irradiation and wound injury. Six week old male WAG/RijCmcr rats were randomly divided into three experimental groups, 9 animals per group as follows (group 1: irradiated + EUK-207; group 2: irradiated + vehicle; group 3: non-irradiated + EUK-207). Unanesthetized rats were given a single radiation dose of 30 Gy defined at the upper dermis level. Within one hour after irradiation, rats were anesthetized and wounded. Control animals were sham irradiated and wounded in the same manner. In order to mimic a terrorist scenario where medical help is often delayed we started with drug administration two days post-irradiation. The Alzet pumps filled with EUK-207 (1.8 mg/kg/day) or vehicle control were implanted into the rats. All animals were treated with EUK-207 for 90 days and followed to 180 days. The extent of radiation-induced skin damage was scored weekly. To evaluate wound healing, we traced the wound, calculated the surface area and compared it to the original wound size at day 0. Within one month, rats treated with EUK-207 demonstrated significantly less skin injury in comparison to irradiated and vehicle-treated animals ($p<0.03$). Wound measurement at 21 days post-irradiation showed significantly smaller wounds ($p<0.001$) in animals treated with EUK-207 (32% of original wound size) in comparison to the irradiated and vehicle-treated group (58% of original wound size). These studies demonstrated that the synthetic SOD/catalase mimetic EUK-207 mitigates radiation-induced skin injury and improves wound healing in irradiated skin.

013

High-fat diet promotes skin inflammation by vascular abnormality and accelerates UVB-induced skin damage

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Previously, we have shown that UVB irradiation induced cutaneous angiogenesis and lymphatic hyperpermeability resulting in the acceleration of skin-damage. These results demonstrated an important role of the cutaneous blood vessels in photo-aging. Since cutaneous blood vessels are also the main conduit of nutrients to the skin, we hypothesized that high-fat diet could affect the microcirculation and thus the condition of the skin. Indeed, the H&E staining revealed that HR-1 hairless mouse fed with high-fat diet (60% of calories as fat) showed thicker dermis and epidermis as compared to mouse fed with control diet after the 11-weeks. Surprisingly in the skin of high-fat diet mice, CD11b-positive macrophages and inducible NO synthase (iNOS)-positive cells were pronouncedly recruited when compared to control mouse skin. Additional double immunofluorescence analysis for the blood vessel marker, Meca32, and lymphatic marker, podoplanin, revealed enlarged blood vessels in the skin with high-fat diet, whereas the lymphatic vessels were comparable between two groups. These results suggest that high-fat diet promotes CD11b-positive cell recruitment and upregulates iNOS expression triggering blood vascular abnormality. Moreover, in order to investigate whether high-fat diet influences UVB-induced skin damage, HR-1 mice fed with high-fat diet were exposed to UVB irradiation for 11 weeks (total: 4.3mJ/cm^2). Interestingly, the moisture content of the stratum corneum of the UVB-exposed skin together with high-fat diet was significantly decreased as compared to the UVB-exposed skin with control diet. These results indicate that high-fat diet promotes skin inflammation by vascular abnormality and causes unfavorable skin conditions such as dry skin and photo-aging.

014

Curcumin at nM concentrations limits burn injury progression and causes β-adrenergic receptor-mediated vasodilation

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Injury progression for several days after burns may convert 2nd-degree burns to full-thickness burns leading to excess morbidity, including more scarring and wound contractures, and poor quality of life. No therapy exists that reduces burn injury progression. Recently we found that purified curcumin reduced injury progression in a rat hot comb model when administered iv at 1 and 24 hours after burns. A bimodal dose response curve suggested that curcumin may have different mechanisms of action at low (nM) and high (μM) concentrations. Many reports in the literature suggest that curcumin has anti-inflammatory and anti-oxidant activities at pericytotoxic μM concentrations. Using an *ex vivo* hamster cheek pouch model of arcade and terminal arteriole vasomotor activity, we now report that curcumin induced vasodilation or vasoconstriction depending on the dose (nM-μM) and time from delivery. Videomicroscopic observation sites were terminal arterioles (baseline diameter, 8±2 μm) and arcade arterioles (22±11μm) that supplied them. Curcumin (10-12 to 10-4M) was applied in increasing doses using micropipette delivery for 60 seconds. In both arcade and terminal arterioles, a biphasic response was observed over time with increasing doses (μM). There was an initial dilation, predominant at nM doses, followed by constriction, predominant at μM doses. Peak dilation (+40±8%) was observed at 1nM and peak constriction (-15±5%) was observed at 1μM. The fitted logEC50 and peak responses were similar for arcade and terminal arterioles. Dilation was inhibited by propranolol while constriction was inhibited by pentolamine, indicating β- and α-adrenergic receptor activity, respectively. These findings elucidate a possible mechanism by which curcumin at nM levels prevented burn injury progression. Such mechanistic studies are key to advancing curcumin treatment of burns to clinical trials.

015

Modulation of VEGF expression enhances epidermal and blood vessel protection against UVB and oxidative stress

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VEGF plays diverse roles in different skin cell types, and is also implicated in skin photoaging, hypoxia, and wound healing. In the present study, the expression of VEGF-A and VEGFR-2 (Flk-1), its major receptor, were investigated in different cell lines by RT-PCR. Immunofluorescence staining of VEGF-A and Flk-1 showed that IV09.006, an active ingredient designed to target the VEGF pathway, increased the expression of these two proteins in normal human keratinocytes (NHK) and endothelial cells. Using immunofluorescence staining of VEGF-A on *ex vivo* skin biopsies, the expression of VEGF-A in the epidermis appeared to be mainly located in the suprabasal layers, in accordance with the previously reported correlation between VEGF expression and keratinocyte differentiation. UVB irradiation and H2O2-induced stress were shown to increase VEGF-A expression in the epidermis. In parallel, pre-treatment with IV09.006 was shown to protect skin structure from stress-induced damage. In order to further investigate the effect on angiogenesis, *in vivo*, we used chicken chorio-allantoïc membrane (CAM). In this model, the IV09.006 active was applied directly on the CAM for 24h, and then a stress induced by UVB irradiation (60 mJ/cm2) or H2O2 (10 mM) was applied on the CAM. A time course observation of the blood vessel network was performed after each stress condition. Our study showed that pre-induction of VEGF-A and Flk-1 enabled a better maintenance of the blood vessel network, preventing vasodilatation and coagulation induced by stress. Taken together, these results indicate that the positive modulation of VEGF-A and Flk-1 expression could be linked to a better preservation of the epidermis from UVB and oxidative stress-induced damage, as well as a protection of the blood vessel network from these stresses.

016

Rapamycin blocks lymphangiogenesis induced by TSC2-null hamartoma cells

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Rapamycin is a molecularly targeted therapy in trials for cancers including tumors in patients with tuberous sclerosis complex (TSC). Studies of TSC provide insights into the mechanisms of action of rapamycin, since rapamycin specifically inhibits mTORC1, a central regulator of cell growth, and TSC tumors due to loss of TSC2 are characterized by abnormally activated mTORC1. In our earlier study, we showed that rapamycin has anti-neoplastic and anti-angiogenic effects in a xenograft mouse model for TSC skin tumors. Here we used this model to determine the effects of rapamycin on lymphangiogenesis in TSC. Lymphangiogenesis is prominent in TSC internal tumors and may play a role in tumor growth and metastasis, but the extent of lymphangiogenesis in hamartomatous TSC skin tumors was unknown. Human angiofibromas, periungual fibromas, and forehead plaques were stained using D2-40, a marker of lymphatic endothelial cells. TSC skin tumors had greater lymphatic vessel density, vessel size, and total vessel area relative to dermal area than patient normal-appearing skin ($p<0.05$), indicating that TSC skin tumors have increased lymphangiogenesis. TSC2-null fibroblast-like cells from a forehead plaque or fibroblasts from patient normal-appearing skin were incorporated into skin equivalents and grafted onto nude mice. Grafts were allowed to heal for 5 weeks after which mice were treated with rapamycin (14 tumor, 17 normal) or vehicle (13 tumor, 12 normal) by intraperitoneal injection every other day for 12 weeks. Sections stained for mouse D2-40 showed that TSC tumor grafts had greater lymphatic vessel density, vessel size, and total vessel area relative to dermal area than normal grafts ($p<0.05$). Rapamycin almost completely abrogated lymphatic vessel formation in grafts containing either TSC tumor cells or normal fibroblasts. These data indicate that TSC skin tumors are lymphangiogenic, that TSC2-null fibroblasts induce lymphangiogenesis in xenografted skin, and that rapamycin blocks lymphangiogenesis in grafted skin.

017

Lhx2 regulates Sox9 and Tcf4 to supply hair follicle-derived progenitor cells to the wound epithelium

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Lhx family of transcription factors play essential roles in the morphogenesis and patterning of ectodermal derivatives. In the skin, Lhx2 controls the activity of epithelial stem cells residing in the hair follicle during its development and postnatal cycling. We demonstrate here that Lhx2 is also involved in the control of wound healing. In response to the wounding, Lhx2+ cells located in the bulge of the hair follicles begin to proliferate, and their number significantly increased by day 5 post wounding. In heterozygous Lhx2 knockout (+/-) mice, the wound healing process was significantly retarded compared to wild-type controls, thus suggesting a contribution of the Lhx2 to regeneration of the skin epithelium. By microarray analysis, qRT-PCR and immunohistochemistry, expressions of the selected markers of epithelial stem cells (Sox9, Tcf3, Tcf4) decreased in the Lhx2 knockout (-/-) mice versus wild-type controls. Furthermore, ChIP analysis of primary mouse keratinocytes revealed Sox9 and Tcf4 as direct Lhx2 targets. Finally, number of the Sox9+ and Tcf4+ cells was significantly reduced in the infundibulum of the HFs adjacent to the wound in the Lhx2+/- mice versus wild-type control. These data provide evidence that Lhx2 is involved in controlling the supply of the hair follicle-derived progenitor cells to the wound epithelium, at least in part, via direct regulation of the expression of Sox9 and Tcf4. These data also suggest Lhx2 as a potential target for the development of new approaches to modulate stem cell activity in a number of pathological skin conditions associated with their un-controlled activation or suppression.

018

The neuropeptide pituitary adenylate cyclase activating Polypeptide regulates neuro-vascular responses in human skin *in vivo*

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Neuropeptides with vasoregulatory capacities may play an important role in skin diseases like atopic dermatitis, erythromelalgia or rosacea, for example. Pituitary adenylate cyclase activating peptide (PACAP) is a novel neuropeptide and immunomodulator in various tissues. Although this peptide and its receptors are expressed in human skin, its biological role is still unknown. We tested whether PACAP regulates vascular responses in human skin *in vivo* and *in vitro*. When injected intravenously, PACAP dose-dependently induced a significant vascular response (flush, erythema, edema) in humans. PACAP also mediated a significant and dose-dependent increase in intrarectal body temperature. These profound effects were not observed in controls. Immunohistochemistry revealed a close association of PACAP-immunoreactive nerve fibers with mast cells and dermal blood vessels. VPAC1R was expressed by dermal endothelial cells, $CD4^+$ and $CD8^+$ T cells, mast cells and keratinocytes while VPAC2R was positively stained only in keratinocytes. VPAC1R protein and mRNA was also detected on human dermal microvascular endothelial cells (HDMEC). Activation of cAMP in HDMEC by PACAP demonstrated VPAC1R to be functional. Together, PACAP directly induces vascular responses in humans which may be associated with neurogenic inflammation.

019

Transgenic overexpression of keratinocyte-specific VEGF and Ang1 in combination promotes wound healing under nondiabetic but not diabetic conditions: A mechanistic role for Ang1-mediated delay in re-epithelialization

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VEGF and Ang1 are growth factors involved in angiogenic remodeling and improve wound healing outcomes. We previously demonstrated an increased rate of wound healing in KC-Ang1 animals under nondiabetic conditions and reported a 1.6-fold increase in granulation tissue ($p=0.03$) along with a 60% decrease in re-epithelialization 7d following wounding ($p<0.05$). We hypothesized that combining VEGF and Ang1 would further enhance diabetes-impaired wound healing. KC-VEGF-Ang1, KC-VEGF or KC-Ang1 mice and their littermate controls were made diabetic (DB) with STZ or left as nondiabetic (ND) controls for 6-8 weeks with transgene expression induced just prior to wounding. Following wounding, all ND mice healed more quickly than their DB counterparts ($p<0.05$). Under DB conditions, the overexpression of VEGF, Ang1 or both failed to improve the rate of wound closure compared to control mice. In contrast, under ND conditions, KC-VEGF-Ang1 mice healed more quickly than all other animals beginning 5d post wounding ($p\leq0.001$). Expression analyses of VEGF and Ang1 revealed no change in VEGF but an 80% decrease in Ang1 ($p<0.01$) in DB mice suggesting Ang1 plays an integral role during wound healing under ND conditions in KC-VEGF-Ang1 mice, consistent with our previous report. To examine if Ang1 mediates its effects on KCs directly, we used an *in vitro* wound healing bioassay and stimulated primary KCs from wildtype mice with Ang1 and confirmed a 16% reduction in KC migration into the wound bed ($p<0.01$). Examination of integrin signaling in Ang1-stimulated KCs identified decreases in pMAPK, pNFκB, pAkt, and pStat3. These data suggest that combined VEGF-Ang1 overexpression cannot overcome DB-induced delays in wound healing but is efficacious under ND conditions possibly via Ang1-mediated effects on KCs leading to delays in re-epithelialization, and enhancement of granulation tissue formation, thereby allowing secondary intention healing to occur more rapidly.

020

Regulation of extracellular matrix protein expression by NF-κB and its role in impaired wound healing response

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The normal wound healing process follows a coordinated sequence of events including hemostasis, inflammation, cell proliferation, and tissue remodeling, with each phase involving multiple interactions between cells, extracellular matrix (ECM) and growth factors. However, a prolonged inflammatory phase coupled with slow-forming ECM leads to compromised re-epithelialization at the wound site, resulting in chronic wounds. The NF-κB pathway is known to be key regulator of inflammatory mediators, and leads to excessive activity of matrix metalloproteases (MMPs), such as collagenase and elastase, causing premature degradation of collagen and elastic fibers. Due to the degradation of these matrix proteins, the scaffold on which re-epithelialization occurs gets disrupted, leading to impaired wound healing. However, the direct effects of NF-κB activation on matrix proteins have not been documented. To investigate this, we utilized elastin and collagen1A luciferase promoters in H9c2 cardiomyocytes, and observed a significant inhibition in the levels of elastin and collagen1A by inducing NF-κB via TNF-α. In addition, blocking the *de novo* expression of NF-κB by using p65 specific siRNA resulted in enhanced levels of elastin and collagen1A. Treatment with a NF-κB inhibitor reversed the inhibition of elastin and collagen 1A expression, confirming that the effect was mediated by NF-κB activation. By treating the cells with increasing doses of a NF-κB inhibitor, it was found that a high degree of NF-κB suppression is required for a substantial increase in elastin. Taken together, our results provide evidence that the activation of NF-κB signaling directly suppresses key matrix proteins, elastin and collagen1A, which may lead to disruption of the wound repair process.

021

A 124-amino acid peptide from secreted heat shock protein-90 (HSP90) is a novel wound repair agent

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Hypoxia plays a critical role in pathogenesis of a variety of human diseases such as wound healing, ischemic cardiovascular disease and cancer. After an acute skin injury, the microenvironment is extremely hypoxic due to the clotting of blood vessels and high oxygen consumption by cells in and around the wound site. We have shown that acute hypoxia is a potent stimulus to increase the migration of both epidermal and dermal cells. However, what factor(s) that actually mediates the hypoxia-promoted cell motility was not clear. In primary human keratinocytes (HKs), we found that hypoxia triggers HKs to secrete heat shock protein 90-alpha (hsp90alpha) to the extracellular environment, via hypoxia inducible factor 1 (HIF-1)-dependent pathway (Woodley et al. 2009, JCS, 122:1495). The secreted hsp90alpha then binds to the cell surface receptor, called LDL Receptor-Related Protein-1 (LRP1), and stimulates HK migration via an autocrine signaling loop. Expression of a constitutively activated HIF-1 fully mimicked the hypoxia-driven motility in HKs even under normoxia. In contrast, expression of a dominant negative HIF-1 or shRNA down-regulation of HIF-1 completely abolished the hypoxia-driven cell migration. The addition of recombinant hsp90alpha duplicated the pro-motility effect of hypoxia. In contrast, inhibition of the extracellular hsp90alpha function blocked hypoxia-induced HK migration. Furthermore, genetic silencing LRP-1 completely blocked hypoxia-triggered HK migration. In this study, we have narrowed down the region in secreted hsp90alpha to a 124-amino acid peptide that retained the full pro-motility activity of the full-length hsp90alpha in tissue culture. Most importantly, topical application of this peptide accelerated wound healing by increased re-epithelialization process in mice. In parallel, the FDA-approved RegranexTM (PDGF-BB) showed much less effect than the hsp90alpha peptide. Thus, this study has discovered a novel wound healing agent: a 124-amino acid peptide within secreted hsp90alpha.

022

Psychological stress-induced delays in cutaneous wound healing reversed by inhibitor of the glucocorticoid activating enzyme, 11β-hydroxysteroid dehydrogenase type 1

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Psychological stress (PS) adversely affects cutaneous wound healing, including primarily a slowing of the recovery process. Previously, our lab and others have demonstrated delayed repair of epidermal barrier function following superficial wounding (i.e., repeated tape-strippings) in PS- vs. non-PS mouse and human skin, with increased endogenous glucocorticoids (GC) accounting for the delays in barrier recovery. Here we assess the hypothesis that the PS-induced delay in wound healing requires localized activation of endogenous GC species produced under stress. We first confirmed that PS (overnight motion restriction) elevated serum GC levels, and significantly delayed wound recovery (i.e., 21-26% delay vs. non-PS control wound area) in hairless mice 2-to-6 d following a full-thickness biopsy wound (<7mm). Blockade of either GC production using the corticotrophin-releasing hormone inhibitor, antalarmin (0.4mg in 4% EtOH IP), or GC peripheral action using RU-486 (0.12mg in 4% EtOH IP), normalized wound recovery in PS mice. In addition, inhibition of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme that converts relatively inactive GC (cortisone in humans, 11-dehydrocorticosterone in rodents) to more-active GC (cortisol in humans, corticosterone in rodents), with carbenoxolone applied topically to wound sites twice daily (0.2μmols in PG:EtOH [3:7] vehicle) completely normalized PS-induced delays in wound healing 2-to-6 d following wounding. As an analogous stress model, hairless mice were treated systemically with IP dexamethasone (10μg in 0.5% DMSO) once daily for 6 d after wounding, and topical carbenoxolone normalized the dexamethasone-induced delay in wound healing (days 4-6). Together, these results not only indicate that the conversion of inactive-to-active GC is important in the PS-induced delay of wound healing, but also suggest that reduction of active GC in the skin can override systemically-initiated PS effects that alter cutaneous wound healing.

023

TGFβ dependent differentiation of dermal fibroblasts to myofibroblasts is regulated by CLIC4

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CLIC4 is a highly conserved, multifunctional protein that causes growth arrest and terminal differentiation in skin keratinocytes and other cell types. Recently, we discovered that CLIC4 is a component of the TGFβ pathway. In skin keratinocytes, TGFβ causes CLIC4 to associate with Schnurri-2 and translocate to the nucleus. Nuclear CLIC4 prolongs TGFβ signaling by inhibiting Smad2/3 dephosphorylation. TGFβ is fundamental to the conversion of fibroblasts to myofibroblasts, an important event in skin wound healing, fibrosis and cancer development. This study was designed to determine if CLIC4 participates in TGFβ dependent myofibroblast conversion of dermal fibroblasts. Using primary dermal fibroblasts from CLIC4wt/wt and CLIC4fl/fl mice transduced with adenoviral Cre recombinase to ablate CLIC4 expression, we show here that absence of CLIC4 inhibits myofibroblast conversion as measured by transcription and translation of alpha smooth muscle actin (αSMA). Expression of extracellular matrix components like the collagens, MMPs and thrombospondins that TGFβ induces during fibroblast activation is reduced in TGFβ treated adeno-Cre infected CLIC4 fl/fl fibroblasts. These cells also have higher cell motility compared to wild type controls. Fibroblasts deleted of CLIC4 have reduced activation of Smad2 and p38 upon TGFβ stimulation. Smad2 siRNA and p38 kinase inhibitors prevented TGFβ stimulation of myofibroblast conversion suggesting that CLIC4 might influence fibroblast differentiation through interaction with both Smad-dependent and Smad-independent TGFβ pathways. Thus CLIC4 plays an important role in both epithelial and mesenchymal cell biology by altering TGFβ signaling and is therefore an attractive target for modifying TGFβ in both tissue compartments.

024

Cholesterol synthesis mediates inhibition of wound healing via anti-inflammatory signals whereas statins reverse it

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Cholesterol synthesis, by a dual mechanism (through farnesyl pyrophosphate [FPP] and synthesis of Glucocorticoids [GCs]) regulates wound healing. While it is established that GCs inhibit wound healing, the discovery that FPP, an intermediate product of the mevalonate pathway, binds and activates the glucocorticoid receptor (GR) to also inhibit healing is new. To understand the mechanisms of this inhibition, we used *ex vivo* human skin and *in vivo* porcine wound healing models in conjunction with microarrays. qPCR results verified that human and porcine epidermis express enzymes necessary for steroid synthesis and regulation, suggesting extra-adrenal synthesis of cortisol. In both models, CYP11B1 (enzyme that executes cortisol synthesis) induction was evident at 24h post-wounding and peaked at 48h, correlating with the highest activity of pro-inflammatory cytokines. Therefore, our hypothesis was that epidermal GC synthesis is triggered by pro-inflammatory signals released by injury as part of a negative feed-back mechanism to curb inflammation. We confirmed that IL-1 induces expression of CYP11B1 and cortisol production in epidermis. Conversely, inhibition of cortisol synthesis during healing increases IL-1. Acting through the same mechanism as GCs, an intermediate of cholesterol synthesis, FPP, causes activation and nuclear translocation of the GR. Both endogenous and exogenous FPP inhibit keratinocyte migration and wound epithelialization *ex vivo*. Using chromatin immunoprecipitation (ChIP) and transfection assays, we show that FPP-GR binds to keratin (K6) promoter and inhibits its transcription. These data suggest that inhibition of cholesterol synthesis would simultaneously block both GC and FPP pathways. Mevastatin, a common drug that lowers cholesterol, promoted keratinocyte migration, induced K6 expression and epithelialization *ex vivo* and *in vivo*. We conclude that Statins, as modulators of FPP and cortisol production in epidermis, may be a new approach to stimulate wound healing.

025

Cutaneous wound healing in the EpiDerm-FT™ full-thickness *in vitro* human skin model

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Cutaneous wound healing involves interactions between dermal fibroblasts and epidermal keratinocytes, as well as cell and extracellular matrix interactions. This poster describes wound healing experiments conducted with a full–thickness *in vitro* human skin model (EpiDerm–FT™). Normal human epidermal keratinocytes (KC) and dermal fibroblasts (FB) were cultured to produce the highly differentiated full–thickness skin model. Small wounds of several mm diameter were induced in the epithelial model by means of a battery operated cauterizer or a dermal biopsy punch. The wounded EpiDerm–FT™ cultures were fixed at various time points and H&E stained paraffin sections were prepared to evaluate the wound and the wound healing process. Immediately after burn wounding, necrotic epithelium and denatured collagen dermal matrix were evident. Within one day, the denatured collagen matrix began to degrade and epithelial KC were observed migrating inward from the wound edges. Over a time course of seven days, migrating KC repopulated the wounded area to form a fully covered epithelium. Dermal fibroblasts were also observed to be proliferating within the wound area and generating new dermal matrix material. Biopsy punches were used to produce wounds that removed only the epidermis. These wounds also healed within a timeframe of 3–7 days. Gene expression profiling of the wounded area showed temporally regulated increases in mRNA expression of basement membrane components, collagens and genes involved in extracellular matrix remodeling. Increased FB proliferation in dermal areas directly adjacent to migrating KC was observed. FB proliferation and epidermal healing were severely impaired in the presence of an EGFR tyrosine kinase inhibitor or a TGFα neutralizing antibody. These results demonstrate that EpiDerm–FT™ is a useful *in vitro* skin model for investigating dermal–epidermal interactions during wound healing and evaluation of the role of specific growth factors or new therapeutics in the dermal wound healing process.

026

Structural relationships between dermal blood vessels and the epithelium in mouse skin

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The vascular system is involved in regulating many developmental processes including the creation of microenvironments that help maintain stem cells. A number of examples have been previously reported: hematopoietic stem cells reside adjacent to sinusoidal blood vessels in the bone marrow; mammalian germ line niche in testes is established as a consequence of vascular pattern formation; the vasculature is a key component of the adult subventricular zone neural stem cell niche; and brain cancer stem cells are maintained within vascular niches. These studies provide evidence that blood vessels may contribute or help create microenvironments for stem cells. Since there is an abundance of blood vessels beneath the epidermis, we were interested in examining the structural patterns of the dermal vasculature and blood vessels and wanted to determine if there is a characteristic relationship between dermal blood vessels and the epithelium and epithelial structures. Dermal blood and lymphatic vessels were characterized with a variety of different cell surface markers including PAL-E, CD31, CD146, CD34, LYVE-1 and desmin, and CD31 was found to be a consistently good marker for dermal vasculature in mouse skin. The spatial relationship between epithelium and blood vessels was detected by immunofluorescence staining using fluorescence microscopy and three-dimensional reconstitution via confocal imaging. We found that the dermal blood vessels go up to the bulge area and then form a loop around the bulge. The density of blood vessels surrounding the bulge area is much higher than that adjacent to other epithelial areas. Additionally, within the interfollicular area, the blood vessels form another loop close to where the hair shaft comes out in the epithelium. Given this pattern of blood vessels in mouse skin, we want to determine if the dermal vasculature patterns correlate with the location of keratinocyte stem cells in skin.

027

Overexpression of Hoxb13 in the basal layer of the epidermis results in upregulation of TGF-β activity and an enhanced fibrotic response in cutaneous wounds

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The highly conserved Hox transcription factors are essential for patterning and designation of cell fate in the developing embryo and are thought to play important roles in the maintenance and repair of adult tissues. We have previously determined that loss of Hoxb13 from adult skin results in enhanced cutaneous wound healing with reduced scarring. We have recently demonstrated that mice overexpressing Hoxb13 in the basal layer of the epidermis via the K14 promoter (Hoxb13K14 mice) show upregulation of VEGF and TNF-α and significantly delayed wound healing as exemplified by persistence of the fibrin clot, a prolonged inflammatory response, and enlarged blood and lymphatic vessels. During this study, observation of hematoxylin and eosin stained sections of 12 day old full-thickness Hoxb13K14 cutaneous wound beds had suggested an enhanced fibrotic response. TGF-β has been identified as a key profibrotic molecule primarily through its role in the fibroblast to myofibroblast transition. To investigate a role for Hoxb13 in fibrosis, we evaluated TGF-β signaling, myofibroblast differentiation, and extracellular matrix (ECM) deposition in Hoxb13K14 wounds at specified time points post-wounding. Compared to their wild-type littermates, Hoxb13K14 wounds showed upregulation of TGF-β activity as determined by significantly higher levels of phosphorylated Smad-2, and increased ECM deposition. Histological analysis and staining with α-SMA revealed an enhanced presence of myofibroblasts in Hoxb13K14 wounds. We postulate that the preponderance of inflammatory cells in Hoxb13 K14 wounds is contributing to the fibrotic response by prolonged exposure to TGF- β from these cells, which supports a critical role for inflammation in regulating fibrosis/scar tissue formation. In conclusion, our current data suggest that Hoxb13 is a potent profibrotic molecule and a potentially important clinical target in pathologies characterized by abnormal fibrosis.

028

Human cutaneous squamous cell carcinoma is associated with increased numbers of VEGF-C producing macrophages

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Metastases from primary cutaneous SCC account for the majority of the 10,000 cancer deaths form nonmelanoma skin cancer in the United States each year. Metastasis is potentially linked to increased lymphangiogenesis adjacent to SCC tumors. Human SCC samples were stained by double label immunofluorescence (IF) for the lymphatic endothelial vessel markers LYVE-1 and VEGFR-3. LYVE-1 positive cells were counted in tumors and compared with normal skin by immunohistochemistry. Gene set enrichment analysis (GSEA) was performed on SCC, adjacent non-tumor bearing skin and normal skin to determine differential expression of lymphangiogenesis-associated gene sets. Laser capture microdissection (LCM) was performed to isolate tumor and tumor-associated inflammatory cells for further gene expression analysis, which confirmed the increased expression of VEGF-C in the inflammatory cells. IF was performed to determine the source of VEGF-C in the tumor microenvironment. We found LYVE-1+/VEGFR-3+ cells in the dermis immediately adjacent to SCC tumor nests. GSEA analysis confirmed increased expression of lymphatic endothelial cell (LEC) specific genes in adjacent peritumoral skin compared to normal skin. The presence of CD163+/VEGFC+ and CD68+/VEGFC+ cells by IF suggests that VEGF-C is macrophage derived. Clarification of mechanisms governing SCC-associated macrophage-mediated lymphangiogenesis may identify potential targets for therapeutic intervention.

029

Cutaneous response to long term percutaneous porous/solid biomaterial implants in a mouse model

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The goal of our research is to biointegrate the skin with percutaneous medical devices to preclude infection and create stability at the skin/device interface. In previous studies using a mouse implant model, we showed that keratinocytes migrate into porous poly(2-hydroxyethyl methacrylate) [poly(HEMA)] rods over 7-28 days. In this study, we mimicked a catheter with a surrounding porous cuff using a solid 0.4 mm diameter poly(HEMA) rod, 10 mm in length, engineered with an outer ring of sphere templated porous poly(HEMA) (outer diameter: 1 mm, 36 μm pores, 14 μm interconnecting pore throats). We hypothesized that the keratinocytes would migrate through the pores, eventually reaching the solid core and subsequently stopping, permigrating, or marsupializing along the porous/solid interface. Rods, implanted through the dorsal skin of C57BL/6 mice, were harvested 7, 22, 50, 56 and 112 days post-implantation. Specimens were analyzed immunohistochemically for keratinocytes, nerves, and endothelial cells. Implanted rods remained infection-free at all times. Keratinocytes migrated into the pores but did not reach the porous/solid interface; migration did not continue beyond the distance observed in the 7 day time-points. A vascular network, as indicated by endothelial cell staining, matured and persisted within the dermal component of the porous cuff. We found no evidence of innervation of the porous cuff. None of the implants showed signs of rod encapsulation. We conclude that: 1) keratinocytes migrate for a limited distance into the pores, 2) a robust vascular network forms, 3) innervation does not occur by 112 days of implantation, and 4) the rods are not encapsulated.

030

Comparison study with a honey dressing and collagen powder for healing of deep partial thickness wounds using a porcine model

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Honey has many properties which make it an attractive addition to a wound dressing including potential angiogenic, anti-inflammatory and antimicrobial activities. Collagen has also been incorporated into a variety of products and has been shown to have stimulatory effects on tissue repair. Here we present a study of two formulations, one with the addition of honey into a glycerin base hydrogel and a new hydrolyzed bovine collagen powder. Multiple (720) deep partial thickness wounds were created on the back of 6 pigs. Wounds were randomly assigned to one of four treatment groups: 1) hydrogel with honey, 2) hydrogel alone, 3) collagen powder or 4) untreated air exposed. On days 4-10 post treatment wounds were assessed for complete epithelialization using a well defined salt split technique. All treatments significantly enhanced the rate of epithelialization as compared to untreated controls with an onset of complete healing two days earlier than the untreated controls. The hydrogel dressing with honey enhanced the rate of epithelialization resulting in 6.75% faster healing than hydrogel dressing alone. Interestingly, the collagen powder was the most effective treatment, resulting in 27.40% faster healing than untreated wounds. This data shows that both the honey and a collagen powder treatments are beneficial for epithelialization of acute wounds and additional well controlled studies examining their potential use on burns and chronic wounds is warranted.

031

Tgfb3 decreases myofibroblast recruitment and keratinocyte proliferation during excisional wound repair

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The transforming growth factor beta (Tgfb) family is comprised of three isoforms that share similar structural homologies. Each has cell-specific effects on cellular proliferation, differentiation, and metabolism. However, their specific role throughout the wound repair process remains elusive. Here, we injected either saline, Tgfb3, or Tgfb3 with Tgfb3 neutralizing antibody under six mm bilateral full thickness wounds of adult mice one day post-wounding. Mice were sacrificed and wounds were harvested 7 days post-wounding. Morphometric analyses were performed on serial histological sections to compare the epidermal surface and epidermal and wound volume between the three groups. Despite no statistical significance between mice injected with saline (n=7) and Tgfb3 (n=4) at day 7 post-wounding, Tgfb3-injected wounds had decreased levels of α-smooth muscle actin (α-SMA) and collagen. Furthermore, Tgfb3-injected wounds showed decreased proliferation in the epidermal compartment. These data suggest that Tgfb3 changes the dynamic mechanism of wound repair by decreasing the contraction potential of the wounds through lowering myofibroblast recruitment and by decreasing cellular epidermal proliferation in the epidermis. In addition to its therapeutic potential in incisional wounds, these data support a role for Tgfb3 in excisional cutaneous wounds.

032

Chloride channels in epithelium and their roles in endogenous wound electric currents

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Endogenous electric fields at wounds are a powerful signal that directs cell migration in wound healing. How the electric fields are generated is not known. We used vibrating probe and ion selective probe systems to investigate the underlying mechanisms. We found that wounds in human and mouse skin produced dynamically-regulated electric currents. After initial inward electric currents of ~10 min duration following injury, the electric currents reversed direction to become outward. The size of the currents kept increasing to a peak at ~120min. We detected large Ca2+, Na+ effluxes with a similar but shorter rising phase as the wound electric currents. There was a large influx of chloride ions, which started to increase after around 2 hours and were very steady. We therefore focused on chloride channels to determine the expression profile of CLC chloride channels in stratified epithelia. We first focused on cornea epithelium due to its simpler structure. We found that the mRNAs of CLC-2, CLC-3, CLC-4, CLC-5, CLC-6, and CFTR were present in human corneal epithelial cells. CLC-1 and CLC-7 were not detectable. Western blot and immunostaining confirmed protein expression and distribution of CLC-2, CLC-3, CLC-4, CLC-6 and CFTR in human corneal epithelium. CLC-2 preferentially labeled the apical and basal layers, while CLC-3 and CLC-4 labeled only the superficial layer. CLC-6 and CFTR labeling showed a unique gradient with strong staining in apical layers which gradually decreased towards the basal layers. We compared the expression profile and pattern in human epidermis. We conclude that both skin and corneal epithelial cells express functional Cl- channels and transporters. CLC-2, CLC-3, CLC-4, CLC-6, and CFTR had distinct expression patterns in the stratified epithelium. Those molecules and their distribution may play important roles in maintaining resting Cl- fluxes and in regulating Cl- flux at wounds, which may be a major contributor to wound electrical signaling.

033

Identification of TRAIL and other molecules that distinguish inflammatory (CD11c+CD1c-) DCs from resident (CD1c+) DCs in human skin

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Previous work has identified CD11c+CD1c- dendritic cells (DCs) as the major "inflammatory" dermal DC population in psoriasis vulgaris and CD1c+ DCs as the "resident" cutaneous DC population. Objective: To further define molecular differences between these two myeloid dermal DC populations. Inflammatory and resident DCs were single-cell sorted from psoriasis lesional skin biopsies, and gene array expression profiling was performed. Results were confirmed with RT-PCR, flow cytometry, immunohistochemistry, and double label immunofluorescence. Pooled human keratinocytes were cultured for functional studies. TNF-related apoptosis-inducing ligand (TRAIL), Toll-like receptors (TLRs) 1 and 2, S100A12/EN-RAGE, CD32, and many other inflammatory products were selectively expressed in inflammatory DCs than in resident DCs. Flow cytometry and immunofluorescence confirmed higher protein expression on CD1c- versus CD1c+ DCs. TRAIL receptor, death receptor 4 (DR4), was expressed on basal keratinocytes and blood vessels, and *in vitro* culture of keratinocytes with rh-TRAIL induced CCL20 leukocyte chemokine. CD11c+CD1c- inflammatory DCs in psoriatic lesional skin express a wide range of inflammatory molecules compared to skin resident CD1c+ DCs. Some molecules made by inflammatory DCs, including TRAIL, could have direct effects on keratinocytes or other skin cell types to promote disease pathogenesis.

034

FcγRIV promotes, while FcγRIIB protects from autoantibody-induced tissue damage in autoimmunity to type VII collagen

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Epidermolysis bullosa acquisita (EBA) is an autoimmune bullous disease mediated by autoantibodies against type VII collagen, the major component of anchoring fibrils of the dermal-epidermal junction. When passively transferred into mice, rabbit IgG against murine type VII collagen induces Fc-dependent complement activation, recruitment of leucocytes into skin, and subepidermal blistering. Since co-expression of activating (FcγRI, FcγRIII, and FcγRIV) and inhibitory (FcγRIIB) Fcγ receptors (FcγRs) is believed to represent an immunoregulatory checkpoint by establishing a threshold for immune cell activation, we determined the role of different FcγRs in autoantibody-induced tissue damage in experimental EBA. Mice lacking the common γ-chain of activating FcγRs were completely resistant to experimental EBA induction. Regarding the contribution of the 3 known activating FcγRs, by use of KO mice or function blocking antibodies we showed a significant role of FcγRIV. More specifically, functional loss of FcγRIV was associated with almost complete protection from EBA induction, whereas all control mice presented with severe skin lesions. In contrast, FcγRI or FcγRIII deficiency had no or little effect on disease expression, respectively. In addition, FcγRIIB deficiency was associated with significantly enhanced disease severity. We also observed that induction of EBA was associated with a shift in the balance of inhibitory and activating FcγRs toward the activating FcγRIV. Our observations suggest targeting of FcγRIV or FcγRIIB as potential therapeutic options in EBA.

035

Appearance in Japanese women correlates with age-related NADH oxidse levels and blood markers of lipid oxidation

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Serum and plasma from a cohort of 46 Japanese women aged 46 to 59 y, approximately half appearing younger (2 to 8.5 years, average 4.5 years) and half appearing older (5 to 11.3 years, average 6.2 years) than their chronological ages, were analyzed for levels of an age-related circulating NADH oxidase (arNOX) that generates superoxide in sera and saliva and for levels of prostaglandins (plasma) and thiobarbituric acid-reactive malondialdehyde-like compounds (serum) potentially indicative of oxidative stress damage. The total apparent age span was approximately 10 years. The ratio of arNOX levels in the older group compared to the younger group was 1.4 for saliva and 1.5 for sera compared to a predicted increase in arNOX of about 1.5. For sera, arNOX levels correlated with the two parameters of lipid oxidation measured in the study. Levels of PGF2a of plasma for the older-appearing subjects were approximately twice those of the younger appearing subjects and thiobarbituric acid-reactive malondialdehyde-like materials of sera correlated nearly exactly with arNOX activity with those of the older-appearing group being 1.55-fold that of the younger-appearing group. Thus, apparent age and indicators of oxidative damage to plasma lipids appear to correlate closely with superoxide dismutase-inhibited superoxide production in the sera, the bulk of which is derived from endogenous circulating arNOX activity.

036

Induction of autoreactive dermatitis by T cells with retrovirally modified T cell receptor specificity to desmoglein 3

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Pemphigus vulgaris (PV) is an autoimmune bullous disease with circulating IgG autoantibodies against desmogleins (Dsg). We recently established Dsg3-specific T cell clones that induced PV phenotype and isolated cDNA clones of their T cell receptors (TCR). Herein, we retrovirally transduced wild type $CD4^+$ T cells with these TCR cDNAs (rvDsg3 T cells) to clarify their pathogenic roles for antigen-specific autoimmune processes in skin. When rvDsg3 T cells were transferred with $Dsg3^{-/-}$ B cells into $Rag2^{-/-}$ mice, the recipient mice neither showed clinical PV phenotype, anti-Dsg3 IgG production, nor histological evidence for acantholysis, but instead developed scaly and erythematous skin lesions. Histology revealed interface dermatitis only in skin, mucous membranes and esophagus, where Dsg3 was expressed. The phenotypes were identical between two different TCR clones, but did not occur in mice that received T cells transduced with retrovirus expressing green fluorescence protein. Moreover, rvDsg3 T cells evoked this phenotype independently of $Dsg3^{-/-}$ B cells. While T cell-derived IL-4 was crucial for PV pathogenesis of parental T cell clones, T cells isolated from skin, draining lymph nodes, and spleen of the present model predominantly expressed IFN-γ. Therefore, T cells harboring identical TCRs evoked two distinct phenotypes: PV phenotype by Dsg3-specific T cell clones, and autoreactive dermatitis by rvDsg3 T cells, where cytokine profiles could be a critical determinant for the disparate phenotypes. Interface dermatitis, as seen in lichen planus or lupus erythematosus, suggests contribution of T cells, but exploration of antigen-specificity has never been challenged. Our results provide striking evidence that T cells specific for a skin-restricted antigen, Dsg3, could mediate interface dermatitis via IFN-γ. Our findings shed light on the understanding of interface dermatitis, and provide impetus for studying T cell mediated inflammatory/autoimmune diseases of unknown antigen specificity.

037

The genetics of actively induced murine epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering disease, characterized by antibodies to type VII collagen (COL7). EBA can be induced in mice by immunization with a fragment of the non-collagenous 1 domain of murine COL7. Contrary, to other autoimmune diseases, e.g. rheumatoid arthritis, little is known about the genetic susceptibility for EBA. We therefore used the EBA mouse model to address the hypothesis that disease induction depends on the MHC haplotype and additional genes outside the MHC. Mice from different inbred strains were immunized with recombinant murine COL7. Five distinct responses were observed: induction of (i) severe disease in SJL/J (H2s) and female MRL/MpJ (H2k), (ii) mild and transient disease in C57Bl/10.s (H2s), (iii) microscopic blistering in DBA/1J (H2q), (iv) only presence of non-pathogenic autoantibodies in C57Bl/6J, NZM2410/J, BXD2 and male MRL/MpJ, and (v) complete resistance to EBA in NOD/ShiLtJ (H2g7) and C57Bl/10.q (H2q) mice. Overall, susceptibility to EBA was strongly associated with H2s. In addition, the diseased phenotype was associated with autoantibodies to specific regions of COL7. We then used a 4-way intercross to map quantitative trait loci, QTL, linked to EBA. Our findings show that induction of pathogenic antibodies with a distinct specificity is linked to the MHC haplotype in experimental EBA. Furthermore, preliminary QTL mapping data from data show that numerous non-MHC genes are involved in EBA susceptibility.

038

Antimitochondrial autoantibodies in pemphigus

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A loss of epidermal cohesion in pemphigus vulgaris (PV) results from autoantibody action on keratinocytes (KCs) activating the signaling kinases and executioner caspases that damage KCs causing their shrinkage, detachment from neighboring cells and rounding up. Both extrinsic and intrinsic apoptotic pathways are activated by PVIgG. While Fas ligand neutralizing antibody could inhibit the former pathway, the mechanism of activation of the latter remained unknown. In this study, we investigated the mechanisms leading to activation of the intrinsic apoptotic pathway upon PVIgG binding to KCs. PV antibodies increased cytochrome c release, suggesting damage to mitochondria. The immunoblotting experiments revealed penetration of PVIgG into the subcellular mitochondrial fraction. The antimitochondrial antibodies (AMA) from different PV patients recognized distinct combinations of antigens with apparent MWs of 25, 30, 35, 57, 60 and 100 kDa. AMA were pathogenic because their absorption abolished the ability of PVIgG to cause keratinocyte detachment both *in vitro* and *in vivo*. The downstream signaling of AMA involved JNK and late p38 MAPK activation, whereas the signaling of anti-desmoglein 3 (Dsg3) antibody—JNK and biphasic p38 MAPK activation. Using KCs grown from Dsg3 -/- mice, we determined that Dsg3 did not serve as a surrogate antigen allowing AMA to enter KCs. The PVIgG-induced activation of EGFR and Src was affected neither in Dsg3 -/- KCs nor due to absorption of AMA. These results demonstrated that acantholysis in PV is a complex process initiated by at least three classes of autoantibodies directed against desmosomal, mitochondrial and other keratinocyte self-antigens. These autoantibodies synergize with the pro-apoptotic serum and tissue factors to trigger both extrinsic and intrinsic pathways of cell death and break the epidermal cohesion, leading to blisters. Further elucidation of the primary signaling events downstream of PV autoantigens will be crucial for the development of a more successful therapy for PV patients

039

A study of culture and toxin genes of staphylococcous aureus in atopic dermatitis

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The skin of atopic dermatitis (AD) has a high susceptibility to Staphylococcus aureus (S.aureus) and toxins produced by S. aureus may aggravate the atopic dermatitis by acting as superantigens. Our purpurse was to evaluate the relationship of skin barrier function, colonization of S.aureus and clinical severity in atopic dermatitis. We also examined the predominant toxin genes related in AD patients. Thirty-nine patients with AD were evaluated the clinical severity and skin barrier function by using SCORAD index and TEWL (transepidermal water loss). S. aureus was isolated from forearm, popliteal fossa and anterior nares of AD patients, and toxin genes were analyzed by using multiplex PCR. TEWL showed a statistically significant correlation with clinical severity in AD patients.($p<0.05$)TEWL was correlated with number of S.aureus colonization site and nasal colonization but these results were not stastically significant. Of the 39 patients, 48.7% was isolated the S.aureus strains and of the 32 sites isolated S.aureus, SEA and TSST-1 were detected 93.7% and 43.8% respectively. Combination of Staphylococcal toxin genes were SEA and TSST-1,40.6% checked. In conclusion, Skin barrier function, measured by TEWL shows a statistically significant correlation with clinical severity in patients with AD. The most common toxin genes was SEA in Korean atopic dermatitis patients and this may be a important role in pathogenesis of atopic dermatitis.

040

Lecinoxoids in the treatment of Psoriasis

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Interleukin-12(IL), Interleukin-23 and their shared sub-unit p40, have been shown to be over expressed in psoriasis plaques, and preclinical studies implicate these cytokine are involved in the pathogenesis of psoriasis. This has been further reinforced by the efficacy of interleukin-12/23 monoclonal antibodies in psoriasis patients. Recent studies have indicated that oxidized phospholipids can down regulate the production of pro-inflammatory cytokines by mature dendritic cells. We have developed the lecinoxoid family of synthetic small molecule oxidized phospholipid analogs.VB-201 is the lead compound of this novel family. VB-201was found to significantly inhibit the production of IL-12/23 common chain p40 by activated dendritic cells. Accordingly, we tested the efficacy of VB-201 in the treatment of a xenotransplant model of psoriasis, which have been showed to mirror many phenotypic and pathogenic aspects of this disease. Normal human skin was transplanted onto Beige- SCID mice. Four weeks following the engraftment, the mice were injected with IL-2 activated PBMC isolated from psoriatic patients. Two weeks after cell transfer, mice were divided and treated orally for 14 days with VB-201 or solvent. Topically applied Dexamethasone was served as positive control. The histological features of psoriasiform were evaluated and skin thickness was measured. 9/10 grafts of solvent treated showed a striking expression of psoriasiform. However, only 4/10 and 5/10 grafts expressed psoriasiform in mice treatment with 4 mg/kg and 0.04 mg/kg of VB-201 respectively. Dexamethasone treatment resulted in a complete recovery of 7/10 grafts and a significant decrease of epidermal thickness. This study implies that oxidized phospholipid analogs store anti inflammatory properties relevant for the treatment of psoriasis. A phase II study of VB-201 in stable plaque psoriasis has recently commenced.

041

Inhibition of Cox-2 suppresses keratinocyte antimicrobial peptide expression

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Cyclooxygenase (Cox) inhibitors are widely used anti-inflammatory agents, but little is known about their effects on innate immunity. Antimicrobial peptides (AMP) such as beta defensins (hBD) are critical to innate immunity and can be induced by activation of TLRs, a pathway that also activates Cox-2 expression. We hypothesized that Cox-2 is induced during TLR activation and is necessary for optimal AMP production. Normal human keratinocytes (NHEK) stimulated with the TLR2 ligand, Malp-2, or the TLR3 ligand, Poly I:C, showed (5 or 8 fold respectively, $p<0.05$) higher Cox-2 mRNA by quantitative PCR. Supporting this, a product of Cox-2, PGE2, also increased (~2 fold, $p<0.04$) measured by ELISA. A Cox-2 selective inhibitor (SC58125) attenuated hBD2, 3, or 4 production in both HaCaT cells and NHEK when stimulated by Malp-2 (-50%, $p<0.03$), Poly IC (-80%, $p<0.02$), or UVB (15mJ/cm2) (-80%, $p<0.004$), but did not inhibit cathelicidin. SC58125 did inhibit cathelicidin expression in mouse macrophages induced by Malp-2- (-50%, $p<0.02$) or Poly I:C- (-50%, $p<0.05$) . The functional significance of AMP inhibition through Cox-2 was seen when NHEK treated with SC58125 showed 2 log reduced anti-staphylococcal activity. These findings demonstrate a critical role for Cox-2 in hBD production and that the use of NSAIDs may adversely influence the risk for bacterial infection.

042

Topical herbal extract exhibits both preventive and therapeutic effects in murine acute irritant contact dermatitis

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Contact dermatitises, including allergic contact dermatitis and irritant contact dermatitis,are among the most common skin disorders in humans. Systemic Chinese herbal medicines (CHM) have been used in treating several types of dermatitis for centuries. Systemic administration of CHM also reportedly improves allergic contact dermatitis induced by 2,4-dinitro fluorobenzene. Since systemic CHM exhibit freguent side effects, we asked here whether topical applications of these herbal extracts can exert beneficial effects on cutaneous inflammation. Here, we investigated the potential benefits of a topical CHM extract containing radix raeonlae rubra, cat nut, phelloden dron, rhizoma alismatis, and rhizoma smilacis glabrae, on cutaneous inflammation in both allergic contact dermatitis and irritant contact dermatitis murine models, induced by topical oxazolone challenge and a phorbol ester, respectively. Then we assessed whether the benefits of topical CHM could be attributed to reduced cutaneous cytokine expression. Our results demonstrate that this topical CHM extract exhibits both preventive and therapeutic effects on cutaneous inflammation in acute irritant contact dermatitis, but it does not improve murine allergic contact dermatitis. Improvement of irritant contact dermatitis further correlates with decreased cutaneous TNF alpha and IL-1 alpha expression. These results suggest that topical CHM extract could provide an alternative regime for the prevention and treatment of irritant contact dermatitis.

043

Th17 cells from human skin require proliferation for IL-17 production

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Th17 cells defend against extracellular pathogens but can also contribute to inflammatory diseases. Psoriasis is thought to result from an overabundance of Th17 cells. We found increased IL-17 producing T cells in psoriatic skin lesions, as reported previously. Culture of psoriatic and normal skin in IL-2 and IL-15 dramatically increased the number of IL-17 producing cells from both tissues; surprisingly, there was then no significant difference in % Th17 cells between normal and psoriatic skin. In normal human skin, the % of IL-17 producing T cells increased from 9% to 33% after culture in IL-2 and IL-15. This increase was not a result of preferential expansion of Th17 cells, nor of repolarization towards the Th17 lineage. Th1, Th2 and Th17 cells expanded equally under these conditions and neutralization of cytokines that contribute to Th17 polarization/expansion did not decrease Th17 numbers. Instead, nonexpanded skin T cells contained a population of $CD4^+$ RORγt expressing T cells that did not produce IL-17 when stimulated. These quiescent Th17 cells were only present in non-expanded T cell populations. Culture of skin in IL-2/IL-15 induced both activation and expansion of T cells whereas TNFα treatment led to T cell activation only. TNFα did not increase the % IL-17 producing T cells. Instead, increases in IL-17 producing T cells paralleled cellular proliferation in skin treated with IL-2/IL-15 and only T cells that had recently proliferated produced IL-17. Even among non-expanded skin resident T cells, most T cells producing IL-17 had recently proliferated. In psoriatic skin lesions treated with IL-2/IL-15, the increase in IL-17 producing T cells suggests that polarized but quiescent Th17 cells exist even in these lesions. Our results suggest that proliferation is required for full IL-17 production by human skin resident Th17 cells. This requirement may serve as a failsafe mechanism, limiting potentially damaging inflammation that can result from aberrant activation of Th17 cells.

044

Immunologic actions of glycosaminoglycans in cutaneous lupus erythematosus & dermatomyositis

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Glycosaminoglycans (GAGs) are long, polyanionic, polysaccharide chains that play structural roles and exert significant effects on the immune system. GAGs have been shown to recruit and activate inflammatory cells, stimulate cytokine production, and in some cases have immuno-inhibitory properties. The GAG content of skin is increased in cutaneous lupus erythematosus (CLE) and dermatomyositis (DM). The focus of our research is to test the effects of specific GAGs that are altered in CLE and DM on immune pathways. We purified peripheral blood mononuclear cells (PBMCs) of healthy controls, CLE patients, and DM patients by Ficoll-paque density gradient centrifugation. GAGs, specifically high molecular weight hyaluronic acid (HMWHA), low molecular weight hyaluronic acid (LMWHA), and chondroitin-4-sulfate (C4S) were added to PBMC cultures and were incubated for 24 hours. TNF-α was measured by ELISA from the supernatant. Of interest, unstimulated control cells from DLE patients had a mean TNF-α released over 32 times larger than DM patients and healthy controls (54.82±11.05 pg/mL, 1.67±0.30 pg/mL, 1.48±0.51 pg/mL, $P<0.001$) respectively. C4S alone increased TNF-α production in DM PBMCs (1.67±0.3 to 3.46±0.94, $P=0.05$), but not significantly in DLE PBMCs (54.82±11.05 to 63.65±12.95, $P=0.1$) or controls. LMWHA alone increased TNF-α production in control PBMCs (1.48±0.51 to 3.15±0.78, $P=0.003$) and DM PBMCs (1.67±0.3 to 3.77±0.71, $P=0.006$), but not significantly in DLE PBMCs (54.82±11.05 to 63.63±13.44, $P=0.06$). The addition of C4S to LMWHA in cultures caused a decline in the amount of TNF-α released by normal PBMCs (3.15±0.78 to 1.29±0.34, $P=0.01$), by DM PBMCs (3.77±0.71 to 1.69±0.47, $P=0.024$), and DLE PBMCs (63.63±13.44 to 46.53±9.7, $P=0.023$). HMWHA did not induce TNF-α production from PBMCs. Our results indicate that C4S modulates the immune system; C4S alone is pro-inflammatory in DM, but C4S is anti-inflammatory in the presence of LMWHA in CLE, DM, and controls.

045

Up-regulation of CCL26 by IL-4 is through Jak-Stat pathway in atopic dermatitis

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Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by skin-infiltrating T cells, mast cells and eosinophils. Chemokine (C-C motif) ligand 26 (CCL26), a chemotactic cytokine for eosinophils, basophils and Th2 lymphocytes, was reported to be significantly elevated in patients with AD, but its detailed signal transduction pathway has not been delineated. We first examined whether CCL26 is up-regulated in the skin of the Keratin 14-IL-4-transgenic (Tg) mice, a mouse AD model we have generated by over-expressing IL-4 in the basal epidermis. Expression of CCL26, measured by real-time RT-PCR, was much higher in the Tg mice than in the wild type mice. We next examined the molecular mechanisms involved in IL-4 regulation of CCL26 in the skin using HaCaT, a human keratinocyte cell line. HaCaT cells, treated with IL-4 (20ng/ml) for 24 hours, have significantly higher CCL26 mRNA expression compared to those treated with vehicle. To delineate signal transduction pathway for IL-4 regulation of CCL26, HaCaT cells were treated with IL-4 for various lengths of time (0, 10, 20, 40 and 60 minutes), and post-treatment cellular proteins were subjected to Western Blot analysis using antibodies against total proteins and phospho-proteins: Stat3, Stat5a/b, Stat6, and the signaling molecules upstream of the Stats, Jak1 and Tyk2. IL-4 treatment induced phosphorylation of Stat3 and Stat6 but not that of Stat5a or Stat5b. Phosphorylation of Jak1, but not Tyk2, was induced by IL-4 treatment. To further confirm the role of Jak-Stat pathway in the regulation of CCL26, the HaCaT cells were simultaneously treated with a fixed amount of IL-4 plus increasing doses of Jak inhibitors and we found that Jak inhibitors suppressed the stimulation of CCL26 mRNA by IL-4 in a dose-dependent manner. Taken together, our data indicate that CCL26 expression is up-regulated in the skin of the IL-4-Tg AD mice and that the regulation of CCL26 by IL-4 involves Jak-Stat pathway.

046

***In vivo* conversion of CD4+FoxP3- to FoxP3+ T cells**

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The purpose of this study was to investigate the conversion of CD4+ FoxP3- T cells to FoxP3+ regulatory T cells *in vivo* and confirm suppressive function of these induced regulatory T cells. FoxP3+ naturally occurring regulatory T cells (nTreg) are potent suppressors of autoreactive CD4+ T cells that escape negative selection in the thymus. The forkhead transcription factor FoxP3 is indispensable for the differentiation, maintenance, and function of nTreg. TCR stimulation of conventional CD4+FoxP3- T cells *in vitro* in the presence of TGFβ and IL-2 results in the induction of FoxP3 expression. These TGFβ-induced FoxP3+ T cells (iTreg) are identical to nTreg in that they are anergic and suppressive *in vitro* and both, polyclonal and antigen-specific iTreg, have demonstrated anti-inflammatory potential in animal models of organ-specific autoimmune disease. To address the question if the conversion to CD4+FoxP3+ T cells also takes place *in vivo*, we transferred sorted CD4+GFP- cells from the Foxp3-GFP 'knock-in' mice into RAG1-/- mice and found that a substantial number of cells (1.15% ±0.18% FoxP3+ within the CD4+ T cells) converted to GFP+ cells within 4 weeks. In contrast no conversion was detectable after transfer of CD4+GFP- cells into WT recipients. The percentage of converted GFP+ T cells was higher in the peripheral lymph nodes than in the spleen or the skin, with the highest percentage in the mesenteric lymph node (2.1% ±0.32% FoxP3+ within the CD4+ T cells). The *in vivo* converted GFP+ T cells were suppressive *in vitro* when sorted directly *ex vivo* 4 weeks after original transfer into RAG1-/- recipients. In summary, these data suggest that certain conditions (e.g. lymphopenia) favor the conversion of FoxP3- cells to FoxP3+ Tregs and that these peripherally converted CD4+FoxP3+ T cells may contribute to the maintenance of tolerance *in vivo*.

047

Syndecan-4 is an important negative regulator of T cell immunity and a potentially useful target for treating diseases resulting from such immunity.

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Antigen presenting cells (APC) and T cells interact to produce immune responses that can protect or damage the host; such interaction is regulated by stimulatory and inhibitory molecules on both cells. We discovered binding of DC-HIL on APC to syndecan-4 (SD-4) on effector T cells to inhibit T cell activation. Now, using SD-4 knockout (KO) mice and $SD\text{-}4^{-/-}$ T cells, we compared responses of those cells vs wild-type (WT) T cells *in vitro*. Both cell types secreted IL-2 and proliferated at similar levels in response to anti-CD3 Ab or ConA; but, unlike controls, $SD\text{-}4^{-/-}$ T cells failed to bind DC-HIL, and had exaggerated IL-2 and proliferative responses to Ag presented by APC. We then compared contact hypersensitivity (CH) responses of KO vs WT mice using oxazolone (Ox) as hapten. $SD\text{-}4^{-/-}$ mice showed markedly augmented ear swelling (2-fold enhancement) after 2nd and 3rd challenges. Infusion of $SD\text{-}4^{-/-}$ T cells from Ox-sensitized KO mice into naïve WT mice (adoptive transfer) led to exaggerated CH in the latter. In an acute graft-vs-host disease (GVHD) model, we compared effects of $SD\text{-}4^{-/-}$ vs $SD\text{-}4^{+/+}$ T cells from B6 mice infused into BALB/c mice subjected to γ-irradiation and bone marrow transplantation. $SD\text{-}4^{-/-}$ T cells exacerbated GVHD with more severe diarrhea, higher mortality, and greater proliferative capacity of infused cells in liver and spleen. Finally, we compared induction of experimental autoimmune encephalitis (EAE) in KO vs WT mice by immunizing with MOG peptide/adjuvant. The former developed worse morbidity scores (3.6 ± 0.4 vs 1.9 ± 0.7), more infiltrating cells in spinal cord, and their lymph nodes harbored more $IFN\text{-}\gamma^+/IL\text{-}17^+$ T cells. We conclude that SD-4 is an important negative regulator of T cell immunity, and a potentially useful target for treating diseases resulting from such immunity.

048

IgG4 depletion as a therapeutic strategy in pemphigus

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Previous studies have suggested an IgG4>IgG1 predominance of pathogenic autoantibodies in pemphigus, a potentially fatal blistering disease of the skin and mucous membranes. Because IgG4 is induced by chronic antigen stimulation, we hypothesized that IgG4 is a marker of the pathogenic autoantibody population in pemphigus. IgG4 is an attractive target for therapy as it is the least prevalent subclass of serum IgG, and selective IgG4 deficiency does not appear to result in significant immune deficiency. In order to determine the feasibility of IgG4 depletion therapy, we sought to investigate 1) whether total IgG4 is elevated in pemphigus vulgaris (PV) and pemphigus foliaceus (PF) patients, 2) what percent of total IgG1 and IgG4 is desmoglein (Dsg) antigen-specific, and 3) whether IgG4 depletion abrogates the pathogenic activity of pemphigus serum. We compared the total immunoglobulin subclass levels in 42 PV and 28 PF serum samples, compared to sera from 24 and 12 non-affected individuals, respectively. Of IgG1, IgG2, IgG3, and IgG4, only IgG4 levels were significantly different in non-affected versus pemphigus sera ($p=0.028$ for PV and $p=0.006$ for PF). We next grafted IgG1 and IgG4 constant regions onto monoclonal Dsg-specific variable regions cloned from a pemphigus patient. Using these recombinant antibodies as a quantitative standard for a novel antigen-specific isotype ELISA, we determined the total amount of Dsg3-specific IgG1 and IgG4 in PV patients and Dsg1-specific IgG1 and IgG4 in PF patients. The median percent of total antibody that was Dsg-specific was 7.7% for PV IgG4 and 0.9% for PV IgG1 ($p<0.001$), and 3.3% for PF IgG4 and 1.5% for PF IgG1 ($p=0.025$). These studies indicate that Dsg-specific antibodies comprise a significant percentage of the total IgG4 population in pemphigus patients. Current studies are investigating the pathogenicity of IgG4-depleted serum. By targeting autoimmune rather than immune antibody populations, isotype-targeted therapies may offer potentially safer treatment options for pemphigus.

049

An IgE monoclonal antibody to BP180-NC16A replicates the effects of bullous pemphigoid IgE *in vitro*.

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IgE class autoantibodies are pathogenic in bullous pemphigoid. In order to further elucidate their pathomechanism a murine hybridoma, 395F, producing an IgE monoclonal antibody (mAb) specific for the NC16A domain of BP180 was generated. To confirm the isotype of this mAb, poly-A+ RNAs isolated from 395Fand a control IgG hybridoma (HD-18) were subjected to RT-PCR using appropriate combinations of a VH1(forward) primer with one of the following reverse primers: CHE1(ε-specific); CHG1(γ1-specific); CHG2,3(γ2 and γ3). When the ε-specific CHE1 primer was used to amplify the clone 395F RNA, a cDNA of the appropriate size was obtained and subcloned into the pScript vector (Invitrogen). Sequence analysis confirmed the cDNA was derived from an ε chain mRNA. Amplification of the 395F RNA with γ-specific primers yielded no product. 395F mAb was further characterized by immunoblotting against a GST-NC16A fusion protein or control GST. NC16A reactivity was observed using a secondary antibody specific for murine IgE, but not with anti-IgG. Epitope mapping revealed that 395F mAb targets the previously described region 2 of NC16A. Indirect immunofluorescence demonstrated that affinity-purified 395F mAb reacted with the basement membrane zone and mast cells of human skin. When 395F was used to coat RBL-SX cells (gift of JP Kinet), NC16A triggered histamine release. After exposure to the 395F mAb (60 ng/ml), normal human keratinocytes (NHK) responded by synthesizing and secreting IL-6 and IL-8, similar to that observed with human BP IgE autoantibodies. An isotype control mAb had no such effect on NHK. These studies clearly demonstrate that a murine IgE antibody targeting a single epitope within BP180-NC16A can trigger pro-inflammatory responses in both keratinocytes and mast cells, thus replicating the effects previously seen with human BP IgE autoantibodies.

050

Norepinephrine (NE) and adenosine-5′-triphosphate (ATP) synergize in inducing IL-6 production by human dermal microvascular endothelial cells (HDMECs)

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Endothelial cells (ECs) play an important role in cutaneous inflammation, in part, by release of inflammatory chemokines/cytokines. Since dermal blood vessels are innervated by sympathetic nerves, the sympathetic transmitter NE and the co-transmitter ATP may regulate expression of EC inflammatory factors. We focused on regulation of IL-6 expression as IL-6 has many inflammatory and immune functions including participation in Th17 cell differentiation. We examined this question with the transformed HDMEC line HMEC-1 as well as primary HDMECs. HMEC-1 cells were cultured in various concentrations of NE (10^{-9}M–10^{-5}M) in the presence or absence of 1-100 μM ATP. Supernatants were harvested at 8, 16 and 24 hrs and IL-6 content was measured by ELISA. NE and ATP synergistically induced release of IL-6 over a wide range of concentrations and at all times examined. By real-time PCR, levels of IL-6 mRNA were also synergistically induced in cells treated with 10^{-6}M NE and 100 μM ATP at 1 hr and 2 hrs. Cells were treated with various concentrations of NE and ATP in the presence or absence of α and β adrenergic receptor antagonists. Propranolol (non-specific β) and ICI 118,151 (β_2-specific) significantly blocked the synergistic response while phentolamine (non-specific α) had no substantial effect. Thus, the NE effect is primarily mediated by β_2-adrenergic receptors. This synergistic effect of NE and ATP could be reproduced in primary HDMECs and was also mediated by β_2-adrenergic receptors. Under conditions of stress, activation of the symphathetic nervous system may lead to release of ATP and NE by sympathetic nerves surrounding dermal blood vessels with induction of IL-6 production by ECs. IL-6 may then participate in immune and inflammatory processes including generation of Th17 cells. As Th17 cells appear to play an important role in psoriasis, production of IL-6 in this manner might help to explain stress-induced exacerbation of psoriasis and, perhaps, other skin disorders involving Th17-type immunity.

051

Laser-capture microdissection of psoriasis vulgaris suggested potential for self-organizing lymphoid tissue in lesisonal dermis

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We applied this technique to psoriasis vulgaris lesional and non-lesional tissue to determine specific cell types/regions that produce specific molecules in the "psoriasis transcriptome." We compared the sensitivity of gene expression detection in captured tissues to whole tissue extracts and found more sensitive (increased) detection of disease-related genes in focal tissue regions. In addition, we validated measures of gene expression in double-amplified samples by comparing gene array with RT-PCR detection (r= 0.81 for tested genes). Psoriasis vulgaris lesions contain unique accumulations of mature dendritic cells intermixed with T-cells in dermal foci that are incompletely characterized. We thus compared molecules synthesized by immune cells within dermal aggregates to molecules made in psoriatic lesional epidermis or non-lesional dermis. We found increased expression of mRNAs encoding CCL19 and CCR7 with dermal lymphoid aggregates of psoriasis lesions and confirmed increased expression of cognate proteins using IHC and immunofluorescence analysis. Mature DCs expressed both CCL19 and CCR7, whereas T-cells mainly expressed CCR7 in these regions. In model systems, engineered over-expression of CCL19 is sufficient to induce/organize ectopic lymphoid tissue and we propose that increased expression of CCL19 by mature DCs in psoriasis may lead to chemoattraction and organization of CCR7+ lymphoid cells to create self-sustaining lymphoid tissues in psoriasis lesions. Active cytokine signaling within these aggregates is suggested by increased expression of STAT1 and chemokines CXCL9 and CXCL11 (STAT1 regulated), as well as other active signaling molecules. Thus, these data extend our understanding of how immune tissues in psoriasis lesion may be essential to disease persistence. We demonstrate and validate use of critical new methods that can be used for "fine" dissection of pathogenic elements in this disease and other skin diseases.

052

Efficacy and safety of Canakinumab (ILARIS®) in Cryopyrin-Associated Periodic Syndrome (CAPS): Interim results of an onging phase III study

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CAPS is associated with IL-1β overproduction. Canakinumab, a fully human monoclonal antibody, achieves selective and prolonged blockade of IL-1β. Method: Patients were canakinumab-naïve or rolled-over from Phase II/III studies. Patients received canakinumab s.c. 150 mg or 2 mg/kg (≤40 kg) every 8 weeks. Primary objective was to assess long-term safety and tolerability of canakinumab in CAPS patients. Secondary objectives included assessment of complete response (CR) (for naïve patients), maintenance of response, and dose adjustment. Relapse was defined as serum CRP and/or SAA levels >30 mg/L and physician's global assessment of disease activity (PGDA) >minimal or PGDA =minimal plus skin assessment >minimal. Results: Of 98 (19 pediatric) enrolled patients (19 FCAS; 69 MWS; 9 MWS/ NOMID; 1 cold urticaria/protocol deviation) aged 5-69 years, 44 were canakinumab-naïve and 54 received canakinumab previously. Median duration of exposure was 113 days. Mean number of injections per patient was 2.9. A CR by Day 8 was observed in 41/44 (93.2%) canakinumab-naïve patients. 77 had no relapse (90.6%), 5 experienced a relapse (5.9%), 3 naïve patients did not achieve a CR (1 achieved CR after dose adjustment) and 13 patients had missing relapse assessment data at the analysis cut-off. At least one dose adjustment was required in 16 patients (16.3%). Predominant AEs were infections (31.6%), mostly mild-to-moderate in severity. SAEs reported in 5 patients resolved on-treatment. 94.9% had absent injection site reaction. None developed anti-canakinumab antibodies. Conclusion: 8-weekly administration of canakinumab provided rapid improvement of symptoms and sustained remission in nearly all CAPS patients across all disease severity phenotypes.

053

Biomimetic electricity generated by an elemental bi-mineral complex produces anti-inflammatory activity

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The human body has its own innate electrical system that regulates the body's functions via communications among organs through the well known neural system, and some less understood cellular activities such as the bioelectricity associated with tissue regeneration. The bioelectricity during wound healing has been attributed the endogenous 'skin battery' that pumps sodium ions using the Na/K-ATPase located on epidermal cells and can generate a potential outward electric current of about 10-100 μA/cm and an electric potential gradient about 60 mV/mm. While the effect of low level electrical stimulation on wound repair has been reported, few studies have examined the effect biomimetic electricity on non-wounded skin. Physiological levels of biomimetic electricity may be derived from the unique combination of elemental zinc and copper. In the current study, we developed an elemental zinc-copper bi-mineral complex to determine the effect of low level electrical stimulation on skin physiology. Treatment with the bi-mineral complex significantly reduced release of pro-inflammatory cytokines from activated human T-cells and inhibited release of cytokines from keratinocytes and macrophages exposed to bacteria. Furthermore topical application of a lotion containing the bi-mineral complex reduced the UV-induced damage to human skin equivalents. Taken together these results demonstrate that biomimetic electricity reduces inflammatory responses and therefore may protect skin from the numerous external aggressions encountered daily by skin.

054

Passive transfer of dermatitis herpetiformis serum high in IgA anti-TG3 produces granular IgA deposition in the papillary dermis of human skin engrafted on SCID mice

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Dermatitis herpetiformis (DH) is characterized by deposition of IgA in the papillary dermis. However, indirect immunofluorescence is routinely negative, raising the question of the mechanism of formation of these immune deposits. We have previously demonstrated that goat IgG anti-human epidermal transglutaminase (TG3) passively transferred to a SCID mouse bearing a xenograft of human skin produces immune deposits in the characteristic pattern of DH. We now report that passive transfer of serum from DH patients to such mice produces deposits of IgA and TG3 in the papillary dermis in a pattern identical to that seen in DH. Sera from 7 DH patients and 7 controls were tested for IgA anti-TG3 by ELISA. 150 microliter aliquots of each serum were injected subcutaneously into 2 areas of each graft 8 times over 3 weeks (2.4 ml total/mouse). Biopsies were removed at baseline, and at weeks 4,5 and 6 and processed for routine histology as well as direct immunofluorescence for IgA and TG3. Three of 7 sera had elevated levels of IgA anti-TG3. Only those three sera produced deposits of granular IgA and TG3, which co-localized in the papillary dermis in a pattern identical to that in seen in skin of DH patients. None of the DH patients with low levels and none of the controls developed any IgA or TG3 deposition. The deposits of human IgA could not be removed with sodium citrate solution, similar to the deposits in patient skin. We hypothesize that the IgA class anti-TG3 antibodies are directly responsible for the immune deposits and that the TG3 is from human epidermis, as this is its only source in our model. These deposits seem to form over weeks in a process similar to an Ouchterlony immunodiffusion precipitate. We further hypothesize that the TG3 covalently modifies the IgA, explaining its long-term persistence in patient skin. This process of deposition explains the negative indirect immunofluorescence results in DH.

055

A xenograft model of human skin to study the role of dendritic cells in inflammatory skin disease

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Cutaneous dendritic cells (DCs) are potent antigen-presenting cells (APCs) and play a pivotal role in T-cell mediated inflammatory skin disease and allogeneic skin reactions like graft-versus-host disease (GVHD). Manipulation or depletion of antigen-presenting cells may be vital to prevent skin inflammation. We generated a model system using human skin xenografts transplanted to NOD/LtSz-scid IL2Rg-null (NSG) mice. We analyzed the distribution of LCs as well as dermal HLA-DR positive cells in the engrafted human skin. LCs vanished after the second week and remained undetectable until 6-8 weeks after transplantation. In contrast, HLA-DR positive dermal APCs were present at any time after transplantation. In further experiments, in some mice LCs were present in weeks 4, 7 and 9 after transplantation, while in others they were not present 4 weeks after transplantation, but clearly detectable two months after transplantation. To induce T-cell mediated inflammation, we used DCs generated from peripheral blood of the skin-donors as stimulators in mixed lymphocyte DC cultures (MLDC) with CD8 T cells of a HLA-class I mismatched healthy donor. Allo-reactive T cells were injected into mice transplanted with healthy skin in escalating doses. An erythematous reaction in the xenograft was detected approximately 1 week after the first dose of MLDC T cells. We detected histological signs of acute skin GVHD as well as infiltrating T cells and a loss of LCs. This observation is consistent with studies showing that infiltrating allo-reactive donor T cells deplete persisting host APCs. In summary, we introduce a model-system for inflammatory skin disease using human xenotransplants on NSG mice. Our data support the hypothesis of a local LC-repopulating precursor population in human skin. In addition, we establish a model of allo-reactivity in xenografts to analyze the role of DCs in T-cell mediated inflammatory skin disease.

056

AIM2 is overexpressed in psoriasis and an AIM2 inflammasome is active in human epidermal keratinocytes

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Although not professional immune cells, epidermal keratinocytes are at the first line of defense against invading pathogens and can initiate the activation of immune responses. In order to do so, they are equipped with danger sensors such as TLRs and inflammasome components. Inflammasomes are cytoplasmatic multi-protein complexes that upon activation lead to the processing of the proinflammatory cytokine IL-1β. Only recently a novel inflammasome was characterized: The cytosolic HIN200 family member "absent in melanoma 2" (AIM2) was shown to be responsible for intracellular recognition of double stranded DNA (dsDNA) and subsequent inflammasome activation. In this study we analyzed the activation of the AIM2 inflammasome in resident human epidermal keratinocytes and its potential role in skin inflammation. We found increased AIM2 expression in lesional psoriatic skin compared to healthy or non-lesional skin. Additionally, an inflammasome was active in lesional psoriatic skin as demonstrated by caspase-1 activity and IL-1β production. Upon dsDNA stimulation keratinocytes secreted IL-1β indicating inflammasome activity. IL-1β release was abrogated when AIM2 was blocked or dsDNA was pretreated with DNAse. *In vivo*, cutaneous expression of IFN-γ correlated with the expression of AIM2 in psoriatic specimens and *in vitro* IFN-γ increased AIM2 in primary epidermal keratinocytes. This data suggests that an AIM2 inflammasome is active in human keratinocytes after dsDNA treatment resulting in the secretion of IL-1β. As its expression is high in lesional psoriatic AIM2 might be a critical contributor to the skin inflammation in this chronic disease.

057

The TLR4 ligands Mrp8/14 play a critical role in the development of autoreactive CD8+ T cells

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Autoimmunity results from a conflict of regulatory mechanisms controlling self-tolerance but the molecular pathomechanisms underlying the loss of immunotolerance against self remain largely unknown. The regulation of the adaptive immune system is initiated by antigen-presenting cells (APC) that can activate naive T cells. In particular the interaction of the receptor CD40 on APC with its ligand CD40L plays an important role during immune responses. Within the skin, transgenic (tg) overexpression of CD40L in keratinocytes leads to systemic autoimmunity as evidenced by autoantibodies, nephritis, and autoimmune dermatitis, which can be adoptively transferred by injecting CD8+ T cells into naive recipient mice. However, the mechanisms linking the local micromilieu and the systemic development of autoreactive CD8+ T cells in CD40L-induced autoimmunity are poorly understood. Myeloid-related proteins (Mrp) such as Mrp8 and Mrp14 are damage associated molecular pattern molecules (DAMPs) highly up-regulated in various autoimmune disorders. Hence, we investigated the relevance of Mrp8 and Mrp14 for the development of functional autoreactive T cells and could show that local Mrp8/14 production is essential for the induction of autoreactivity in CD8+ T cells and the development of systemic autoimmunity. This effect is mediated via TLR4 signaling and up-regulation of the transcription factors Runx-1 and RORc in CD8+ T cells leading to increased IL-17 expression. Notably, Mrp8 and Mrp14 expression was increased in cutaneous lesional lupus erythematosus (LE) and enhanced concentrations of Mrp8/14 were detectable in patient's serum. Strikingly, stimulation of CD8+ T cells from LE patients with Mrp8 and Mrp14 proteins resulted in a significant up-regulation of IL-17 expression indicating that Mrp8/14 play also an important role during human MHC class I-mediated cellular autoimmunity. Together, these data present the first link between local expression of a DAMP-molecule and the development of systemic autoimmunity.

058

Gene expression, cytokine profiling and pharmacological evaluation of IL-23 induced skin inflammation in mice-an animal model of psoriasis.

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Psoriasis is a common chronic inflammatory skin disease suggested to be driven primarily by Th1 and/or Th17 cells. It is characterized by immune cell infiltration, epidermal hyperplasia, up-regulation of a variety of inflammatory mediators and increased dermal angiogenesis. Psoriasis plaques have an elevated level of interleukin 23 (IL-23) and recent studies show that intradermal injection with IL-23 cause psoriasis-like symptoms in the skin of mice. The differentiation of Th17 cells has been shown to be critically dependent of TGF-β and IL-6 (and /or IL-21), whereas IL-23 stabilizes differentiation and induces complete Th17 maturation. The aim of this study was to characterize IL-23 induced skin inflammation as an *in vivo* model for evaluation of anti-psoriatic drugs with investigative focus on: (i) inflammation and infiltration of immune cells, (ii) epidermal hyperplasia (iii) gene expression of biomarkers relevant for psoriatic disease, (iv) cytokine expression pattern, (v) requirements of T cells and interleukin 6 respectively for disease progression and (vi) the effect of well established anti-psoriatic drugs on disease outcome. Our results confirm that intra-dermal IL-23 injections in mice cause a sustainable inflammation with psoriasis characteristics including epidermal hyperplasia and hyperparakeratosis. Disease initiation is critically dependent on T cells, since nude mice were protected from disease whereas IL-6 was proven to be important but not essential for disease progression. Importantly, gene expression data correlated to human disease and strongly suggest that the pathogenesis is driven by Th17 cells. Finally, systemic administration of cyclosporin A or Dexametazone significantly reduced the IL-23 induced skin inflammation

059

Characterization of photosensitivity and poor quality of life in lupus

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The purpose of this study was to validate measures of self-reported photosensitivity (PS) in an ongoing cutaneous lupus database study and to evaluate the impact of PS on quality of life (QoL) in a U.S. lupus population. Patients enrolled in the study completed the Skindex 29+3 which included two lupus specific, PS-related items (I worry about going outside because the sun might flare my disease; My skin disease prevents me from doing outdoor activities). These items, scored on a Likert 5 point scale, were averaged to generate a photosensitivity subscale. Patients were asked about a history of PS and about current PS symptoms. Patients that reported a history of and current symptoms of PS were categorized into the PS group. The NOT PS group was comprised of patients who answered 'no' to both PS questions. Of the 131 patients in the study, 48% had discoid lupus (DLE), 10% had tumid lupus (LET), 27% had subacute cutaneous lupus (SCLE), 10% had acute cutaneous lupus (ACLE), and 40% met criteria for systemic lupus (SLE). Overall, 70% of patients reported both a history of and current PS. The distribution of diagnoses among the PS group did not differ significantly from the total sample (DLE, 38%; LET, 12%; SCLE, 34%; ACLE, 13%; SLE, 44%). Overall, 56% of DLE, 78% of LET, 88% of SCLE, 85% of ACLE, and 45% of SLE patients reported PS. PS patients had significantly more lesions in sun exposed areas (p=0.005) and more frequently used sunscreens and/or protective clothing (p=0.002). PS patients scored significantly worse on the Skindex 29+3 photosensitivity subscale (p=0.0001) and on the three established subscales (emotion, p=0.0007; symptoms, p=0.005; function, p=0.0001). In conclusion, our two simple PS questions were able to distinguish between lupus patients with self-reported PS. Patients with ACLE, SCLE, and LET report PS most often. PS patients score worse on all skin-related QoL scales including emotions, symptoms, functioning, and photosensitivity.

060

Novel secosteroids inhibit collagen and hyaluronan synthesis by human fibroblasts

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1α, 25-dihydroxyvitamin D3 [1,25$(OH)_2D_3$]regulates calcium homeostasis and bone remodeling. It is also known to inhibit collagen synthesis by some types of fibroblasts, has been shown to inhibit interstitial fibrosis in a renal model and may improve bone marrow fibrosis in patients with myelofibrosis. Use of 1,25$(OH)_2D_3$ as a therapeutic antifibrotic agent is limited by its inherent ability to induce hypercalcemia. Recently our group identified a novel secosteroidogenic pathway that generates novel steroidal 5, 7-dienes and their secosteroidal derivates that are biologically active and have reduced or no hypercalcemic property. We found that 1,25$(OH)_2D_3$ and novel compounds including 20-hydroxyvitamin D3; (5Z,7E-3,20-9,10-Secocholesta-5,7,10(19)-triene-3,20-diol) (20(OH)D3; 7-dehydropregnenolone (3β-hydroxypregna-5,7-dien-20-one) (7DHP); 20-oxypregnacalciferol (5Z,7E-3β-hydroxy-9,10-secopregna-5,7,10(19)-trien-20-one) (pD); 17α,20S-dihydroxy-7DHP (3β,17α,20S-trihydroxypregna-5,7-diene) (17,20S(OH)27DHP; 17α,20S-dihydroxy-pD (5Z,7E-3β,17α,20S-trihydroxy-9,10-secopregna-5,7,10(19)-triene) (17,20S(OH)2pD), 17α,20S-dihydroxy-lumisterol-like (3β,17α,20S-trihydroxy-9β,10α-pregna-5,7-diene) (17,20S(OH)2pL); 17α,20R-dihydroxy-7DHP (17,20S(OH)27DHP); 17α,20R-dihydroxy-pD (17,20R(OH)2pD); 17α,20R-dihydroxy-lumisterol-like (17,20R(OH)2pL) at 10^{-10} and 10^{-9} M significantly inhibited (from ≥ 90%) total collagen and hyaluronan synthesis by cultured human dermal fibroblasts stimulated with TGF-β1 (5 ng/ml). These steroidal 5,7-dienes and corresponding secosteroids had no cytotoxic effect on the cultured fibroblasts. These data suggest that novel secosteroids identified by us with no hypercalcemic property retain the antifibrotic properties of 1,25$(OH)_2D_3$ and provide a basis for further testing of these compounds in preclinical models of fibrosis.

061

Cutaneous regulatory T cells can produce IL-17: a possible source of autoreactive Th17 cells in human skin

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Foxp3$^+$ regulatory T cells (Treg) are essential for inducing and maintaining tolerance to self antigens. In contrast, IL-17 producing Th17 cells have been implicated in a number of autoimmune diseases including psoriasis, multiple sclerosis and Crohn's disease. Though initially thought to be independent populations, a recently characterized subset of human blood Treg make IL-17. Additionally, a subset of blood Treg stimulated with allogeneic APC and IL-2 & IL-15 can be induced to make IL-17. Low numbers of Treg and Th17 T cells are found in blood but larger numbers of both subsets reside in human skin. We examined the population of skin resident Treg for their ability to make IL-17. A discrete population of Foxp3$^+$ cutaneous T cells produced IL-17 at baseline (2.4% ± 1.4). Similar to findings in blood, expansion of skin T cells with IL-2 and IL-15 resulted in a dramatic increase in Foxp3$^+$ IL-17 producing T cells (8.4%±4.1). Addition of IL-1β, IL-6, IL-21, or IL-22 did not further increase the number of these cells. IL-17 producing Foxp3$^+$ T cells were CD4 CD45RO$^+$ memory T cells that expressed HLA-DR but largely lacked expression of GITR, CTLA-4, CD161 and CD127. Skin addressins (CLA, CCR4) were expressed, but cells lacked expression of central memory markers L-selectin and CCR7, suggesting a skin resident phenotype. Virtually all Foxp3$^+$ IL-17 producing cells also produced TNFα, 25.9±15.5% produced IFNγ, but most lacked production of IL-22, IL-21, TGFβ, and IL-10. In summary, a significant population of skin resident Foxp3$^+$ Treg can be induced to make IL-17 when treated with IL-2 and IL-15, two cytokines prevalent in chronically inflamed skin. This suggests that under certain inflammatory conditions, Treg can be converted into potentially autoreactive Th17 cells, leading to a loss of peripheral tolerance. A better understanding of the signals that induce IL-17 production in Treg could lead to insights on how autoreactive pathogenic Th17 cells arise in cutaneous autoimmune diseases, and how this conversion could be inhibited.

062

Systems level high-throughput and multiparametric analyses to elucidate cell death associated molecules involved in Pemphigus Vulgaris

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The pathogenic mechanisms involved in Pemphigus Vulgaris (PV) relate primarily to a loss of cell adhesion which proceeds, albeit in part, through activation of apoptotic pathways. We have developed an original paradigm of external perturbation where keratinocytes are treated *in vitro* with IgG and sera in order to mimick PV and then applied a high throughput, systems biology analysis to this model. Our aim was to combine a top-down cell-wide network analysis with bottom-up experimental verification. The results from the in silico analysis and mathematical modelling, together with a genome-wide investigation of the changes following autoimmune perturbation, were merged to select molecules that were causally involved in PV. DNA microarray screening demonstrated that apoptotic pathways were enriched in PV, findings that were investigated further using protein microarrays. Both extrinsic and intrinsic cascades were activated, together with anti-apoptotic molecules. The altered genes and proteins (nodes) associated with the apoptotic biomodule were used to build a functional map of a PV-associated apoptosome which was characterized according to biophysical criteria. The apoptosome formed a non-random network with considerable connectivity and internal communication, suggesting a relevant biological role in PV. The overall cell behaviour in response to PV sera was then examined by combining high throughput siRNA screening (n=92) with multiparametric image analysis. The results demonstrated that some of the apoptotic molecules involved in acantholysis, including Caspase 3 and HSP70, were also part of the cell adhesion interactome (developed by us previously). New molecules such as Prohibitin were also identified as being crucial for PV. Our data further elucidate the mechanisms of acantholysis and raise the possibility that specific therapeutic modalities other than high dose steroids and immunosuppressants might be developed for the treatment of this most debilitating of disorders.

063

Human IgG1 mAb against human COL17 NC16A developed from BP patients induces blisters in experimental bullous pemphigoid model

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Bullous pemphigoid (BP) is an autoimmune blistering disease caused by IgG autoantibodies targeting the NC16A domain of human collagen 17 (hCOL17), which trigger blister formation via complement activation. IgG1 autoantibodies are the predominant IgG subclass in BP; however, their pathogenic role has not been fully demonstrated. We constructed a recombinant IgG1 monoclonal antibody (mAb) against hCOL17 NC16A from the BP patients. In COL17-humanized mice, which express human COL17 at dermal-epidermal junction in place of mouse COL17, this mAb effectively reproduced a BP phenotype that included subepidermal blisters, deposition of IgG1, C1q and C3, neutrophil infiltration, and mast cell degranulation. Subsequently, alanine substitutions at various C1q binding sites were separately introduced to the Fc region of the IgG1 mAb. Among these mutated mAbs, the one that was mutated at the P331 residue completely failed to activate complement *in vitro* and drastically lost pathogenic activity in COL17-humanized mice. These findings indicate that P331 is a key residue required for complement activation, and that IgG1-dependent complement activation is essential for blister formation in BP. This study is the first direct evidence that IgG1 antibodies to hCOL17 NC16A can induce blister formation *in vivo*, and it raises the possibility that IgG1 mAbs with Fc modification may be used to block pathogenic epitopes in autoimmune diseases.

064

Systemic epinephrine alters neutrophil trafficking and prolongs their persistence in wounds: Implications for stress-mediated impairment of wound healing

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Stress-induced hormones are known to alter the inflammatory response to tissue injury. Neutrophil (PMN) trafficking to sites of injury is an essential inflammatory response for innate immune protection, but extended persistence can result in chronic inflammation and impaired tissue repair. Here we aimed to examine mechanistic interactions between the stress hormone epinephrine, kinetics of neutrophil trafficking and the efficiency of wound repair. These phenomena were non-invasively observed *in vivo* by real time tissue fluorescence imaging technique to detect the kinetics of genetically tagged EGFP-neutrophil (EGFP-PMN) infiltration into an excisional skin wound of mouse, with implanted osmotic pumps for sustained delivery of either epinephrine (5 mg/kg/day) or saline (control) immediately after skin wounding. The PMN infiltration at early phase (~24 hr) was not significantly altered between saline and epinephrine-treated mice. However, although EGFP-PMNs were cleared from the wound at day 2 in control saline-treated mice, they persisted for up to 8 days in the epinephrine-treated mice, and this correlated with a delay in wound closure. The increase in PMN persistence was not associated with an increase in bacteria at the wound site. Multiplex cytokine assay revealed substantial increase in tissue level of IL-6 (by 75%) at late phase of day 5. Inhibition of PMN infiltration into the wound following the 24 hr time point by infusion of anti-neutrophil mAb Gr-1, significantly improved the rate of wound closure for epinephrine-treated mice (by 57%, $p<0.05$ vs epinephrine only), but with negligible effect on saline-treated mice. Our results suggest that prolonged exposure of epinephrine alters kinetics of neutrophil trafficking to sites of wound via an IL-6 mediated mechanism and this in turn could delay wound repair.

065

Targeted depletion of IL-23 by IL-4 *in vivo* impairs T cell mediated DTHR

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Over decades, the scientific debate on the pathogenesis of the T-cell mediated delayed-type hypersensitivity reactions (DTHR) was focused on Th1/Tc1 and Th2/Tc2 cells. IFN-γ producing Th1 cells and Tc1 were considered to be the primary effector cells and IL-4 producing Th2 cells to be the counter regulator of DTHR responses. Indeed, systemic IL-4 treatment was shown to reverse established DTHR. More recently, experimental evidence on the role of Th17/Tc17 in DTHR increased, but the role of the Th17 sustaining IL-23, especially in the context of successful IL-4 treatment, remained elusive. We used a model of TNCB-induced DTHR in C57Bl6 mice. TNCB challenge in sensitized mice induced pronounced ear swelling and skin inflammation. Interestingly, the inflammatory infiltrate was dominated by both Th17 cell-associated IL-23 and IL-17 cytokine expression. Systemic IL-4 treatment of these mice resulted in reduction of ear swelling and normalized skin morphology. Moreover, we found that IL-4 therapy selectively suppressed cutaneous IL23A but not IL12A mRNA expression. In agreement with the critical role of IL-23 in sustaining Th17 responses, efficient IL-4 treatment not only suppressed lesional IL23A but also IL17A mRNA in mouse ears. To further analyze this unique and selective suppression of IL-23 by IL-4, mouse bone marrow-derived dendritic cells (mBMDC) were allowed to mature in an IL-4 dominated milieu. In these mBMDC IL-4 suppressed IL-23 to 40% or less of its original levels while promoting 200% up regulation of IL-12. Unraveling the molecular mechanism, we found that IL-4 exerts its regulatory effect over both IL-23 and IL-12 in mBMDC in a STAT6 dependent manner. Our findings not only assign cutaneous DTHR as IL-23 and Th17 dependent. We also demonstrate a novel and unique strategy to selectively target IL-23, which might be beneficial in the treatment of other Th17-mediated autoimmune diseases such as psoriasis, Crohn disease, rheumatoid arthritis or multiple sclerosis.

066

Spatiotemporal progression of tissue death surrounding burns

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Burn injury progression is a well-known; extension from 2nd-degree, deep dermal burns to full-thickness 3rd-degree burns occurs commonly and leads to increased morbidity and mortality. The relative roles and the exact spatiotemporal progression of cell necrosis and apoptosis are not known. To this end, we employed immunohistochemistry to characterize cell necrosis and apoptosis surrounding burn wounds in a porcine model at various time points after initial injury. Eight mm punch biopsies, taken at 1, 4, 24 and 48 hrs. post-burn from the interspaces of hot comb burns, were fixed, paraffin-embedded and cut into 5μ sections. Specimens were probed with anti-high mobility group box-1 protein (HMGB-1, Abcam) or anti-Caspase 3a (CC3a, Cell Signaling) for evidence of necrosis or apoptosis, respectively, and nuclei were counterstained with hematoxylin. The burn edge was defined as the margin of heat-induced collagen denaturation. From the burn edge, each tissue section was divided into five 0.5 mm intervals. Within each interval, all nuclei were counted and cells were evaluated for the presence of CC3a in the cytoplasm, or HMGB-1 in the nucleus, cytoplasm or pericellular space. We defined early changes of cell necrosis as evidenced by HMGB-1 shift from the nucleus to cytoplasm; intermediate changes by HMGB-1 leakage into the pericellular matrix; and late changes by HMGB-1 absence in the tissue. HMGB-1 staining shifted from cell nuclei to cytoplasm, to pericellular space, to total absence in a progressive spatiotemporal fashion over 24 hrs after burn injury. Cytoplasmic CC3a was found in cells at the periphery of the HMGB-1 changes. We interpreted these findings as indicating that burn injury progression was mostly attributable to cell necrosis with apoptosis only observed at the tissue boundary between cell necrosis and viable tissue. The delineation of the pathobiology of burn injury progression, we believe, will be fundamentally important in screening for agents that might limit such tissue injury clinically.

067

Effector T cells and local innate immunity need to act in concert for development of autoimmune myositis

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A C-protein induced myositis (CIM) is a murine model of polymyositis (PM), which is a chronic autoimmune inflammatory myopathy driven by CD8 cytotoxic T lymphocytes (CTL). We have established a murine PM model, skeletal muscle C-protein induced myositis (CIM), which also mediated by CD8 CTL. This is in sharp contrast with traditional myosin-induced experimental autoimmune myositis (EAM), which is driven by CD4 T cells and humoral immunity. As in other inducible autoimmune models, CIM resolves spontaneously in a few weeks after immunizing C-protein fragments in complete Freund's adjuvant (CFA). Re-immunization of the recovered animals with the same immunogen re-induced the myositis, and depletion of CD25 Treg or IL-10 did not alter the disease course, showing no tolerance established. Of note, CFA injection re-induced the myositis. When, the draining lymph node cells of CIM mice were adoptively transferred to naïve mice with or without each footpad treated with CFA, myositis was only observed in the CFA-treated legs. These facts showed that CIM development requires both activated muscle-specific T lymphocytes and local inflammatory conditioning by CFA. Footpad CFA injection recruited macrophages, producing IL-1, IL-6 and TNF-alpha. When their ligand/receptor interaction was inhibited prior to the adoptive transfer, blockade of IL-1 and TNF-alpha abrogated transfer of the myositis. Thus, Local immune activation, as well as T cell activation, should be a therapeutic target of PM.

068

IL-17-producing dendritic epidermal T cells represent a subset of murine T cells involved in cutanous immunity and wound healing

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Dendritic epidermal T cells (DETC) are a resident population of T cells in murine skin and express an invariant γδTCR. DETC produce cytokines and growth factors that contribute to skin homeostasis, modulate inflammatory responses, and are crucial in wound healing. IL-17 has been demonstrated to be an important regulatory cytokine in the periphery and many tissues. Splenic γδT cells can produce large amounts of IL-17A demonstrating that this cytokine is not exclusively produced by Th17 cells. IL-17 has been reported to be elevated in patients with inflammatory skin diseases, however its role in the cutaneous wound healing response is less clear. Therefore, we investigated whether IL-17 is produced by skin-resident T cells and might contribute to the cutaneous immune response following wounding. We found that cultured DETC produce IL-17A rapidly and in a dose dependent manner upon activation with anti-CD3 or anti-TCRγδ. IL-17A production by DETC was confirmed by real time RT-PCR, FACS analysis, and quantitative ELISA. Furthermore, IL-23 and IL-1β exposure led to IL-17A secretion by DETC and these cytokines synergized with anti-CD3 and anti-TCRγδ mediated signals to augment IL-17A production. These *in vitro* findings were confirmed and extended by *in vivo* studies. Using an *in vivo* wounding model we found substantial and transient IL-17 production by DETC in response to skin injury. Current experiments comparing wildtype and IL-17-/- mice will further elucidate the role of IL-17-producing DETC in the wound healing response. Together, our results highlight a novel role for IL-17-producing DETC in cutaneous immunity and wound healing.

069

Identification and regulation of specific chondroitin sulfate species in discoid lupus erythematosus and dermatomyositis

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The extracellular matrix of DLE and DM accumulates mucin that appears blue by Hale stain, but its components had not been identified molecularly. We previously showed that this material contains chondroitin sulfate (CS) and that the amounts of CS in DLE and DM are significantly greater than in healthy control skin (SID 2008). We now sought to further characterize CS in these lesions. We found that CS in the matrix of DLE and DM is predominantly the 4-sulfated isoform (C4S), consistent with our prior finding that the 6-sulfated isoform (C6S) localizes mainly to vessels in these diseases and is largely absent from matrix. The amounts of C4S in DLE ($p<0.0001$) and DM ($p=0.0021$) lesions were significantly greater than in healthy control biopsies. Dermis in SCLE, which we previously found to contain normal amounts of total CS, did not differ in C4S content from controls. Consistent with this finding, DLE dermis contained significantly more C4S than SCLE dermis ($p=0.0173$), indicating distinct molecular patterns between these two types of cutaneous lupus. To explore mechanisms for C4S accumulation, we examined the regulation of two key enzymes involved in C4S assembly: CS synthase-1 (CSS-1) and the carbohydrate sulfotransferase-11 (CHST-11), which attaches sulfates onto the 4-position of unsulfated chondroitin. Treatment of cultured dermal fibroblasts with five cytokines implicated in DLE and DM (TNFα; IFNα, β, γ; & IL-1α) failed to alter CSS-1 mRNA levels. In contrast, CHST-11 mRNA showed robust four-fold increases upon exposure of fibroblasts to IFNγ ($p<0.0001$) or IL-1α ($p<0.0001$). Induction of CHST-11 mRNA was specific to these two cytokines. Overall, our results identify specific molecular components of the mucin that accumulates in DLE and DM lesions, and we implicate IFNγ and IL-1α as key enhancers of CHST-11 expression by dermal fibroblasts. Because C4S and C6S have different immunologic effects, their dysregulation in DLE and DM may contribute to the pathogenesis of these disorders.

070

Atomic force microscopy-based investigation of autoimmune pathology

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Despite significant progress in our understanding of autoimmune development, there is a major gap in our knowledge regarding the detailed mechanisms by which autoimmune damage is mediated at the tissue level. For example, it is not known exactly how the subset of pathogenic anti-desmoglein (Dsg)3 specificities cause blister formation in Pemphigus vulgaris (PV), but non-pathogenic autoantibodies do not. Atomic force microscopy (AFM) has become an increasingly versatile tool in the biological sciences in recent years. AFM can provide three-dimensional images of cell-associated structures in physiologically stable environments. We have recently used AFM to reveal details of intercellular adhesion structures between adjacent keratinocytes (HaCaT cell line) in real-time. We now extend our analysis with integrated immunofluorescence and AFM imaging of fixed keratinocytes to more precisely target intercellular adhesion sites and to monitor the effect of anti-Dsg3 antibody binding. Additionally, we have developed a novel technology utilizing a polymer micromesh with 3 μm openings to immobilize specified cellular areas that allows ultra-high resolution imaging (< 1 μm scan size) of intercellular structures in living (non-fixed) cells. Finally, we have used AFM force measurements to quantitatively determine changes in cell stiffness/elasticity under physiologic and disease conditions. We show that the addition of pathogenic anti-Dsg3 antibodies to HaCaT cell cultures leads to an initial decrease in cellular stiffness, followed by a significant long-lasting increase in stiffness starting 2 h after antibody treatment. In contrast, the addition of non-pathogenic antibodies leads only to the initial decrease in stiffness that then returns to baseline levels after 2 h. Our data demonstrate the utility of AFM technology for the study of keratinocyte behavior and autoimmune pathology.

071

Plasmin is involved in the pathogenesis of psoriasis by inducing CCL20 expression in macrophages

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Skin-homing pathogenic T cells play a key role in the immunopathogenesis of psoriasis. However, mechanisms underlying recruitment of pathogenic T cells to skin lesions are poorly understood. Plasmin is a serine protease that is released as plasminogen from the liver into the circulation, and activated by tissue plasminogen activator, urokinase plasminogen activator, and factor XII. Recently, accumulating evidence demonstrates that plasmin is also recognized as a potent signaling molecule in particular monocytes/macrophages by inducing a pro-inflammatory response. Here, we show that expression of the CC chemokine, CCL20, is partially confined to dermal macrophages, and both skin-homing CLA+ memory T cells and TH17 cells display high levels of CCR6, the receptor of CCL20 in psoriatic lesions. Importantly, we find that the expression levels of plasminogen activators are highly elevated in psoriatic skin. As a consequence, plasminogen is significantly decreased in psoriatic skin compared with normal skin. *In vitro*, plasmin triggers CCL20 induction in human monocyte-derived macrophages, and its release requires activation of NF-kappa B, and p38 MAPK, but not of JAK1 and ERK1/2 MAPK signaling pathway. Finally, we demonstrate that simultaneous injection of plasmin together with recombinant murine MCP-1 resulted in the induction of psoriasiform skin inflammation around the injection sites with recruitment and activation of macrophages and TH17 cells. Taken together, our data reveal that plasmin at sites of inflammation in psoriasis stimulate CCL20 production mediated by NF-kappa B or p38 MAPK signaling pathway that plays an essential role in the recruitment of pathogenic T cells, and thus provide a potential mechanism of how skin-homing pathogenic T cells maintain their continual presence in psoriasis through a positive chemotactic feedback loop.

072

Protective effects of quercetin on oxidation-induced damage of cultured human keratinocytes

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To study the effects of quercetin on oxidation-induced damage in cultured normal human keratinocytes. Cultured HaCaT cells were treated with different concentrations of hydrogen peroxide. In some experiments, the HaCaT cells were treated by quercetin (0, 10, 25, 50uM)before exposure to hydrogen peroxide. Morphological changes of the cells were observed by light and electron microscopy. The cell proliferation was measured by MTT method. Cell apoptosis and mitochondrial membrane potential (MMP) were measured by flow cytometry. An oxidative stress model of HaCaT cells was established by treatment of HaCaT cells with 250uM hydrogen peroxide for 2 hours.Treatment of HaCaT cells with hydrogen peroxide induced a dose-dependent and time-dependent decrease in cell viability. The apoptosis cells were significantly more in hydrogen peroxide-treated groups (250uM and 500uM) than in control group while the MMP level was lower than in control group In quercetin-treated group, both cell proliferation and MMP level increased,while the cell apoptosis decreased. The results above indicate that quercetin might have some protective roles on hydrogen peroxide-induced cell oxidative damage.

073

Effects of defensamide, a newly synthesized epidermal AMP stimulating molecule, on oxazolone-induced atopic dermatitis animal model

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Previously, we reported that topical application of anti-microbial peptides (AMPs) significantly accelerated the epidermal permeability barrier recovery rate after acute barrier disruption. Similar effects were also observed in K6-L19, an epidermal AMPs stimulating compound. While, in previous studies, the K6-L19 showed significant beneficial effects on epidermal barrier function, the compound was not practically applicable to topical products due to its inappropriate chemical properties. In a continuation of the previous study, we have further screened new compounds and found that the methyl caprooyl tyrosinate (defensamide), a derivative of K6-L19, can also stimulate the epidermal AMPs synthesis *in vitro* and *in vivo*. In this study, we first investigated the effects of defensamide on the atopic dermatitis-like skin eruption seen in the oxazolone induced atopic dermatitis animal model. Topical sensitization and chronic induction with oxazolone resulted in AD like skin lesions in hairless mouse, and topical application of 0.1% defensamide accelerated the recovery of the permeability barrier function and skin hydration, assessed by transpidermal water loss and stratum corneum hydration, respectively. Epidermal hyperplasia, which was observed after oxazolone induction, was also reduced in the defensamide treated skin sites, verified by PCNA staining. In order to measure the anti-microbial barrier function, S. aureus binding into stratum corneum was evaluated using *ex vivo* culture model. Significant reduction in S. aureus binding was observed in defensamide treated skin sites, compared with vehicle treated sites. In summary, the newly developed AMPs stimulating compound, defensamide, improves the AD-like dermatitis seen the in oxazolone induced AD model. These results suggest therapeutic benefits of defensamide for AD treatment.

074

Ketoconazole activates nuclear factor-erythroid 2-related factor-2 (Nrf2) via aryl hydrocarbon receptor signaling pathway. : A possible mechanism of anti-inflammatory effect

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Extensive studies have demonstrated that ketoconazole (KCZ) exhibits anti-inflammatory effects in addition to the potent inhibitory effect against fungi, however, the molecular mechanism is poorly understood. Nuclear factor-erythroid 2-related factor-2 (Nrf2) is a key transcription factor that plays a central role in cellular defense against oxidative and electrophilic stress, consequently exerts anti-inflammatory effects. Aryl hydrocarbon receptor (AhR), known as a dioxin receptor, has been shown to be coordinately regulated with Nrf2 in hepatocytes. We hypothesized that KCZ activates Nrf2 via AhR signaling pathway in normal human epidermal keratinocytes (NHEKs). KCZ administration to NHEKs induced 1) Nrf2 translocation from cytoplasm into nuclei, 2) up-regulation of mRNA and protein levels of Nrf2 and NAD(P)H:quinone oxidoreductase 1 (NQO1) in a dose-dependent manner, 3) translocation of AhR from cytoplasm into nuclei, and 4) up-regulation of CYP1A1 mRNA and protein levels in a dose-dependent manner. These data indicate that KCZ has a potent ability to activate Nrf2 and AhR signaling pathway. NHEKs transfected with Si RNA targeted against AhR failed to demonstrate translocation of Nrf2 into nuclei and to induce Nrf2 expression in spite of KCZ treatment, indicating that activation of Nrf2 by KCZ is AhR signaling-dependent. Furthermore, KCZ antagonistically inhibited up-regulation of 8-hydroxydeoxyguanosine and IL-8 synthesis induced by classical AhR-activating chemical, benzo(a)pyrene. KCZ also inhibited TNF-α-induced IL-8 production from NHEKs, which was cancelled by transfection of Si RNA against AhR. To our knowledge, these findings provide the first molecular basis for the anti-inflammatory effect of KCZ on the skin.

075

Distinct roles of IL-23 and IL-17 in the development of psoriasis-like lesion in a mouse model

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Psoriasis is an autoimmune disease with interactive alterations between the immune system and the skin. It has been demonstrated that the IL-23/Th17 axis plays an important role in the pathogenesis of psoriasis. However, it remains unclear as to detailed contributions of IL-23 and IL-17 *in vivo*. K5.Stat3C transgenic mice, in which keratinocytes express constitutively activated Stat3, develop skin lesions that resemble psoriasis with similar cytokine expression profiles. In this study, we studied the effects of anti-IL-17A, anti-IL-12/23p40, anti-IL-23p19 and anti-IL-6R antibodies on the development of psoriasis-like lesions by K5.Stat3C mice. Treatment with anti-IL-12/23p40 or anti-IL-23p19 antibody greatly inhibited TPA-induced epidermal hyperplasia in the ears of K5.Stat3C mice, whereas the inhibitory effect of anti-IL-17A antibody was relatively less prominent. Moreover, anti-IL-6R antibody had no inhibitory effect. Treatment with anti-IL-12/23p40 or anti-IL-23p19 antibody markedly lowered the transcript levels of Th17 cytokines as well as b-defensins and S100A familiy members in the skin lesions. However, anti-IL-17A antibody did not affect the mRNA expression of Th17 cytokines in the lesion. Crossing IL-17A-deficient mice with K5.Stat3C mice resulted in the partial attenuation of TPA-induced psoriasiform lesions, which were further attenuated by the administration with anti-IL-12/23p40 antibody. Taken together, this mouse system provided a psoriatic model for *in vivo* screening to assess the efficacies of antibodies, and demonstrated distinct roles of IL-23 and IL-17.

076

An environmental contaminant, benzo(a)pyrene induces inflammatory responses in human keratinocytes via aryl hydrocarbon receptor signaling pathway

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Benzo(a)pyrene (BaP) is one of polycyclic aromatic hydrocarbons and an environmental contaminant found in cigarette smoke, burnt food and smoke from the burning of fossil fuels. Although BaP has been shown to exert various effects via aryl hydrocarbon receptor (AhR) signaling pathway, inflammatory effects of BaP on skin remains unanswered. To examine the effect of BaP on the skin, normal human epidermal keratinocytes (NHEKs) were stimulated with BaP. Time course study using immunostaining clearly demonstrated translocation of AhR from the cytoplasm into the nuclei of NHEKs after incubation with BaP for 6 hours. Induction of CYP1A1 mRNA and protein by BaP was observed in a dose-dependent manner. ROS generation and IL-8 production were strongly induced by BaP treatment, whereas the production of IL-1a, IL-6, TNF-α, or GM-CSF was not induced. NHEKs failed to generate ROS and IL-8 production, when transfected with Si RNA targeted against AhR, suggesting that these responses are strongly dependent on AhR signaling pathway. Furthermore, addition of N-acetyl cysteine, a ROS inhibitor, resulted in inhibition of IL-8 production by BaP, indicating that ROS is responsible for IL-8 production. Overall, these data highlight an AhR-dependent up-regulation of ROS and IL-8 in response to BaP in NHEKs, which may profoundly contribute to skin inflammatory reactions exerted by this environmental contaminant. These findings may explain the mechanisms, at least in part, why smoking aggravates IL-8-associated skin diseases such as psoriasis and acne.

077

Antimicrobial peptide – DNA complexes are implicated in initial pathogenesis of rosacea

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Rosacea is a common chronic skin disease that starts with fleeting inflammation and subsequently disfigures patients by induction of sebaceous gland and fibrous hyperplasia. Recently, antimicrobial peptides have been shown to be expressed in rosacea and to play a major role in induction of inflammation. Here we show that antimicrobial peptide overexpression in rosacea is accompanied by intake of nucleic acid into plasmacytoid dendritic cells and production of type I interferon. This was associated with expression of the interferon-signalling surrogate markers MxA protein and IRF-7 in rosacea. In addition, presence of plasmacytoid dendritic cells correlated with T cell numbers, but not with grade of rosacea or number of blood vessels, indicating that these events play a major early role in inducing typical fleeting inflammation, leading to flares and disease exacerbation in rosacea.

078

Triggering of psoriasis-like disease by PPARβ/δ in the epidermis involves activation of STAT3.

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The nuclear hormone receptor peroxisome proliferator activator receptor beta/delta (PPARβ/δ) regulates differentiation and wound-healing and is upregulated in psoriasis. In psoriasis lesions, PPARβ/δ is most strongly expressed in the upper spinous layer. In mice, PPARβ/δ is not expressed in the epidermis. Targeted activation of PPARβ/δ in murine suprabasal epidermis is sufficient to trigger a psoriasis – like disease, including keratinocyte hyperproliferation and activation of Th17 cells. Here we report that STAT3 is activated upon PPARβ/δ activation. Inhibition of STAT3 phosphorylation significantly attenuates disease development, confirming that this pathway is relevant for PPARβ/δ - mediated inflammatory changes. Expression profiling showed that interferon response genes are repressed in PPARβ/δ mice, as opposed to psoriasis. Strikingly, repression of IFN response genes is at least partially mediated by STAT3, by a STAT3-induced program previously termed "anti-inflammatory response". Thus, our data unexpectedly suggest the existence of a signalling block downstream of STAT3 enabling the continuous upregulation of IFN response genes in psoriasis. Importantly, our data show that on-going upregulation of interferon-response genes is not required for downstream immune activation, including IL1-signalling and Th17 activation, since these events are present in PPARβ/δ mice.

079

ATP: a tissue site specific activator of naïve regulatory T cells during contact hypersensitivity reactions

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The two phases of the contact hypersensitivity (CHS) are initiated at different tissue sites. The first antigen contact induces T cells in the draining lymph nodes, whereas the second application of the antigen initiates immune reactions in the tissue and in the blood. Nevertheless, adoptively transferred naïve CD4+CD25+Foxp3+ regulatory T cells (Treg) are able to suppress both phases. Treg require activation to convey their suppressive capacity, therefore we analyzed the activation status of adoptively transferred Treg during CHS responses. Isolated naïve, fluorescently labelled Treg were injected either 2h before sensitization or 15 min before challenge. 24h later, the re-isolated Treg showed increased expression of the activation markers CD69, Foxp3 and CD44 as compared to "before" injection. Because we have recently shown that the suppressive function of Treg is partly mediated by adenosine, generated by degradation of ATP via CD39/CD73, we hypothesized that ATP might also act as an activator for Treg. We demonstrated *in vitro* that ATP activates naïve Treg as indicated by upregulation of CD69. Moreover, ATP-activated Treg showed increased suppressive capacity, comparable to anti-CD3/CD28 activated Treg. Experiments applying PPADS, a P2 ATP receptor antagonist, showed that activation as well as the suppressive function of ATP stimulated Treg were abrogated. The *in vivo* relevance of this mechanism was established by findings showing that application of hapten augmented the ATP concentration in specific tissue. That is, during sensitization ATP was increased in the draining LN, whereas challenge elevated ATP levels selectively in the serum. These data strictly correlate with the observed pattern of Treg activation *in vivo*. Moreover, the suppression of the CHS response by Treg was dependent on functional P2 ATP-receptors, as blockade of this receptor on Treg abrogated their suppressive function *in vivo*. Thus, these data suggest a critical role of ATP in tissue-specific activation of Treg via P2 receptors during allergic and inflammatory reactions.

080

Proteomic analyses for probing biomarkers of atopic dermatitis from the serum-derived samples

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Atopic dermatitis (AD) is a chronic relapsing eczematous skin disease, which requires multiple, combined treatment strategies in the setting of limited therapeutic options. Serum proteomics supplies an optimal source for discovery and analysis of biomarkers. Proteins derived from diseased tissue compartments may leak into lymph and blood, eventually becoming detectable in plasma and serum. Such characteristic alterations may give rise to clinical biomarkers. Serum proteins are usually prefractioned on immunoaffinity column for depletion of abundant proteins and performed by general 2D-PAGE for acidic and neural proteome separation. However, we optimize basic proteome fractionation by micro-Rotofor, separation by acetic acid-urea polyacrylamide gel electrophoresis (A-U PAGE) and followed LC-ESI-MSMS analysis for identifying possible differences in protein profiles in AD patients compared with control subjects. As a result, we found specific cleavage patterns of complement component 3 and 4 in AD patients-derived serums, which were different from those of other inflammatory diseases including systemic lupus erythematous, rheumatoid arthritis, and asthma. These results implied that cleaved fragments from complement component 3 and 4 proteins could be used for specific AD biomarkers at the serum level.

081

IL-21 in the pathogenesis of psoriasis

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Psoriasis is a common chronic immune-mediated skin disease that affects 1–3% of the population worldwide, with an enormous physical, functional impact on the everyday lives of many patients who suffer from the condition. Although the exact pathogenetic mechanisms underlying the development of this disease are not fully understood yet, T cells seem to be crucial mediators of the skin damage in psoriasis. T-cell induced damage is mediated by the secretion of several cytokines including TNF-α, IFN-γ, IL-22 and IL-23. Recently Th17 cells were described to be overexpressed in dermis of psoriatic plaques. These cells are thought to be a major player in psoriasis pathogenesis being important producers of IL-22 and other inflammatory cytokines in response to IL-23. Interestingly IL-21, a " γ chain cytokine " was recently involved together with IL-6 and TGF-β in differentiation of naïve CD4 positive T cells to Th-17 cells acting upstream IL-23 and found to be important mediator in pathogenesis of autoimmune inflammatory disorders. Therefore, we investigated the role of IL-21 in the pathogenesis of psoriasis by studying its expression in skin and peripheral blood of psoriasis patients and by blocking its activity in human psoriasis skin grafts transplanted onto severe combined immunodeficient (SCID) mice. We have found that IL-21 is highly expressed in the skin and peripheral blood of psoriatic patients, and causes psoriasis-like hyperplasia when injected intradermally into mice. IL-21 directly induces keratinocytes proliferation in a MAPK dependent manner. In the human psoriasis-xenograft mouse model antibody-mediated blockade of IL-21 activity leads to a resolution of inflammation and reduces keratinocyte proliferation. Moreover IL-21 mediated proliferation is independent from IL-23/IL-22 axis. These results taken collectively support that a key role of IL-21 in the pathogenesis of psoriasis, suggesting that this cytokine could be a potential therapeutic target.

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Endothelial cells augment the suppressive function of CD4+CD25+Foxp3+ regulatory T cells: Involvement of PD-1 and IL-10

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Blood endothelial cells (EC) act as gatekeepers to coordinate the extravasation of different T cell subpopulations. To do so, EC express defined panels of adhesion molecules, facilitating interaction with blood circulating T cells. Besides the mere adhesion, this cellular interaction between EC and transmigrating T cells may also provide signals that affect the phenotype and function of the T cells. To test the effects of EC on regulatory T cells (Treg) we set up cocultures of freshly isolated murine Treg and primary EC and assessed the phenotype and function of the Treg. We show that (Treg) upregulate PD-1 expression, as well as Interleukin (IL)-10 and TGF-β secretion after contact to EC. These changes in phenotype were accompanied by an increased suppressive capacity of the Treg. Blockade of the PD-1 and/or the IL-10 secretion in *in vitro* suppression assays abrogated the enhanced suppressive capacity, indicating relevance of these molecules for the enhanced suppressive activity of Treg. In aggregate our data show, that endothelial cells increase the immunosuppressive potential of activated Treg by upregulation of PD-1 and stimulation of the production of high levels of IL-10 and TGF-β. Therefore one can speculate that Treg during transendothelial transmigration become "armed" for their suppressive function(s) to be carried out in peripheral tissues sites.

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Detection of IgE by direct immunofluorescence in a large cohort of bullous pemphigoid patients

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Bullous pemphigoid (BP) is an autoantibody-mediated blistering disorder of the skin involving immunoglobulin attachment and subsequent epidermal separation at the dermal-epidermal junction (DEJ). Histochemical appearances range from subepidermal bullae with or without neutrophils and eosinophils to no bullae with numerous eosinophils. Regardless of the histological appearance, direct immunofluorescence (DIF) demonstrating IgG and C3 at the DEJ is virtually pathognomonic for this disease. The involvement of subtypes IgG1 and IgG4 in blister fluid and sera are well described. A number of groups have elucidated the role of IgE in the pathogenesis—i.e. mast cell, basophil, and eosinophil degranulation leading to neutrophil elastase and gelatinase B expression. Prior studies of IgE have relied on analysis of its presence in sera, DIF, and blister fluid in limited numbers. We present a large cohort, of 56 BP patients analyzed by histochemistry—grading the amount of eosinophil infiltration from minimal, moderate to heavy—and by DIF, noting IgE deposition along the DEJ. 43 had classical BP and the remaining 13 had prodromal stage BP. 29 patients with classical BP had moderate to heavy degree of eosinophil infiltration—24 revealed IgE, while the remaining 5 were negative. 14 patients with classical BP had minimal eosinophil infiltration. Of the 13 patients with prodromal BP, 5 had moderate amount of eosinophils, while the other 8 had minimal infiltration. In total, 29 of the 56 patients (45%) revealed positive IgE linear staining at the DEJ, and all of them had classical BP. 10 of 31 (32%) of the IgE-negative samples revealed eosinophils, whereas 24 of the 25 (96%) of the IgE-positive patients showed moderate to heavy eosinophils involvement. To date this is the largest study of BP patients with analysis via DIF implicating IgE with likely recruitment of eosinophils as the mediating mechanism.

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Pharmacological levels of staphylococcal protein A are found in infected atopic dermatitis lesions

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Staphylococcus aureus infections are known triggers for skin inflammation and can modulate immune responses. Patients with atopic dermatitis are particularly sensitive to staphylococcal skin infections, which are known to worsen their skin disease. One mechanism by which staphylococcal bacteria can worsen atopic dermatitis could involve bacterial products, including staphylococcal protein A (SPA). Of note, SPA can bind to immunoglobulin, yet recent studies indicate that it can also act on the tumor necrosis factor receptor 1 (TNFR1). The objective of the present studies was to assess quantitatively the amount of SPA present on clinically infected atopic dermatitis lesions and compare with the numbers of bacteria present and the amount of skin inflammation. We enrolled 89 children with clinically impetiginized atopic dermatitis and evaluated the amount of S. aureus bacteria, SPA, lipoteichoic acid, and a panel of 12 cytokines in wash fluid from a quantitative culture of an infected lesion. The lesional inflammation was quantitated by the Ezcema Area and Severity Index (EASI), and the subject treated with appropriate care including topical corticosteroids and oral antibiotics. The subjects were then re-evaluated after two weeks and the clinical EASI evaluation and wash fluid studies repeated. Spearman rank correlation analysis revealed that the clinical EASI score signficantly correlated with the staphylococcal bacteria numbers and SPA levels. The amounts of SPA also correlated with the amounts of lipoteichoic acid and cytokines including TNF-alpha, IL-1-beta and IL-8. It should be noted that the effective concentration of SPA found *in vivo* on 31% of the subjects at first visit (> 10 ng/ml) was adequate to induce skin inflammation and upregulation of epidermal TNF when injected in the ears of C57BL/6 mice. These studies suggest that infected atopic dermatitis lesions contain biologically relevant amounts of SPA which could in part provide a mechanism for the increased inflammation seen during a staphylococcal skin infection.

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A role for the novel pro-inflammatory cytokine IL-17C in the pathogenesis of psoriasis

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The interleukin(IL)-17 family of cytokines contains 6 members (A-F) with the prototype, IL-17A, serving as a potent proinflammatory cytokine associated with rheumatoid arthritis (RA) and psoriasis. IL-17C is a novel member of this family; it shares 26% homology with IL-17A but utilizes a novel receptor (IL-17RE) and is functionally distinct. In RA patients IL-17C is increased in joints and is associated with destructive inflammation. We hypothesized that IL-17C also plays a role in the pathogenesis of psoriasis. We evaluated IL-17C expression in normal (NN), uninvolved (PN) and lesional psoriatic (PP) skin biopsies using QRT-PCR and ELISA. IL17C mRNA and protein were increased 30-fold ($p<0.01$) and 5-fold ($p<0.04$), respectively in PP skin compared with PN skin. IL17C mRNA expression was also significantly increased (100-fold, $p=0.039$) in the skin of KC-Tie2 mice by 4 wk of age, coinciding with psoriasiform phenotype development. Using normal human keratinocytes (NHK), we determined that IL17C is robustly induced following exposure to both IL-1α and IL-17A (14 and 160-fold, $p<0.02$ both). Alone, TNF-α or IL-22 did not induce IL17C; however, IL-22 synergized with IL-17A boosting IL17C expression 300-fold ($p=0.03$). The purported receptor for IL-17C, IL-17RE was expressed in normal epidermis and NHK, and IL-17C treatment of NHK dose-dependently induced expression of CCL20 and HBD2 mRNA. Unlike IL-17A and F, IL-17C expression by T cells was undetectable; however IL-17C mRNA was identified in monocytes and is capable of inducing TNF-α expression. Interestingly, KC-Tie2 mice treated with macrophage depleting liposomes had a 46% decrease in IL-17C ($p=0.038$) and this corresponded with a 68% decrease in TNF-α ($p=0.047$). Our results suggest that IL17C may be part of an IL-17-TNF-α positive feedback loop involving T cells, monocytes and keratinocytes and thus have an important role in maintaining inflammation in psoriatic lesions.

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Myeloperoxidase as a marker to assess the propensity of psoriasis patients to develop cardiovascular disease in relation to systemic inflammation, lipids and disease severity

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Current literature supports an association between psoriasis and cardiovascular disease (CVD). A cross-sectional study of 100 psoriasis patients and 53 controls demonstrated that serum myeloperoxidase (MPO) levels, after adjusting for age, gender, waist to hip ratio, smoking, systolic blood pressure, and HDL, increased 2.22x in psoriatics compared to controls (95% CI: (1.61, 3.06); $p<0.0001$). Serum MPO levels decreased with increasing high density lipoprotein (0.99; 95% CI:(0.98, 1.00); $p=0.023$), known to be protective against CVD. Immunofluorescence staining of cutaneous MPO showed high expression in lesional skin but minimal expression in non-lesional and normal skin. MPO was detectable mainly in the papillary dermis and co-localized with bone marrow-derived $CD45^+$ cells. $CD11b^+$ cells were a major source of MPO in the dermis and dermal-epidermal junction of lesional skin. Serial sections from involved tissue showed a distinct expression pattern for MPO and the neutrophil marker neutrophil elastase. In murine models $CD11b^+$ circulating leukocytes mediate CVD progression, we therefore perfomed flow cytometry of MPO-producing peripheral blood mononuclear cells. However, psoriatic patients with the highest levels of MPO and controls revealed no difference in MPO producing cell subsets including those co-expressing CD11c, CD14 and CD11b (79.6%, 98.6% and 93.95% respectively). MPO expression among $CD14^+CD16^-$ and $CD14^-CD16^+$ cells was 94.3% and 4.74% respectively. Our cross-sectional findings suggest that inflammation associated with psoriasis may contribute to systemic levels of MPO released from psoriatic plaques. Whether MPO+ leukocytes in psoriatic skin contribute directly to CV risk or are a marker of systemic activation (in vessels and skin), MPO may serve as a surrogate marker for therapeutics in psoriasis patients at risk for atherosclerosis.

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A systems biology approach to evaluate psoriasis as a systemic disease

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Upregulation of the innate and specific immune response in the skin of psoriatic patients has been implicated in the immunopathogenesis of psoriasis. The specific contribution of various inflammatory cells and inflammatory mediators in the blood of psoriasis patients remains to be fully elucidated. Previous studies have used RNA microarray analysis to transcriptionally profile the skin of psoriasis patients but no study has profiled differential gene expression in psoriasis patients compared to healthy controls using microarray analysis of whole blood. There is growing evidence that psoriasis is a systemic disease with associated comorbidities including an increased risk of cardiovascular mortality and metabolic syndrome. We have used a systems biology approach to evaluate psoriasis as a systemic disease. This study assessed baseline immunogenetic signatures in psoriasis patients (n=27) compared to healthy controls (n=20) in whole blood using Illumina HT-12v3 Bead Chips and GenespringTM software. We also applied a novel modular analysis framework which is based on the identification of transcriptional modules formed by genes coordinately expressed in multiple disease datasets.1 These results are currently being validated in a distinct patient cohort using microarray analysis, and verified at a protein and cellular level using flow cytometry and Luminex. A total of 1468 genes were differentially expressed 1.5 fold or more by psoriasis patients compared to healthy controls, resulting in a distinct immune signature for psoriasis. In particular, there was considerable differential expression of genes encoding interleukins, interleukin receptors, dendritic cell function, macrophage receptors and toll-like receptors. The findings of this study provide new insights into the immune signalling pathways in psoriasis, and identify potential biomarkers to follow patients in the clinical setting.

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Molecular and morphometric characterization of neuroinflammatory and neurovascular changes in the development of rosacea

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Rosacea is a frequent chronic inflammatory skin disease with a high impact on the quality of life in these patients. Neurogenic inflammation is defined as an inflammatory response induced by sensory nerves which release neuromediators at the site of inflammation resulting in vasodilatation (erythema), plasma extravasation of proteins (edema) and recruitment of inflammatory cells (inflammatory infiltrate). Rosacea is characterized by a transient to persistent erythema, edema, teleangiectasia, burning pain and a lymphomonocytic infiltrate resulting – at later stages – in facial skin fibrosis. Because rosacea reveals all characteristics of neurogenic inflammation, a central role of sensory nerves in the pathophysiology can be anticipated. However, the underlying mechanisms for the onset and maintainance as well as the crucial mediators involved in the pathophysiology of this disease are poorly understood. Therefore, we analysed the molecular and immunohistochemical characteristics in the different stages of this disease by immunohistochemistry, double-immunofluorescence, morphometry, RNA gene arrays and RT PCR as compared to healthy skin and lupus erythematodes. Our results show that in all stages, rosacea is a disease predominant of TH1 helper cells, T17 cells, macrophages and mast cells. Blood vessels and lymphatic vessels are dramatically dilated but show no signs of angiogenesis. Gene array and RT PCR data confirm the upregulation of genes involved in vasoregulation, neurogenic inflammation, immune defense and receptors which can be stimulated by trigger factors of rosacea such as the transient receptor ion channels, for example. Thus, dysregulation of mediators and receptors implicated in neuro-vascular and neuro-immune communication may be critically involved at early stages of rosacea. Thus, drugs which act on the peripheral sensory nervous system may be beneficial for the treatment of rosacea.

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Constitutively active inflammasome in human melanoma cells mediating autoinflammation via caspase-1 processing and secretion of interleukin-1β*

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Interleukin-1β (IL-1β) is a pleiotropic cytokine promoting inflammation, angiogenesis and tissue remodeling as well as regulation of immune responses. Although IL-1β contributes to growth and metastatic spread in experimental and human cancers, the molecular mechanisms regulating the conversion of the inactive IL-1β precursor to a secreted and active cytokine remains unclear. In the present study, we analyzed 9 melanoma cell lines, 15 melanoma specimens and 6 fresh melanoma cells derived from patient tumor specimens. We demonstrated that NALP3 inflammasome is constitutively assembled and activated with cleavage of caspase-1 in human melanoma cells. Late stage human melanoma cells spontaneously secrete active IL-1β via constitutive activation of the NALP3 inflammasome and IL-1 receptor signaling, exhibiting a feature of autoinflammatory diseases. Unlike human blood monocytes, these melanoma cells require no exogenous stimulation. In contrast, NALP3 functionality in intermediate stage melanoma cells requires activation of the IL-1 receptor to secrete active IL-1β; cells from early stage of melanoma require stimulation of the IL-1 receptor plus the co-stimulant muramyl dipeptide. The spontaneous secretion of IL-1β from melanoma cells was reduced by inhibition of caspase-1 or the use of siRNA directed against ASC. Supernatants from melanoma cell cultures enhanced macrophage chemotaxis and promoted *in vitro* angiogenesis, both prevented by pretreating melanoma cells with inhibitors of caspases-1 and -5 or IL-1 receptor blockade. These findings implicate IL-1-mediated autoinflammation as contributing to the development and progression of human melanoma, and suggest that inhibiting the inflammasome pathway or reducing IL-1 activity can be a therapeutic option for melanoma patients.

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Clinical and experimental evidence that psoriasis is exacerbated by tobacco smoke via an enhanced generation of Th17 cells

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Environmental factors are thought to contribute to the increased prevalence of autoimmune diseases via the activation of Th17 cells. Tobacco smoking increases the risk of psoriasis, but the mechanisms are not clear. Tobacco smoke contains low levels of aryl hydrocarbon receptor (AhR) agonists. AhR is expressed in human Th17 cells, and AhR activation during Th17 generation markedly increases the production of Th17 cells. We investigated whether tobacco smoking exacerbates skin lesions via Th17 cell activation in patients with psoriasis. Peripheral blood mononuclear cells (PBMC) were obtained from 33 smoking and 21 non-smoking psoriasis patients. The proportion of Th17 among $CD4^+$ cells in PBMC from smokers, nonsmokers, and healthy volunteers was 3.46±2.02%, 3.24±1.55%, and 2.58±0.80%, respectively. To evaluate the correlation between smoking and Th17, groups of smokers and nonsmokers were further divided into two groups (Th17-high and Th17-normal) based on the proportion of Th17 cells among $CD4^+$ cells. A greater proportion of smokers (19/33) than nonsmokers (6/21) had high Th17 levels (Th17/CD4 > 3.38%, mean+1SD of healthy volunteers). Frequency of Th17 cells among PBMC was significantly increased by tobacco smoking in patients with psoriasis (χ^2, $p=0.0372$). We further investigated whether tobacco smoke extract (TSE) generated Th17 production from the central memory T cell population *in vitro*. Central memory cells were isolated from PBMC by magnetic sorting using CD4 and CD62L antibodies. TSE (7 μl/ml) induced central memory cells to produce Th17 cells. TSE further increased interleukin (IL)17 and IL22 expression as compared with inducible cytokines, such as IL1 and IL6/TGFβ, and IL21. These findings provide clinical and experimental evidence that tobacco smoke exacerbates psoriasis via the induction of Th17 in the peripheral blood.

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Development of alopecia areata may be associated with heart disease

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Alopecia areata (AA), a non-scarring, inflammatory hair loss disease, is believed to involve an autoimmune mechanism. In other autoimmune skin conditions, such as psoriasis, there is evidence that affected individuals have an increased risk of heart disease and heart failure. We investigated the potential for a relationship between AA and heart disease. The mortality rate in 18 month old C3H/HeJ mice with induced AA of 12+ months was higher (28.6%) but statistically insignificant compared to controls (15.4%). However, the heart sizes/wet weights in surviving AA-affected mice (n=10) were statistically significantly greater than age and sex matched normal-haired littermates (n=11). We conducted quantitative PCR (qPCR) analysis of AA mouse heart and skin tissues for selected genes involved in apoptosis and inflammation. We observed IL18, IL18 receptor-1 (IL18r1) and IL18 binding protein (IL18bp) increased significantly (4.6, 2.8 and 5.2 fold respectively) in the heart tissue (respectively 2.4, 3.1 and 5.2 fold in skin) compared to controls. It has been shown by others that IL18 levels, in both circulation and resident myocardial tissues are increased in patients with heart diseases. Experimentally, administration of IL18 increases heart mass and wall thickness leading to cardiac dysfunction. ELISA analysis for cardiac troponin I (cTnI) levels, a marker of cardiac tissue damage, in peripheral blood from AA patients, revealed highest cTnI levels were associated with advancing AA compared to stable and regressing AA. However, this result failed to achieve statistical significance. Based on our observations, the development of AA may be associated with increased IL18 activity which may confer a modest increased risk of heart disease.

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Comparison of index values of Dsg3-ELISA and those of EDTA-treated Dsg3-ELISA is a reliable monitor for pathogenic atuoantibody in pemphigus

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Autoantibodies (Abs) against desmoglein (Dsg) 3 have a pathogenic role in pemphigus vulgaris (PV). Some patients however retain high levels of anti-Dsg3 Ab even in a remission phase. We hypothesized that those Abs might be non-pathogenic because of their reactivities against linear epitopes of Dsg3. Our study was designed to distinguish anti-Dsg3 Ab directed against the conformational epitopes using commercially available ELISA plate. First, we determined the alterations in titer of monoclonal anti-Dsg3 Abs carrying different epitope reactivities with or w/o EDTA treatments of the ELISA plate. The reactivity of the pathogenic AK23 and 19 mAbs (EC1-2) and non-pathogenic AK18, 15 and 20 mAbs (EC2-5) were determined with EDTA treated ELISA plate with modification of the Ca2+-dependent conformational epitopes. Pathogenic AK23, 19 and non-pathogenic 18mAb showed 65.5-87.4% reduction in the EDTA ELISA as compared with untreated ELISA index values (ivs), indicating a high Ca2+-dependent reactivity. AK15 mAbs showed a medium reduction. AK20 mAb show no reduction at all, indicating a Ca2+-independent reactivity. These results suggest that difference in index values between conventional ELISA and EDTA-ELISA is nearly equivalent to pathogenic autoAb titer against Ca2+-dependent conformational epitopes of Dsg3. Then, we tested 8 PV sera with high anti-Dsg3 Ab levels (>100 iv) even though in inactive phase. In the sera, 40.9-77.2 % of anti-Dsg3 Ab were directed against the linear epitope, and PV-IgG containing 81% Ab against the linear epitope, did not induce acantholysis. The proportion of Ab binding to the conformational epitope were greater in an active than in an inactive phase in 6 out of 8 cases. Calculated conformational ELISA ivs were more correlated to the disease activity. To determine the ratios of conventional and EDTA-ELISA, i.e., reactivity of Abs against conformational and linear epitopes, may be a useful tool to find out false ELISA ivs in terms of pathogenecity and also to clarify the cause of disease in PV.

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The majority of patients with Neumann type pemphigus vegetans show IgG autoantibodies to human desmocollins 1-3, particularly desmocollin 3

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The major autoantigens for pemphigus are desmogleins (Dsgs). We reported that SPD type IgA pemphigus shows IgA antibodies to human desmocollin 1 (Dsc1). However, IgG antibodies to Dscs were rarely detected. Although the pathogenic mechanism for vegetating skin lesions in pemphigus vegetans (PVeg) is still unknown, non-Dsg IgG antibodies may play a role in PVeg. To investigate further the pathogenesis in PVeg, we examined IgG reactivity with Dscs in PVeg. We collected 12 patients with Neumann type PVeg, whose diagnoses were made by clinical, histological and immunofluorescence findings. By our immunoblot analysis using normal human epidermal extracts, 3 of the 12 PVeg cases showed IgG reactivity with the 160 kDa Dsg1, 4 cases with the 130 kDa Dsg3, and 5 cases with the 110 kDa a-form and the 100 kDa b-form of Dscs. Dsg ELISAs showed anti-Dsg1 and Dsg3 IgG antibodies in 7 and 5 cases, respectively. Four cases did not react with either Dsg1 or Dsg3. We further examined IgG reactivity with Dscs by so-called cDNA transfection method. In this system, eukaryotic vectors containing full length cDNAs of human Dsc1-3 were transfected into COS-7 cells, and IgG reactivity to each Dsc expressed on COS-7 cell surface was detected by living cell immunofluorescence. By this method, 6 of the 12 cases reacted with Dsc1, 7 cases with Dsc2, and 7 cases with Dsc3. We also previously developed ELISAs using recombinant baculoproteins of human Dsc1-3. By these ELISAs, only one case showed IgG reactivity with Dsc1, 2 cases with Dsc2 and 7 cases with Dsc3. These results clearly indicated that Dsc1-3, particularly Dsc3, are major autoantigens in Neumann type PVeg. IgG autoantibodies to Dscs may be pathogenic, because some cases did not react with Dsgs. Furthermore, cDNA transfection method is more sensitive than baculoprotein ELISA to detect autoantibodies to Dscs.

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Anti-epiplakin autoantibodies are present in paraneoplastic pemphigus

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Epiplakin, a cytoskeletal linker protein, was originally identified as an autoantigen for a patient with unique subepidermal blistering disease. Epiplakin is expressed in both the stratified epithelium and the simple epithelium. Molecular cloning studies showed that epiplakin was a 552 kDa large protein of plakin family. Paraneoplastic pemphigus (PNP) is characterized immunologically by autoantibodies directed to various target antigens, mainly plakin family proteins. Particularly, we have shown that all PNP sera detect a clear reactivity with a doublet of the 210 kDa envoplakin and the 190 kDa periplakin by immunoblot (IB) analysis using normal human epidermal extracts. However, it was unknown whether epiplakin is recognized by PNP sera. The aim of this study was to examine the prevalence of autoimmunity against epiplakin in PNP. We performed both IB analysis and immunoprecipitation-IB analysis (IP-IB) using sera from 50 PNP patients whose diagnoses were confirmed by various immunofluorescence and IB tests. By IB analysis using normal human epidermal extracts, 6 of the 50 PNP sera showed a clear protein band which was identical to that shown by polyclonal antibody specific to epiplakin. In IP-IB analysis, the RIPA buffer extracts of non-radiolabeled cultured KU8 cells, a cell line of squamous cell carcinoma, were first immunoprecipitated using PNP sera and protein-G sepharose beads. Then, the immunoprecipitated proteins were immunostained using anti-epiplakin polyclonal antibody. This IP-IB analysis clearly confirmed that a significant number of PNP sera contained anti-epiplakin IgG autoantibodies. The IP-IB analysis was a much more sensitive method than IB analysis to detect the anti-epiplakin autoantibodies. In this study, we showed and concluded for the first time that epiplakin is one of the PNP antigens.

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Granzyme B is a novel interleukin-18 converting enzyme

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Granzyme B (GrB) is a caspase-like serine protease found in the granules of cytotoxic T lymphocytes and natural killer cells. GrB is known to induce apoptosis; however, little is known about its possible involvement in other biological events. Interleukin-18 (IL-18), a potent inflammatory cytokine, is produced as an inactive precursor (proIL-18). Several cells, including monocytes/macrophage lineage and non-hematopoietic cells, produce proIL-18. ProIL-18 needs appropriate processing to become active. Caspase-1 is the authentic IL-18 processing enzyme and is essential for IL-18 release from monocyte/macrophage lineage cells. However, caspase-1 is absent in non-hematopoietic cells, allowing us to investigate whether exogenous GrB cleaves proIL-18 into the biologically active form. The amino acid sequence of the recombinant proIL-18 after treatment with GrB was identical to that cleaved by caspase-1. Furthermore, the cleaved fragment had the same biological activity as authentic mature IL-18. GrB-induced proIL-18 cleavage was completely abrogated by the GrB-specific inhibitor. Moreover, culture supernatant from GrB(+)/ caspase-1(-) human CD8+T cells was able to cleave proIL-18 into IL-18 mature form. Interferon-γ induction was also detected in normal human keratinocyte treated with cultured CD8 T cells. These results clearly demonstrate that GrB is a potent IL-18-converting enzyme and suggest that GrB secreted by cytotoxic T lymphocytes and/or natural killer cells may initiate IL-18 release from target cells, leading to the development of inflammation.

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Cross-reactivity at the T-cell level between human and Malassezia sympodialis thioredoxin(s) in adult patients with atopic dermatitis

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Objective: To demonstrate T-cell mediated cross-reactivity between the human (HuTRX) and Malassezia sympodialis (Mala s 13) thioredoxin(s) in adult patients with atopic dermatitis. Methods: T-cell lines (TCL) were generated in the presence of Mala s13 from the peripheral blood of five healthy individuals, three psoriasis patients and three AD patients with specific IgE to Mala s13 and HuTRX. Mala s 13 specific T-cell clones (TCC) were generated both from the peripheral blood and biopsies of positive patch test lesions to Mala s 13 from the three AD patients and from the peripheral blood of two healthy persons. Mala s 13 specific and HuTRX cross-reactive TCC were phenotyped according to the cell surface expression of CD4, CD8 and CLA and by their cytokine secretion profile upon stimulation with HuTRX. Human keratinocytes were stimulated with IFN-gamma, TNF-alpha and IL-4 and the release of human thioredoxin was assayed by ELISA. Results: Mala s 13 specific TCC both from the blood and skin compartment were wholly cross-reactive with HuTRX. Cross-reactive TCC were CD4+ and co-expressed the skin homing receptor CLA . In addition to Th1 and Th2 TCC, we could also identify TCC that were of the newly described subsets Th17 and Th22. Following 48 h stimulation with IFN-γ and TNF-α keratinocytes released substantial amounts of thioredoxin. Conclusion: Thioredoxin auto-reactive skin homing T-cells secreting inflammatory cytokines such as IFN-gamma, IL-17 and IL-22 may have relevance in cutaneous inflammation in AD. Keratinocytes release thioredoxin upon prolonged IFN-γ and TNF-alpha stimulation, that can then perpetuate inflammation in the AD skin by providing an autoallergen which cross-reacts with thioredoxin from skin colonizing Malassezia spp at the site of cutaneous inflammation.

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Anti-inflammatory effect of rocket seed isothiocyanates on cultured macrophages and inflamed skin *ex vivo*

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Skin inflammatory diseases (e.g. psoriasis, contact dermatitis and photodamage) are regulated by a complicated network of interactions between keratinocytes and the immune system cells. In light of the recent trend to use "natural" plant extracts from popular ethnic medicine, plant material active in quenching skin inflammation was sought. The rocket seed (*Eruca sativa*) extract, containing the isothiocyanate (ITC), 4-methylthiobutylisothiocyanate (MTBI), demonstrated anti-inflammatory characteristics, in monocytes [*Yehuda et al. Biofactors 2009,* ***35****:295-305*]. Thus, the anti-inflammatory effects of MTBI and its oxidized derivative, sulforaphane (SFN), found at lower quantities in rocket seeds, were further assessed *in vitro* in lipopolysaccharide (LPS)-activated macrophage-like cells, and *ex vivo*, in inflamed skin in organ culture. Both ITCs significantly reduced the induction, as well as the ongoing process of inflammation in macrophage-like THP-1 cells, as indicated by the reduced expression of the mRNA of the LPS-induced proinflammatory cytokine genes IL-6, IL-8, IL-12/23p40 and TNF-α. MTBI also downregulated IL-1β transcription during the inflammation process, and SFN pretreatment prevented the LPS-induced IL-1β mRNA increase. In LPS-treated skin in organ culture, MTBI was found to reduce the release of the proinflammatory cytokines TNF-α, IL-1α, IL-6 and IL-8. Generally, the ITCs have a stronger inhibition effect on cells involved in the inflammation process than on non-induced cells. The study indicates that rocket seed ITCs could serve as potential natural biological agents in the therapy of skin inflammation.

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Erlotinib stimulates epidermal hyperplasia in KC-Tie2 mice and human skin organ culture

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Skin rash is a dose-limiting toxicity common to several EGFR tyrosine kinase inhibitors. EGFR KO mice also develop a scaly rash characterized by marked immunocytic infiltration (JID 2009 Nov 5). However, we previously found that treatment of organ cultures of psoriatic skin with an EGFR blocking antibody (225 IgG) partially ameliorated the psoriatic phenotype (Pathobiology 66:253). K5tTA:TetOSTek/Tie2 bigenic mice with TET-dependent overexpression of Tie2 in KC (KC-Tie2 mice) develop psoriasiform skin lesions (AJP174:1443). In an effort to integrate these observations, we characterized EGFR ligand expression and downstream signaling during lesion development in KC-Tie2 mice, and treated both KC-Tie2 mice and organ cultures of normal human skin with erlotinib. As assessed by QRT-PCR, expression of EGFR ligands AREG, HB-EGF, and EPGN all increased markedly in KC-Tie2 skin lesions beginning at 4 weeks of age. As measured by western blotting, EGFR tyrosine phosphorylation and ERK phosphorylation were also increased in adult mice. Paradoxically, treatment of adult mice with an established skin phenotype with erlotinib (50-200 mg/kg by gavage 5 d/wk for 4 wk) failed to failed to reverse the acanthosis. Indeed, erlotinib treatment prior to development of skin lesions (10 mg/kg IP daily for 25 d beginning at p10) dramatically exacerbated the phenotype, with a doubling of epidermal thickness in erlotinib-treated KC-Tie2 mice relative to age-matched KC-Tie2 controls (72 vs. 34 μ), but no effect in wt littermates (11 μ). While erlotinib markedly inhibited human KC proliferation in monolayer culture, human skin organ cultures developed marked acanthosis with increased elaboration of IL-8 and GRO-a (after 2d) as well as MCP-1 (after 4-8 d). Acanthosis and MMP-1 elaboration were markedly inhibited by IL-1 receptor antagonist. Taken together, these results support an IL-1-mediated anti-inflammatory role for autocrine EGFR signaling in mouse and human skin *in vivo*.

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Substance P is modifies antigen presentation in skin draining lymph nodes in a mouse model of stress and allergic dermatitis

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Close interaction between the nervous and immune systems greatly contributes to inflammatory diseases especially in organs at the self-environment interface such as the skin. During the cutaneous stress response the sensory neuropeptide substance P (SP) conducts an acute local stress-response via neurogenic inflammation. Using a mouse model, that combines allergic inflammation and noise stress we here show that repeated stress-exposure markedly attenuates allergic inflammation in a neurokinin 1-SP-receptor (NK-1 R) dependent manner. We found that repeated stress exposure prior to allergen sensitization increases dendritic cell (DC)-nerve fiber contacts, decreases SP+ nerve fiber numbers, enhances DC migration out of the skin and their maturation in blood and skin draining lymph nodes, alters cytokine balance and increases levels of IL-2 and T regulatory cell numbers in local lymph nodes and inflamed tissue. All of these changes were inhibited by NK-1 R blockade and allergic inflammation returned to levels detectable in stressed animals. We conclude that repeated stress prior to immune-activation acts pro-tolerogenic and thereby beneficial in inflammation in a SP dependent fashion and can therefore be exploited to train resistance to allergy exacerbation and potentially development.

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Peptide protection of the pinna: a model for studying the role of CD8+ T cells in mice

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We previously reported that when OT-1 CD8+ T cells (which express Vα2/Vβ5 chains of the T cell receptor and are specific for OVA peptide 257-264) are adoptively transferred into no 15 K14-sOVA mice expressing high levels of soluble chicken ovalbumin (OVA) under control of the keratin-14 promoter, death ensues in 5-8 days. Mating these K14-sOVA mice with OT-1 mice results in death in 80-90% of the progeny by 21 days, and the death can be obviated by administration of the TCR-recognized peptide (SIINFEKL) on days 5 and 9 after birth. We have now assessed other of our K14-sOVA expressing strains (no 5 and 17) and find that when these mice are crossed with OT-1 mice, the progeny are uniformly born without ears but otherwise develop normally. The goal of this study was to elucidate the mechanism of selective tissue destruction in the double transgenic (DTg) mice and to attempt to prevent this destruction. Histopathology of the ear buds exhibited an intense inflammatory infiltrate present as early as day 1 after birth, suggesting that the process began in utero. FACS analysis and immunohistochemical staining revealed that a large percentage of the inflammatory infiltrate was CD8+. When we injected anti-CD8 antibody i.p. into pregnant no 5 or 17 sOVA/OT-1 DTg mice, there was normal ear development in all pups. Subsequently, we injected pregnant DTg mice with varying doses of SIINFEKL peptide i.p. and found that 100μg of peptide had no effect but that repeated larger i.p. doses (300μg to 1mg) resulted in pups being born with either normal-looking ears or partial ears. A total of 62 (no 5 and 17) DTg mice have been born, 5 with fully-formed ears and 9 with partial ears (8% and 15%, respectively). We therefore postulate that our transgenic mice undergo peptide-induced tolerance in the attenuation of ear loss. This model will continue to inform us about local CD8+-induced tissue destruction and may provide insight into the treatment of tissue-specific autoimmune disease.

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Nerve growth factor partially recovers inflamed skin from stress-induced worsening

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Atopic disease is characterized by neuro-immune dysregulation but its nature and clinical impact still remain ill-defined. The neurotrophin nerve growth factor (NGF) may worsen cutaneous inflammation and can be released during a stress experience ranging from physical to emotional. The role of NGF in the cutaneous stress response was therefore studied in a model for atopic dermatitis-like allergic dermatitis (AlD). By microarray analysis increased NGF associated growth factor and inflammatory mRNAs were detected in the skin of stressed mice. qPCR, Elisa and immunohistochemistry confirmed that stress and AlD increased cutaneous, serum, but not hypothalamic NGF. NGF-neutralizing antibodies marked reduced epidermal thickening in stress-worsened AlD, together with NGF, neurotrophin receptor (TrkA and p75NTR) and tumor growth factor beta (TGFβ expression by keratinocytes but did not alter transepidermal water loss. Also, mast cell NGF expression was reduced which corresponded to reduced cutaneous tumor necrosis factor alpha (TNFα mRNA levels but not mast cell degranulation or TH1/TH2 cytokine balance. Moreover, TNF receptor type 2 (TNFRII) was detected on eosinophils which corresponded to reduced eosinophil infiltration after treatment with NGF-neutralizing antibodies. We thus conclude that NGF signaling contributes to worsening of cutaneous inflammation primarily by enhancing epidermal hyperplasia, pro-allergic cytokine induction and allergy characteristic cellular infiltration and thereby acts as a local stress mediator in perceived stress and allergy.

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Modifying the heavy chain third complementarity determining region (H-CDR3) of pemphigus antibody to prevent pathogenicity but not binding suggests a novel approach to targeted therapy

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Current therapy for pemphigus involves general suppression of the immune system. More targeted therapy directed toward the anti-desmoglein (dsg) autoantibodies will depend on characterizing the specific antibody (Ab) sequences that impart pathogenicity. Because the H-CDR3 is thought to contribute most to antigen binding, we analyzed the H-CDR3 regions of 8 unique pathogenic monoclonal Abs (mAbs) compared to 23 nonpathogenic mAbs cloned by phage display from 2 pemphigus vulgaris (PV) and 2 pemphigus foliaceus patients. We found a consensus amino acid (AA) motif of D/E-x-x-x-W (D/E-acidic AA; W-tryptophan) in 6 (2 with long and 4 with shorter H-CDR3 regions) pathogenic Abs; AK23, a pathogenic murine PV mAb, also expressed this consensus sequence, but the 23 nonpathogenic mAbs did not. Swapping among mAbs, randomization, and site directed mutagenesis of the H-CDR3 regions showed that: restricted H-CDR3 sequences are critical for binding to Dsg3; W in the long sequence is necessary for pathogenicity but not binding to dsg; mutation of W in the short CDR3 resulted in an unstable Ab, but mutation of 4 AA immediately upstream blocked Ab pathogenicity but not binding. MAbs in which H-CDR3 alterations resulted in the uncoupling of pathogenicity from binding were able to block, in ELISA, dsg binding of unaltered pathogenic mAbs. These data show the important role of W in H-CDR3 in promoting autoAb-induced loss of adhesion for some Abs, perhaps by interfering with dsg's W2 interactions within the binding pocket of its intercellular partner, the area to which these mAbs have been shown to bind. Our data suggest that strategies to target consensus H-CDR3 regions of pathogenic Abs with peptides or small molecules are feasible and would have dual benefits of blocking pathogenicity and preventing binding of newly produced pathogenic Abs in serum.

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Possible pathogenic role of CD4+CD25highFoxp3+ and CD4+IL-10+ regulatory T cells in patients with pemphigus vulgaris

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Pemphigus vulgaris (PV) is a group of organic specific autoimmune blistering skin and mucosa diseases characterized by circulating pathogenic autoantibodies directed against desmoglein (Dsg) 1 and Dsg3. Abnormalities in regulatory T cells might play an important role in the loss of self-tolerance, which may be one of the major pathogenic courses of patients with PV. We sought to investigate the changes of natural CD4+CD25high Foxp3+ regulatory T (nTreg) cells in ratio, and inducible CD4+IL-10+ regulatory T (Tr1) cells in the blood of patients with PV, and to analyze their correlation with disease severity. 40 of PV patients and 20 of age and sex matched healthy controls were enrolled in the study. The frequency of nTreg and Tr1 cells in peripheral blood was determined by flow cytometry. The proportion of CD4+CD25high Foxp3+ nTreg cells in PV patients was markedly reduced, but the population of CD4+IL-10+ Tr1 cells was significantly elevated compared with the healthy controls. However, these variations were unrelated to disease severity. We concluded that reduced ratio of CD4+CD25highFoxp3+ T cells and increased percentage of CD4+IL-10+ Tr1 cells might be involved in the pathogenesis of PV.

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The cathelicidin response to wounding in psoriatic lesional and non-lesional skin

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Cathelicidin has been shown to be strongly expressed in lesional psoriatic (PS) skin, and may play an important role as an antimicrobial peptide, as well as a proinflammatory mediator in the pathogenesis of psoriasis. The purpose of this study was to compare cathelicidin induction in response to wounding in PS plaques and non-lesional skin, and the skin of normal (NL) controls. 2mm biopsies were taken from 10 healthy controls and 8 PS at baseline with a subsequent 4mm biopsy 3-5 days later over the site of the 2mm punch in order to assess changes in cathelicidin expression brought about by wounding. All sites (PS lesional and non-lesional and NL skin) showed an increase in cathelicidin expression following wounding. Mean cathelicidin expression measured using quantitative real time PCR techniques increased 24.2 fold in NL controls (1.0 Relative copy units (RCU) to 24 RCU), 16.1 fold (0.58 RCU to 9.38 RCU) in the non-lesional skin of PS, and 2.4 fold (69.98 RCU to 170.0 RCU) in PS plaques. All subjects showed induction of cathelicidin with wounding, although NL and PS non-lesional subjects induced cathelicidin to a greater degree than PS lesional skin. Cathelicidin has recently been shown to play an important role as an autoinflammatory mediator in psoriasis through the Th17 pathway. It has also been shown to stimulate TNF-α production and inhibit apoptosis via Cox-2. Although all subjects showed induction of cathelicidin with wounding, as evidenced by the Koebner phenomenon (the development of psoriasis in areas of wounding), only subjects with PS typically develop psoriatic lesions. Thus, the pathology of psoriasis may be due to a dysregulation in cathelicidin production in PS subjects in which the normal wounding mechanism continues as a positive feedback loop for cathelicidin induction while NL subjects are able to suppress further cathelicidin production. Such evidence suggests that a better understanding of this dysregulation of cathelicidin may shed important insight in the pathogenesis of psoriasis.

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IL-23-mediated epidermal hyperplasia is dependent on IL-17A in mice

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Numerous dendritic cells producing IL-23 and Th17 cells producing IL-17A and IL-22 are found in psoriasis lesions. Previous studies have shown that IL-22 mediates the psoriasis-like changes of keratinocyte proliferation and epidermal hyperplasia following IL-23 injection into skin of wild-type (WT) mice (Zheng et al, Nature, 2008). Here, we explored the role of IL-17A in mediating IL-23-induced epidermal hyperplasia using WT mice, *IL-17A* knockout (KO) mice, and *IL-22* KO mice. Mouse ears were injected with 1 μg IL-23 and adjacent ears were injected with saline daily for 4 days. On day 4, a blinded investigator measured ear swelling using an engineer's caliper, and a second blinded investigator measured epidermal thickness by non-invasive confocal microscopy. Ears were then collected for routine histology and mRNA expression analyses by real-time RT-PCR. In WT mice, IL-23 induced ear swelling, epidermal thickening (p=0.04 vs saline), and increased expression of both *IL-17A* and *IL-22* mRNAs. As reported by Zheng et al, IL-23 injections into skin of *IL-22* KO mice resulted in relatively little ear swelling and epidermal hyperplasia (p=0.64 vs saline), even though levels of *IL-17* mRNA were elevated. Notably, IL-23 injections into skin of *IL-17A* KO mice also failed to produce ear swelling and epidermal hyperplasia (p=0.89), although *IL-22* mRNA was induced in these mice. These results show that IL-17A, like IL-22, is a downstream mediator for IL-23-induced changes in keratinocytes, and that both of these Th17 cytokines are necessary to induce IL-23-mediated skin pathology. IL-17A may represent an attractive therapeutic target in individuals with psoriasis by blocking downstream effects of IL-23.

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Cyotkine and chemokine protein levels of dorsal skin in the C3H model of alopecia areata

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Alopecia areata (AA) has been discussed in the literature for over 100 years. Neither the cause nor the basic immunology of this autoimmune disease are well understood. While there is a large body of work on AA in the literature exploring the immunobiology of the disease, the vast majority of the reports have focused on mRNA and not protein levels. As cells of the immune system can pre-make and store cytokine and chemokine mRNA, determining the expression of these proteins in AA is of great importance. To determine the level of various cytokine and chemokine proteins in AA, we utilized the C3H/HeJ model of the disease. In this model, skin biopsies from mice that spontaneously develop the disease are transplanted onto unaffected mice. The unaffected mice then develop AA, which is not confined to the transplanted skin biopsy. Mice that had their own skin biopsies taken and rotated 180 degrees and reattached serve as controls. We harvested mice at 5, 10, 15, and 20 weeks after transplant. Dorsal skin was snap frozen and stored at -80 prior to protein isolation. ELISAs were performed on skin homogenates to measure cytokine and chemokine levels. At 10 weeks, CTLA4 and CXCL9 were significantly increased (p=0.006 and 0.036, Mann-Whitney). CXCL9 and CCL5 were significantly increased at 15 weeks as well (p<0.01). At 20 weeks IL10, IL17, IL21, IL22, and IFNG were all significantly decreased (p<0.02) in mice with AA, while CXCL9 and CCL5 were significantly increased (p<0.02). This is contrary to reports in the literature showing that IFNG mRNA is increased in the disease. There were no significant differences in the levels of IL13. The increases in CXCL9 and CCL5 protein and decrease in IFNG protein are similar to reports of cytokine and chemokine protein levels in human patients suffering from AA, supporting the use of the C3H/HeJ model for the study of AA.

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A subpopulation of CD163 positive macrophages is classically activated in psoriasis

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Macrophages are important cells of the innate immune system, and their study is essential to gain greater understanding of the inflammatory nature of psoriasis. We used immunohistochemistry and double-label immunofluorescence to characterize CD163+ macrophages in psoriasis. Dermal macrophages were increased in psoriasis compared to normal skin and were identified by CD163, RFD7, CD68, LAMP2, Stabilin-1, and MARCO. CD163+ macrophages expressed C-lectins CD206/MMR and CD209/DC-SIGN, as well as co-stimulatory molecules CD86 and CD40. They did not express mature DC markers CD208/DC-LAMP, CD205/DEC205 or CD83. Microarray analysis of in vitro derived macrophages treated with IFNg showed that many of the genes upregulated in macrophages were found in psoriasis, including STAT1, CXCL9, Mx1 and HLA-DR. CD163+ macrophages produced inflammatory molecules IL-23p19 and IL-12/23p40 as well as TNF and iNOS. These data demonstrated that CD163 is a superior marker of macrophages, and identify a subpopulation of "classically activated" macrophages in psoriasis. We conclude that macrophages are likely to be contributing to the pathogenic inflammation in psoriasis, a prototypical Th1 and Th17 disease, by releasing key inflammatory products.

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Myeloid cells, and not T cells, are the main source of TNF-α in plaque-type psoriasis

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The spectrum of tumor necrosis factor (TNF)-α-producing cells is not clearly defined in psoriasis. The elucidation of this question should allow us to better understand the mode of action, efficacy and, perhaps, also the risks of an anti-psoriatic therapy with TNF-α-antagonists. Using conventional immunofluorescence methods, we were not able to detect TNF-α in sections of lesional psoriatic skin, but by the application of a tyramide amplification system we obtained reproducible and firm stainings. TNF-α was exclusively found on dermal leukocytes coexpressing CD11c and HLA-DR and, to a lesser extent, CD163. This marker profile is consistent with that of mDCs and macrophages. We did not find TNF-α colocalization on mast cells (CD117+), T cells (CD3+), neutrophils (CD15+HLA-DR-), endothelial cells (vWF+), pDCs (BDCA-2+) or Langerhans cells (langerin+). Consistently, we found corresponding populations of TNF-α-producing mDCs and monocytes in unstimulated PBMCs of psoriatic patients. More importantly, their number closely correlated with disease activity. In healthy persons, anti-TNF-α-stainings of skin and blood yielded essentially negative results. *In vitro*, we confirmed that TNF-α-antagonists are able to induce apoptosis in, as well as complement killing and antibody-dependent cellular cytotoxicity of TNF-α producing cell lines. *In vivo*, infliximab therapy reduced the number of TNF-α-producing cells in the peripheral blood of psoriatic patients 24 hours after administration. Our data strongly suggest that myeloid cells (dendritic cells, monocytes/macrophages) are the main source of TNF-α in stable plaque-type psoriasis. This highlights the importance of these cells in disease pathogenesis.

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Signal transduction triggered by IgE class autoantibodies in bullous pemphigoid

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Recently, our laboratory has identified a pathogenic role for BP180-specific IgE autoantibodies in bullous pemphigoid (BP). This pathogenicity has been attributed to the degranulation of skin mast cells after autoantibody binding to the high affinity IgE receptor. However, we have also shown that treatment of cultured keratinocytes with IgE purified from BP sera results in increased secretion of IL-6 and IL-8. To determine which signal transduction pathways were responsible for this modulation of cytokine production in response to IgE, we treated primary cultures of human keratinocytes with BP or control IgE for 0.5, 1, 2, 4, 8 and 20 hr and evaluated cell lysates for the relative phosphorylation of a panel of signaling molecules linked to IL-8 production using a BioPlex. IL-6 and IL-8 were measured in culture supernatants by ELISA. As expected, treatment of keratinocytes with BP, but not control, IgE increased secretion of IL-8 (from 2-20 hr) and IL-6 (from 8-20 hr). The BioPlex revealed that the phosphorylated form of specific signaling molecules were elevated at either early (0-2 hr), intermediate (2-8 hr) times after BP IgE addition, or remained unchanged Specifically, phospho CREB, HSP27, and p38 MAPK were elevated early, c-JUN, ERK1, ERK2, MEK1, and TYK2 were increased at the intermediate time points and STAT3 and STAT6 were not significantly increased. The mainstay of BP therapy remains global immune suppression which is associated with a high rate of morbidity. Identification of the signaling events triggered by autoantibody binding will be useful in the development of targeted therapies, which would significantly improve patient care.

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Identification of cell-restricted gene expression profiles or cellular "barcodes" to de-code complex skin pathology

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Normal human skin contains many types of resident cell types, as well as many types of immunocytes that are present as either resident or re-circulating cell populations. Inflammatory and neoplastic skin diseases frequently have expanded populations of different cell types, but it is rare that only 1 type of cell is affected in a given condition. For example, immune-related skin diseases have increased immunocytes with altered differentiation compared to resident populations and epidermal hyperplasia is frequently associated with inflammation. Conversely, neoplasms of keratinocytes often have reactive immune infiltrates in the skin. While traditional definitions of skin diseases are based on visible cell populations in tissue sections, a new classification of skin diseases could be based on specific alterations in gene expression profiles in these diseases. However, one is faced with the need for core identification of specific cell populations by gene expression measures, as well as potential alterations in gene expression that may be driven by the underlying pathology. We now present a set of cellular barcodes, generated from analysis of global gene expression on Affymetrix U133 2.0+ arrays, which can be used to define the presence of many distinct cell types within human skin biopsies. Our working set of barcodes now includes the ability to distinguish endothelial cells, melanocytes, fibroblasts, macrophages, immature or mature dendritic cells and activated or unactivated T-cells, from epidermal keratinocytes. Examples of de-coding complex skin diseases such as psoriasis or skin cancers will be presented.

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Development of protein microarrays to investigate autoantibody profiles in pemphigus vulgaris

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In addition to the desmosomal glycoprotein desmoglein (Dsg)3, a number of other skin-associated protein targets have been considered to have a possible role in Pemphigus vulgaris (PV), but no clear disease related autoantibody profile has been established. The purpose of our study was to establish comprehensive autoantibody profiles in PV patients by utilizing novel protein microarray technology. Seventeen putative PV-associated autoantigens were printed on glass microarray slides and incubated with serum samples from PV patients and healthy controls. Slides were then incubated with biotinylated anti-human Ig to detect the presence of autoantigen-specific reactivity. The absolute fluorescence intensity was determined using a TECAN scanner and Genepix 5.0 software. To validate the applicability of our technique, we first compared protein array data with standard ELISA protein detection. We found a high array/ELISA correlation for both the anti-Dsg1 ($R^2 = 0.83$) and -Dsg3 ($R^2 = 0.77$) response. In addition to a significantly increased reactivity in the serum of active PV patients vs. healthy controls for Dsg1 and -3, we also find significant reactivity for several non-desmoglein targets including muscarinic acetylcholine receptor (mAChR)1, mAChR2, mAChR4, mAChR5, pemphaxin, and thyroid peroxidase ($p<0.05$, Student's t-test). The functional significance of autoimmune reactivity to non-desmoglein targets in PV remains to be determined. These data are relevant for our further understanding of the specificity of the autoimmune response in PV and offer insights into disease mechanisms. Determining comprehensive autoantibody profiles in patients may facilitate improved systems for diagnosis and ultimately more individualized and effective treatments in the future.

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Analysis of anti-desmoglein 1 autoantibodies in 76 healthy mother/neonate pairs from a highly endemic region of Fogo Selvagem in Brazil

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We have determined that the Amerindian reservation of Limao Verde (LV), Brazil shows a 3% prevalence of Fogo Selvagem (FS), an endemic phenotype of pemphigus foliaceus (Hans, JID, 107:68, 1996). The healthy population of LV exhibits a high prevalence of IgG and IgM anti-Dsg1 autoantibodies (Diaz, JID, 128:667, 2008); however, IgG4 subclass anti-Dsg1 autoantibodies are predictors of FS (Qaquish, JID, 129:110, 2009). It has been hypothesized that the triggering antigen(s) is harbored within LV and decreases in communities distant from this settlement. The purpose of this investigation was to determine the anti-Dsg1 response in a cohort of 76 healthy Terena mother/neonate pairs attended in a hospital of Aquidauana, Brazil, which is located at 30 kilometers from LV. The sera were tested by indirect immunofluorescence (IF) and ELISA using recombinant Dsg1. We tested IgG, IgG1, IgG2, IgG3, IgG4 and IgM anti-Dsg1 response in these individuals. Sera from the 76 pairs were negative by indirect IF. There were 13 mothers (17%) and 11 neonates (14.4%) that showed positive total IgG Dsg1 ELISA. Only two healthy mothers (2.6%) and one normal neonate (from one of these mothers)(1.3%) exhibit positive IgG4 anti-Dsg1. The mother/neonate pair with positive IgG4 anti-Dsg1 ELISA is under continuous surveillance for the development of FS. IgM anti-Dsg1 autoantibodies were found in 12 mothers (15.7%) and none of the neonates (0%). Mother/neonate sera showed no differences on IgG1, IgG2 & IgG3 anti-Dsg1 antibodies. These results suggest that the immunization process leading to FS in endemic regions of Brazil begins in the post neonatal period of life, i.e. early childhood.

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Therapeutic removal of amyloid deposits by anti amyloid antibodies

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Amyloidoses are a large group of conformational diseases in which protein aggregates accumulate either systemically or locally in certain organs or tissues. Primary cutaneous amyloidosis is a local amyloidosis in which amyloid deposits are restricted to the skin. Patients usually present with itching, hyperkeratotic papules and plaques, skin hyperpigmentation and lichenification, or in the most severe, rare variant, with waxy nodules that can ulcerate and hemorrhage, resulting in disfiguration. Several treatments have been proposed for localized cutaneous amyloidosis that improves cosmetic appearance of the lesions; these include surgical excision, carbon dioxide laser, cryotherapy, dermabrasion, curettage, and electrocoagulation, but none has been demonstrated effective to date. In this study, we were able to characterize amyloid aggregates in the skin nodules from cutaneous amyloidosis patients, using conformation specific antibodies. We show for the first time that both amyloid fibrils and oligomers are present in these deposits, in addition, we reproduced cutaneous amyloidosis with similar deposits in mice by intra-dermal administration of amyloid enhancing factor (AEF). Moreover we demonstrated that conformational antibodies were effective in clearing amyloid deposits caused by localized intralesional injections without the necessity of an immune response. Given the accessibility and amyloid localization in this disease, direct intra-dermal injections of conformational antibodies could be a convenient and direct method for treatment.

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Neutrophil elastase cleves BP180 and its degradation products are chemotactic and therapeutic in skin autoimmune blistering disease in mice

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Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease associated with autoantibodies against the hemidesmosomal proteins BP180 and BP230. In IgG passive transfer model of BP, blister formation is triggered by anti-BP180 IgG and depends on complement activation, mast cell degranulation, and neutrophil recruitment. Mice lacking neutrophil elastase (NE) are protected from experimental BP. Here, we demonstrated that NE degrades the recombinant BP180 within the immunodominant extracellular domain at amino acid positions 506 and 561, generating peptides p506 and p561. The peptide p561 is chemotactic for neutrophils both *in vitro* and *in vivo*. Local injection of NE into B6 mice recruits neutrophils to the skin, and neutrophil infiltration is completely blocked by co-injection with the NE inhibitor α1-proteinase inhibitor. The BP180 peptide containing p506 NE cleavage site shows no chemotactic activity, but, inhibits neutrophil infiltration and protects mice from subepidermal blistering. These results suggest that NE is critical to development of BP through both directly damaging the extracellular matrix and generating neutrophil chemotactic peptides from BP180. Non-chemotactic NE-cleavage peptides may be therapeutic for NE-mediated inflammatory and autoimmune diseases.

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Human 6-Sulfo LacNAc+ (slan) dendritic cells are powerful inducers of Th17/Th1 T cells and prevail among inflammatory dermal dendritic cells in psoriatic skin

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Psoriasis is an autoinflammatory skin disease that is considered to result from the activity of Th17/Th1 T cells stimulated by inflammatory dermal dendritic cells (DCs). The classification and origin of these inflammatory DCs is still elusive. In this study we identify DCs that bear the selective marker 6-sulfo LacNAc (slanDCs) as a major proinflammatory DC type in psoriatic skin lesions expressing IL-23, TNF-α and iNOS. Their local recruitment may have been induced into psoriatic skin lesions by their preferential expression of functional CX3CR1 and C5aR. Previously, we reported on slanDCs to have a marked production of IL-12 and TNF-α. When compared to classical CD1c+ blood DCs, slanDCs are by far more powerful in programming Th17/Th1 T cells that secrete IL-17, IL-22, TNF-α and IFN-γ and less effective in inducing IL-10-producing T cells. Our finding that slanDCs are a principal source of IL-23, IL-6 and IL-1β may explain their efficiency in programming Th17 cells. Thus, an ongoing stimulation of Th17/Th1 cells by proinflammatory slanDCs may critically contribute to the chronic inflammation in psoriatic skin.

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Mechanisms of pruritus: Potential relationship between IL-31, IFN-γ, cathepsins and protease-activated receptors

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IL-31 and IFN-γ are cytokines associated with inflammation and pruritic conditions including atopic dermatitis and prurigo nodularis. The link between these cytokines and itch is not clear. Cathepsins can elicit itch and signal via protease-activate receptors, the activation of which has been linked to pruritus. We have used qRT-PCR to examine the induction of cathepsin mRNA following cytokine stimulation of normal human keratinocytes. Stimulation with IL-31 resulted in the 5- and 75-fold induction of cathepsins B and G respectively. Stimulation with IFN-γ resulted in the 6- and 7-fold induction of cathepsins G and S respectively. These results are consistent with the hypothesis that cytokines induce the expression of cathepsins that serve as mediators of itch via interaction with protease-activated receptors on keratinocytes or sensory nerves. Cathepsins or their receptors may be therapeutic targets for the treatment of conditions associated with inflammation, including pruritus.

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IgG4 and IgE anti-Dsg1 auto-antibodies recognize Lutzomyia longipalpis salivary gland antigens

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The endemic phenotype of pemphigus foliaceus, Fogo Selvagem (FS), is thought to be precipitated by an environmental antigen(s) harbored in certain endemic states of Brazil. Anti-desmoglein 1 (Dsg1) IgM, IgG and IgE autoantibodies are present not only in FS patients but also in normal individuals living in FS endemic regions (Diaz, JID, 128:667, 2008 & Qaquish, JID, 129:110, 2009). Hypervariable V genes of FS autoantibodies indicate that they are antigen selected, and this selection occurs even before the onset of clinical FS (Qian, JID, 129:2823, 2009). These studies strongly suggest the presence of an inciting environmental antigen(s) in FS endemic regions. It has been hypothesized that hematophagous insects involved in transmission of parasitic diseases such as Chagas disease, onchocercasis and leishmaniasis may be associated with the etiology of FS (Diaz, JID, 123:565, 2004). The purpose of this study was to test if salivary gland antigens from Lutzomyia longipalpis (SG-LL), the vector of Leishmania, react with FS autoantibodies. Soluble antigens from SG-LL were tested by ELISA for reactivity with FS IgG4 and IgE autoantibodies and two human monoclonal IgG4 antibodies derived from FS patients (Qian, JID, 129:2823, 2009). A significant number of sera from active FS patients possess anti-SG-LL IgG4 (96%) and IgE (48%) antibodies as compared with normal controls from non-endemic areas (15% and 6% respectively). Moreover, the two IgG4 monoclonal antibodies also reacted with SG-LL antigens. Importantly, this reactivity was blocked in a dose-dependent manner by human recombinant Dsg1 ectodomain. These findings suggest that insect bites may deliver allergens that drive the production of cross-reactive anti-Dsg1 antibodies.

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Isoprenylcysteine (IPC) analogs suppresses 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced cytokine production in primary human keratinocytes and mouse skin *in vivo*

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Reduction of epidermally derived inflammatory cytokines contributing to pathophysiological mechanisms associated with various inflammatory dermatoses has been proposed as a therapeutic strategy. Isoprenylcysteine (IPC) analogs including N-acetyl-S-farnesyl-L-cysteine (AFC) are structural mimics of the lipidated C-termini of the Gγ subunit of all heterotrimeric G proteins, as well as that of small GTPases such as Ras, Rho and Rac. G protein coupled receptors participate in eliciting inflammatory responses such as the release of proinflammatory mediators, and the migration and activation of inflammatory cells. We previously demonstrated using the *in vivo* TPA-induced acute contact irritation mouse model that topically applied AFC is comparable to corticosteroids in its anti-inflammatory efficacy (Gordon et al, 2008, JID, 128: 643). Chemical modifications to the structure of AFC yielded 2nd generation IPC analogs with a substantially higher potency in the acute irritation mouse ear model. In this study we investigated if IPC analogs inhibit TPA induction of keratinocyte-derived cytokines. Cultured primary normal human keratinocytes (NHEKs) exposed to TPA upregulated the production of IL-6, IL-8, TNF-α, and IL-12. IPC analogs dose-dependently inhibited production of each of these chemokines/cytokines in the micromolar range at non-cytotoxic doses with a particular potency towards IL-8 and TNF-α inhibition. When topically applied, IPC analogs inhibited TPA induction of these cytokines in the skin with the similar potency at which they were active *in vitro* and reduced edema, neutrophil infiltration and erythema, suggesting an excellent *in vitro* versus *in vivo* correlation. Overall, these results indicate IPC analogs represent a novel pharmaceutical class of therapeutic small molecules to treat inflammatory skin diseases through its anti-cytokine effects.

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The psoriasis associated IL12B risk haplotype influences IL-12 and IL-23 expression and secretion

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To date three variants in the IL-23 cytokine axis; IL12B, IL23A and IL23R, have been shown to increase the risk of psoriasis. Although these variants individually increase the risk of psoriasis by the order of 1.3 to 1.6-fold the biological mechanisms behind this are unknown. The aim of this study was to address how the IL12B associated risk variants influence the risk of psoriasis and the biological mechanisms involved. We identified 36 individuals who are either homozygous carriers or non-carriers of the IL12B risk haplotype AGG (SNPs rs3212227, rs6887695 and rs20822412) (n=20 and n=16 respectively). Monocytes were isolated from peripheral blood using negative selection and purity was evaluated by FACS. Monocytes were maintained in growth medium for 72 hours with or without IFN-γ and subsequently stimulated with varying doses of LPS for 12, 24 and 48 hours. IL12B risk allele carriers had significantly higher expression of IL12B mRNA (which encodes the p40 subunit of IL-12 and IL-23, 6.7-fold, $p<0.05$), whereas non-carriers expressed significantly higher levels of the IL12A mRNA (which encodes the p35 subunit of IL-12, 1.9-2.8 fold, $p<0.001$). IL-12 and IL-23 cytokine levels were consistent with QRT-PCR, showing that the IL12B risk allele carriers produced higher levels of IL-23 (3.2-fold, $p<0.001$) and lower levels of IL-12 (1.5-3.8-fold, $p<0.05$) compared to non-carriers. There was no difference in the expression of IL-1beta mRNA between the two groups. Interestingly, these changes were more pronounced in the IFN-γ pre-treated monocytes compared to non-treated monocytes. Given the role of IL-12 and IL-23 in the polarization and generation of Th1 and Th17 cells, our data indicate that the IL12B risk allele may cause a shift away from a Th1-priming towards Th17 priming, particularly in an IFN-γ rich environment. These data integrate genetic and functional data on IL12B for the first time and emphasize the importance of the IL-23 axis in psoriasis pathogenesis.

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Generation of a hyper iNOS-expressing macrophage leads to a severe delay in wound healing

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Excessive nitric oxide (NO) leads to local tissue damage and has been implicated in numerous destructive inflammatory diseases. Macrophages (MΦ), cellular responders to injury capable of NO production via induction of inducible NO synthase (iNOS), are significant effector cells mediating inflammation and tissue repair. Using *in vitro* LPS stimulated bone-marrow-derived MΦ, we generated a polarized phenotype of hyper iNOS-expressing (HiNOS) MΦ through activation of PPAR-γ receptors in the absence of IL-6. To determine the function of HiNOS MΦ *in vivo*, we developed an excisional wound healing model in IL-6-/- mice treated with a PPAR-γ agonist under inflammatory conditions. We hypothesized that accumulation of HiNOS MΦ at wounds will result in local tissue destruction leading to delayed wound repair. Indeed, we found a 30-fold increase in iNOS expression by RT-PCR from wounded skin of IL-6-/- mice compared to controls (n=3, $p<0.001$). Triple-color confocal microscopy demonstrated co-localization of intense iNOS staining with cells expressing both MΦ markers CD11b and F4/80 (n=3). IL-6-/- mice under experimental conditions demonstrated: dramatic enlargement of wounds up to 136+4% of the initial size between 0-2 days; failure to initiate wound healing with sustained enlarged wounds for 7 days; delayed complete re-epithielialization compared to controls (23 vs. 12 days, n=15, $p<0.001$). Administration of a blocking antibody (anti-CD11b) targeting monocytes and MΦ resulted in decreased skin iNOS expression by RT-PCR (n=3, $p<0.001$), decreased numbers of iNOS+ MΦ by immunostaining (n=3, $p<0.001$), and most importantly restored wound healing to 12 days (n=3, $p<0.001$). Finally, treatment with a specific iNOS inhibitor (1400W), prevented wound enlargement between days 0-2 and restored animals to a normal healing curve demonstrating the criticality of iNOS in tissue destruction (n=4, $p<0.001$). In summary, our data demonstrate a novel mechanism of delayed wound repair mediated by HiNOS MΦ which suggests a role for HiNOS MΦ in destructive inflammatory diseases.

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A non-redundant role for HSP70i in autoimmune depigmentation

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In vitiligo, cytotoxic T lymphocytes mediate autoimmune depigmentation, and stress has been postulated to accelerate the process by means of HSP70i expression. Indeed HSP70i accelerates depigmentation in mice vaccinated with melanocyte antigens. Here, we investigated whether HSP70i is necessary or redundant in the depigmentation process to establish HSP70i as a potential target for vitiligo therapy. In duplicate experiments, groups of 10 wildtype and hsp70-1 knockout mice were gene gun vaccinated weekly for 5 weeks with 6.4 μg plasmid DNA encoding modified TRP-1 with heteroclytic epitopes designed to stimulate CTL activity, in presence or absence of HSP70i encoding DNA. Depigmentation was evaluated by scanning and image analysis 3 weeks after the final vaccination. Involvement of CTL activity was measured by modified *in vivo* cytotoxicity assays using splenocytes pulsed with TRP-1 and TRP-2 derived peptides to simultaneously investigate epitope spreading. Wildtype mice displayed a 5-fold increased depigmentation as compared to knockout mice in response to TRP-1, which was not restored by addition of HSP70i. In addition, an increase in cytotoxicity was observed towards TRP-1 pulsed splenocytes in the absence (26%) and presence (60%) of HSP70, comparing wildtype to knockout mice. Significant epitope spreading towards TRP-2 was detectable in all vaccinated mice. In a separate experiment, depigmentation was not significantly reduced in mice knocked out for constitutive HSP70. In conclusion, inducible HSP70 appears to play a unique and crucial role in autoimmune depigmentation and may be a suitable target for preventing depigmentation.

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Epidermal depigmentation in a mouse model of vitiligo is IFNγ-dependent and associated with local chemokine expression

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The goal of this study was to develop a mouse model of vitiligo with isolated epidermal depigmentation (akin to the human disease) and to determine the local factors that contribute to disease progression. Vitiligo is an autoimmune skin disease defined clinically by patchy depigmentation and histologically by the absence of melanocytes. Disease progression is associated with circulating and lesional melanocyte-specific cytotoxic T lymphocytes (CTL). Lesional CTL have the capacity to destroy melanocytes in non-lesional skin *ex vivo*. Existing mouse models of vitiligo consist of hair depigmentation, a characteristic not consistent with human disease. Here, we have developed a new mouse model of vitiligo that results in epidermal depigmentation with sparing of hair pigmentation. Using a transgenic host with increased epidermal melanocytes, depigmentation occurs 5-7 weeks following sublethal irradiation of the host, adoptive transfer of melanocyte-specific CTL and activation *in vivo* with recombinant vaccinia virus expressing CTL target antigen. Depigmentation in this model requires IFNγ, as neutralization *in vivo* abrogates disease. In addition, the IFNγ-dependent chemokines CXCL9 (MIG) and CXCL10 (IP-10) are induced at high levels in lesional skin as disease progresses. IFNγ neutralization prevents both CTL accumulation in the skin as well as CXCL9 and CXCL10 expression. In conclusion, we have developed the first mouse model of vitiligo with isolated epidermal depigmentation. Using this model, we have determined for the first time that vitiligo disease progression requires IFNγ and correlates with expression of the IFNγ-dependent chemokines CXCL9 and CXCL10. IFNγ neutralization abrogates depigmentation, CTL accumulation in the skin, and local expression of CXCL9 and CXCL10. These results provide evidence that IFNγ influences the ability of CTL to accumulate in the skin, likely through local chemokine secretion.

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Abnormal DNA methylation in peripheral blood mononuclear cells and skin lesions from patients with psoriasis vulgaris

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Aberrant DNA methylation has been demonstrated to be associated with autoimmune diseases, such as systemic lupus erythematosus. However, it is unknown whether global DNA methylation levels are altered in patients with psoriasis, a T-cell mediated autoimmune disease, is characterized by keratinocyte hyperproliferation. Using an enzyme-linked immunosorbent assay-like reaction with a 5-methylcytosine antibody to detect methylated DNA, we present evidence to show that global methylation levels are increased in peripheral blood mononuclear cells (PBMCs) of patients with psoriasis vulgaris. Immunohistochemical analysis also revealed that DNA is hypermethylated within sites of psoriatic skin lesions, and the degree of hypermethylation in psoriatic skin tissue samples positively correlates with the patient's Psoriasis Area and Severity Index (PASI) score ($p< 0.05$). Furthermore, we also found that transcription levels of the DNA methylating enzyme DNMT1 were upregulated in PBMCs from psoriatic patients compared with healthy controls, whereas the methylated DNA-binding transcriptional repressors MBD2 and MeCP2 were downregulated. In addition, the genomic locus encoding tumor suppressor gene p14 was hypermethylated and p14 mRNA expression was decreased in psoriatic skin lesions compared with normal skin tissue.These data suggest that aberrant regulation of DNA methylation in PBMCs and within skin lesions contributes to the pathogenesis of psoriasis.

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CD 161+ NKT or NKT-like cells are major producers of IL-17 in psoriasis skin and blood

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A recent study indicated that circulating Th17 cells in human blood originate from CD161+ precursor cells in umbilical cord blood. This study demonstrated that IL-17+ T cells in human blood express CD 161, a marker of NKT and NKT-like cells, and that IL-17 in inflammatory diseases is produced largely by CD161+ NKT-like cells. However, the relative prevalence of CD161+ cells in normal appearing skin and lesional skin of psoriasis remains unquantified, as does their relative role in IL-17 production. We isolated lymphocytes from blood, uninvolved skin, and lesional skin from psoriasis patients and controls and analyzed IL-17, CD3, CD4, CD8, and CD161 expression by intracellular flow cytometry without *in vitro* culture. CD 161 positive T cells were present in increased numbers in psoriasis lesions, compared to normal-appearing skin. In psoriasis lesions up to one third of CD4+CD161+ NKT cells produce IL-17, compared to less than 10% of CD4+CD161+ T cells in normal skin. Indeed a majority of CD4+IL-17+ or CD8+IL-17+ T cells in psoriasis lesions express CD161. In contrast, only a minority of IL-17+ T cells in normal-appearing skin are CD161+. Many CD161+ T cells in normal appearing skin and psoriasis skin and blood also produced IFN-γ with IL-17 or IFN-γ alone. Analyzed without *ex vivo* culture, our findings reveal that a majority of IL-17+ T cells from normal skin, psoriasis lesions, and blood of humans express CD161. While the function of CD161 on these cells remains unknown, further study of this protein on this population of cells indicated, especially in diseases like psoriasis where IL-17 plays a key role in pathology.

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IL-1F5, F6, F8, and F9 represent a novel IL-1 signaling system which is active in psoriasis and promotes keratinocyte antimicrobial peptide expression

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Four new members of the IL-1 family have recently been identified: pro-inflammatory IL-1F6, -F8 and -F9 and the IL-1R6 receptor antagonist IL-1F5. These constitute a novel IL-1 signaling system which is poorly understood in skin. Overexpression of IL-1F6 in IL-1F5 knockout mice leads to a psoriasiform phenotype and expression of these two cytokines is elevated in psoriatic skin. We hypothesized that these IL-1 members play key roles in psoriasis pathogenesis. To test our hypothesis, we assessed the expression of all IL-1 cytokines in healthy control (NN), uninvolved psoriasis (PN) and psoriasis plaque (PP) skin using QRT-PCR. Expression of IL -1F5, -F6, -F8, and -F9 were increased 2-3 orders of magnitude in PP vs PN skin ($p<.001$, all) which was supported immunohistologically, and treatment of psoriasis with etanercept led to significantly decreased IL1-1F5($p=.009$), -F6($p<.0001$), -F8($p<.0001$) and -F9($p<.0001$) mRNA concomitant with clinical improvement. Similarly increased IL-1F5, -F6, -F8 and -F9 was seen in the involved skin of KC-Tie2 mice. To understand the induction of these cytokines in psoriasis, we treated normal human keratinocytes (NHK) with IL-1α, IL-17A, IL-22 and TNF-α. IL-1α and TNF-α induced 4 to 10-fold increases in IL-1F5, -F6, -F8, and -F9 mRNA. IL-17A induced robust IL-1F6 and F9 expression ($p<.05$) and while IL-22 alone was ineffective, it augmented the effects of IL-17A by 6 and 2-fold respectively ($p<.01$). Although IL-1α could drive robust increases in IL-1F9 mRNA and intracellular protein, ATP treatment was required for IL-1F9 secretion, suggesting that like IL-1α, IL-1F9 uses a non-classical secretion pathway . With regard to effector function, IL-1F6 induced NF-κB activation in monocytes and treatment of NHK with IL-1F8 increased HBD2 mRNA 18-fold($p=.0001$) and protein 6-fold($p=.008$). These results suggest important roles for these novel cytokines in the pathogenesis of psoriasis.

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Partial evaluation of pemphigus vulgaris (PV) autoantibody profile using the protein array technology

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Autoantibodies can occur in healthy individuals and patients with autoimmune disorders. In autoimmune disorders, autoantibodies can damage the target cells and/or serve as biomarkers providing an opportunity for diagnosis and therapeutic intervention. PV is an autoimmune blistering dermatosis associated with both organ specific and nonspecific antibodies. While organ-specific autoantigens, such as desmogleins 1 and 3, are well characterized, a little is known about the specificities of nondesmoglein autoantibodies. In this study, we employed the protein array technology platform for autoimmunity screening of sera from 7 PV patients and 5 healthy controls. The sera were probed for the presence of autoantibodies characteristic of rheumatoid arthritis, lupus erythematosus, scleroderma, diabetes and some other autoimmune disorders. Our array targeted 850 human genes amplified using Mammalian Gene Clone Collection with gene specific primers containing 20 bp nucleotide extension complementary to ends of linear pXT7 vector. Fusion proteins tagged with hemagglutinin and polyhistidine were expressed by in vitro transcription translation assay, printed without further purification, and recognized by monoclonal anti-polyhistidine and anti-hemagglutinin antibodies. The array identified 15 antigens significantly differentially reactive with PV sera, including M1 muscarinic acetylcholine receptor, consistent with the facts that blocking of this receptor can cause keratinocyte detachment, and its activation by pilocarpine is therapeutic for PV patients. Both PV and normal sera were also highly reactive with 37 antigens representing the targets for natural antibodies. Thus, using the protein array technology platform of human autoimmunity screening we identified novel subsets of autoantibodies in PV patients that may serve as potential biomarkers and guide future studies of PV pathophysiology.

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Therapeutic efficacy of defensamide in atopic dermatitis

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Increased susceptibility to the microbial infection is an important clinical feature of atopic dermatitis (AD). Among the various underlying factors, reduced expression of epidermal anti-microbial peptides is considered as a major factor. Recent development of epidermal AMPs-stimulating compound provides a new therapeutic approach for AD. In an oxazolone-induced AD animal model, topical application of defensamide, which stimulate the AMPs expression in epidermis, showed significant improves in AD-like symptoms, such as skin barrier functions and epidermal hyperplasia. In this study, we have evaluated the therapeutic efficacy of defensamide for AD. The clinical studies were performed in two-stages and in all the trials, patients were directed to use it as much as needed. In order to evaluate the therapeutic improvement, SCORAD index, as well as objective and subjective satisfaction was evaluated. Skin barrier functions were also evaluated by measuring the trans-epidermal water loss (TEWL) and skin surface hydration, respectively. In the first stage, topical formulation containing 0.1% defensamide was provided to 10 AD patients in an open-labeled manner. As a result, significant improvement was observed in all the evaluated parameters. In the second stage, double-blinded randomized-control trials were performed. 20 patients were randomly allocated into two groups: 10 patients for control group and 10 patients for sample group, respectively. Vehicles were provided to control group and 0.1% defensamide containing products were provided to sample group. In addition to the above mentioned parameters, S. aureus colonization in lesional area was also measured. As a result, significant reduction in the number of S. aureus was observed in sample group. These results suggest that AMPs simulating compound can be a promising therapeutic agent for AD.

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Prevalence of cardiovascular risk factors and obstructive sleep apnea in patients with psoriasis and psoriatic arthritis

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Patients with psoriasis (Ps) and psoriatic arthritis (PsA) often have poor sleep quality. Recently, sleep deprivation and obstructive sleep apnea (OSA) have emerged as independent risk factors for obesity, diabetes, hypertension, and cardiovascular (CV) disease, all considered co-morbidities of Ps and PsA. To explore this association, patients with uncontrolled Ps or PsA who were candidates for systemic therapy were enrolled. Medical history, PASI, BSA, joint assessments and home overnight polysomnography were done at baseline. Of the 15 patients enrolled, 12 had Ps, and 3 also had PsA. Eight (53%) met criteria for OSA (apnea-hypopnea index [AHI] of ≥15 or AHI 5-14.9 with secondary diagnosis of daytime sleepiness, impaired cognition, mood disorders, insomnia, hypertension, ischemic heart disease or stroke). All 3 patients with PsA met criteria for OSA. Mean age in the non-OSA group was 39, vs. 45 in the OSA group (p=0.49). There were no differences in mean PASI score (12.3 vs. 13, p=0.91), BSA (18.9% vs. 16%, p=0.79), or DLQI (14.0 vs. 10.4, p=0.45) in the non-OSA vs OSA groups, respectively. The mean BMI of the non-OSA group was 27.5, compared to 36.2 (p=0.005) in the OSA group. Rates of hypertension (14.3% vs. 50%, p=0.28), diabetes (14.3% vs. 37.5%, p=0.57), and smoking (57% vs. 63%, p=1.00) (Fisher's exact) trended to being higher in the OSA group but were not significant. We conclude that our patients have a high prevalence of OSA compared to 2-4% in the general population. Non-significant differences in other CV risk factors were due to limited power. Enrollment of Ps patients and age, gender, and BMI-matched controls is ongoing to compare CV risk and serum markers of inflammation in these groups.

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WITHDRAWN

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αEβ7 promotes skin inflammation, but is not necessary for leukocyte trafficking, in a mouse model of atopic dermatitis

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Integrins are cell surface heterodimeric receptors that mediate leukocyte migration and activation. αEβ7 is an integrin expressed on T lymphocytes within epithelial tissues, as well as dendritic cells (DCs), and is upregulated in inflammatory states including human atopic dermatitis (AD). Its interaction with E-cadherin on epithelia is thought to mediate tissue localization, but a role in cellular activation has not been excluded. Because αEβ7 appears to be preferentially expressed by CD4+FoxP3+ T regulatory cells, particularly in both steady-state and inflamed skin, we hypothesized that it may play a role in suppressing inflammation. We used an ovalbumin-based mouse model of AD to evaluate the expression patterns and role of this integrin in skin inflammation. CD103 (αE subunit) expression in skin-draining lymph nodes (SDLNs) and skin was determined by flow cytometry and immunofluorescent staining, respectively. Diseased mice showed an increase in CD103+ T cells in both SDLNs and inflamed skin, and this expression correlated with degree of inflammation as determined by inflammatory cell profile and cytokine expression, serum IgE, and histology. Furthermore, while only ~50% of infiltrating CD4+ effector T cells in the skin were CD103+, the majority of CD4+FoxP3+ T regulatory cells demonstrated expression. To determine the functional significance of this upregulated expression, we next evaluated inflammation in CD103-/- mice. Contrary to our hypothesis, in the absence of αEβ7, inflammation was significantly dampened. In addition, tissue localization of CD4+ effector T cells, CD4+FoxP3+ T regulatory cells, and DCs was intact, although the infiltrates were reduced in proportion to the inflammatory response. These findings suggest (i) that preferential expression of αEβ7 on T regulatory cells does not serve to promote their function but either serves in an inhibitory capacity for these cells or plays a more dominant role in regulating other effector cell types to promote inflammation; and (ii) that αEβ7 does not play a primary role in leukocyte trafficking.

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Non invasive *in vivo* small animal Positron Emission Tomography (PET) imaging of Th1 cell trafficking in mouse models for asthma and contact allergy using [64Cu]PTSM labelling

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To get a better understanding about T cell migration kinetics, homing or proliferation in inflammation, high sensitive multimodality imaging is an important tool for preclinical studies. The goal of our studies was to visualize the migration properties of ovalbumin-T cell-receptor transgenic T-helper cells (OVA-Th1) in an experimental model for lung inflammation and contact allergy. We used the lipophilic radioactive marker [64Cu]PTSM as intracellular label of the Th1 cells for *in vivo* PET-imaging. BALB/c mice were sensitized at the abdomen with the hapten TNCB and one week later, cutaneous DTHR was elicited at the right ear. To induce OVA-specific lung inflammation mice were sensitized with OVA (i.p.) and challenged intranasal twice four weeks later to induce lung inflammation. Static PET-scans, CT-scans, autoradiography, and biodistribution-analysis were performed 24 and 48 hours after OVA-Th1 cell injection. Analysing T cell trafficking in contact allergic mice we detected a strongly enhanced [64Cu]Th1 cell accumulation in the cervical draining lymph node of the TNCB-treated right ear compared to the lymph node of the untreated left ear. *Ex vivo* FACS-analysis confirmed accumulation of OVA-Th1 cells in the draining right cervical lymph node. Analysing OVA-specific Th1 cell migration in lung inflammation we detected accumulating [64Cu]OVA-Th1 cells in lung tissue already 24h after the final challenge. Data were further confirmed *ex vivo* by biodistribution and autoradiography, 24h and 48h after OVA-challenge. Thus, non invasive *in vivo* investigation of T cell trafficking using [Cu-64]PTSM labelled T cells and PET provides an important tool gain important information about the mode of T cell trafficking in T cell mediated autoimmune diseases.

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A cohort study of skin cancer risk by immunosuppressive drug type in solid organ transplant patients

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Previous studies have shown an increased risk of skin cancer in solid organ transplant recipients. However, the risk with different immunosuppressive drugs or regimens is unknown. The objective of this study was to analyze skin cancer risk by type and cumulative dose of commonly used drugs. A retrospective cohort study included 182 OTRs followed at a single academic subspecialty dermatology clinic. Drug exposures studied included prednisone (P), cyclosporine (C), tacrolimus (T), azathioprine (A), mycophenolate (M), or rapamycin (R). The outcome of interest was skin cancer formation. Study drugs, doses, and pathologically confirmed skin cancer occurrences were recorded from time of transplantation until the close of the study. Drug exposure data was analyzed using cumulative dose at the time of cancer occurrence and (in Cox modeling) time-varying covariates. There were 1,021 person years of follow-up. Mean cumulative dose of P, C, M and A was significantly higher in those who developed skin cancer as compared to those who did not ($p<0.0001$). Conversely, cumulative doses of R and T were significantly lower in skin cancer patients (p=0.05, 0.03, respectively). Log rank testing of cumulative dose quartiles showed increasing R dose to be associated with fewer skin cancers (p=0.03). In multivariate analysis, only age over 60, Fitzpatrick skin types 1-3, and male gender were significantly associated with an increased risk of skin cancer (adjusted HRs: 5.49, 4.92, and 0.35, respectively with latter being the hazard for women). In univariate analysis, heart and lung transplant patients had a significantly higher skin cancer risk than liver transplant patients (HR=2.66, 95% CI 1.15-6.17). Rapamycin and tacrolimus may be protective against skin cancer formation. A larger study is needed to fully quantify risks of skin cancer associated with immunosuppressive therapy taking into account important cofactors such as age, gender, and skin type.

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The tumour suppressor gene BRM is downregulated in human skin cancer

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Non-melanoma skin cancer is caused by exposure to ultraviolet radiation within sunlight and is the most common type of cancer in fair-skinned Caucasians. Actinic keratosis (AK) are benign precursor lesions that can develop into invasive squamous cell carcinoma (SCC). Little is known about the molecular events that lead to human skin cancer progression from benign to invasive. To determine novel genes that may be involved in skin cancer progression we initially screened human skin cancers with microarrays. This identified the SWI/SNF ATPase subunit BRM as being downregulated in SCC but not AK compared to normal skin. RT-PCR confirmed reduced levels of mRNA coding for BRM but not the alternative SWI/SNF ATPase subunit BRG1 in SCC but not AK. Sequencing of the BRM gene in human skin cancers identified a common non-synonymous point mutation present in one of ten SCC and two of six basal cell carcinoma (BCC) of the skin. This hotspot was not present in germline DNA from the same patients, nor in epithelial precancerous lesions. As we only sequenced part of the gene, other mutations may occur at different locations. Strikingly, both BRM and BRG1 protein was reduced by about 10 fold in 100% of SCC and BCC, but not AK specimens examined as determined by immunohistochemistry. Thus BRM protein may be decreased due to low levels of mRNA, while BRG1 protein loss appears to be post-translational. This may be due to gene mutations for BRM in at least some cases. A functional role for BRM was confirmed by showing that BRM knockout mice have increased susceptibility to photocarcinogenesis. This suggests that BRM and BRG1 may be novel tumour suppressor genes for human skin cancer. They appear to be involved after development of benign lesions, and are downregulated during progression towards invasion.

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ERB-041, an estrogen receptor beta agonist inhibits skin photocarcinogesis in SKH-1 hairless mice

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ERB-041 [7-ethenyl-2-(3-fluoro-4-hydroxyphenyl)-1, 3-benzoxazol-5-ol] acts by modulating various biological responses including inflammation. In this study, we show that ERB-041 reduced UVB-induced skin tumor development in SKH-1 mice. Sixty mice divided into three groups received topically vehicle, vehicle+UVB (180mJ/cm2 twice weekly) or UVB+ERB-041 (2mg/mouse in 200µl of ethanol) for 30 weeks. At this stage, ERB-041-treated mice manifested 40% reduction in tumor number, 74% in tumor volume and 20% in tumor incidence as compared to UVB-alone-treated positive controls. This reduction in tumorigenesis was accompanied by an inhibition in proliferation markers such as PCNA, cyclin D1 and vascular endothelial growth factor (VEGF). ERB-041 treatment of UVB-induced tumors also resulted in the induction of apoptosis as ascertained by a significant increase in Bax:Bcl-2 ratio ($p<0.009$) with an enhanced TUNEL staining. In addition, we observed a significant decrease ($p<0.03$) in SCCs development in ERB-041-treated mice. Tumor progression from benign to malignant stage involves a complex cascade of events that leads to epithelial-mesenchymal transition (EMT). These changes occur due to the loss of E-cadherin which is orchestrated by several transcriptional repressors of E-cadhrein such as Twist, Slug and SNAI. To assess whether ERB-041 reduces malignant transformation by blocking EMT, we stained SCCs for various EMT markers. The expression of E-cadhrein was increased whereas Fibronectin, N-cadherin, Slug, SNAI and Twist were decreased in ERB-041-treated animals. These changes in EMT markers were associated with a remarkable diminution of UVB-induced phosphorylation of MAPK proteins such as extracellular signal-regulated kinase 1/2 (Erk 1/2), and p38 in cutaneous SCCs. Taken together, our data suggest that ERB-041 is a potent chemopreventive agent against skin photocarcinogenesis and its inhibitory effects are associated with its ability to induce apoptosis, repress cell proliferation, and block EMT.

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Nucleotide excision repair is inhibited by Cyclosporin A via calcineurin-mediated downregulation of the xeroderma pigmentosum group A and G proteins

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Cyclosporin A (CsA) inhibits nucleotide excision repair (NER) in human cells, a process that contributes to the skin cancer proneness in organ transplant patients. We investigated the molecular mechanisms of CsA-induced NER reduction by measuring the xeroderma pigmentosum (XP) mRNA and protein expression of all known XP genes (XPA-XPG). Western blot analyses revealed that XPA and XPG protein expression were reduced in the cytosol of GM00637 fibroblasts exposed to 0.1 µM or 0.5 µM CsA, respectively. Nuclear XPA and XPG protein expression was completely stalled. Other XP proteins were not downregulated by CsA. Using RNAi we found that calcineurin knockdown in GM00637 fibroblasts led to the same results suggesting the involvement of calcineurin-dependent signalling in XPA and XPG protein regulation. CsA-induced reduction of NER in GM00637 fibroblasts could be complemented by overexpression of either XPA or XPG protein as assessed by host cell reactivation (HCR) and transfection of XPA or XPG cDNA-containing plasmids. Likewise, XPA-deficient fibroblasts stably corrected with XPA (XP2OS-pCAH19WS) did not retain the inhibitory effect of CsA on NER. In contrast, CsA reduced NER in XPC-deficient fibroblasts (XP4PA-SV-EB) complemented with XPC. Further, CsA treatment of GM00637 fibroblasts reduced XPG but not XPA mRNA expression. Our data indicate that the CsA-induced inhibition of NER is a result of the downregulation of XPA and XPG protein in a calcineurin-dependent manner. The fact that CsA affects XPA protein but not XPA mRNA expression suggests the involvement of at least another protein regulation pathway besides transcriptional regulation.

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Inflammation blocks carcinogenesis in skin with severe barrier defect

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Progressive loss of Notch proteins in epidermal keratinocytes is associated with corresponding decline in barrier function and increased susceptibility to carcinogens. RBP-j is the DNA-binding partner of all Notch paralogs; mice lacking RBP-j in dorsal and ventral skin patches develop a severe atopic dermatitis (AD)-like phenotype. Surprisingly, RBP-j-deficient skin was more resistant to chemically-induced carcinogenesis than Notch1-deficient or even wild-type skin. Resistance to carcinogenesis correlated with gradual reduction in RBP-j-deleted epidermal clones and their replacement by neighboring wild-type cells. RBP-j-deficient cells infected with activated-RAS expressing retrovirus formed tumors in nude mice, establishing that RBP-j was not necessary for RAS-mediated transformation in an immuno-compromised host. Combined, these observations suggested that skin inflammation was eradicating RBP-j-deficient cells and preventing skin carcinogenesis. Interestingly, blunting the AD-like inflammation by blocking the response to thymic stromal lymphopoietin (TSLP), a highly expressed cytokine in RBP-j-deleted epidermis implicated in AD development, reduced skin inflammation. This resulted in an aggressive expansion of RBP-j-deleted epidermal clones, and restored spontaneous carcinogenesis in RBP-j-deficient skin. Taken together, these findings suggest that in contrast to the tumor-promoting role of mild barrier defects, local skin inflammation downstream of severe skin-barrier defects plays a protective role against skin carcinogenesis.

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Increased twist expression in advanced stage Mycosis Fungoides (MF)/Sézary Syndrome (SS)

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Mycosis Fungoides (MF) and the leukemic variant, Sézary Syndrome (SS) are the most common cutaneous T-cell lymphomas (CTCL) but the mechanism underlying progression from early MF to systemic lymphoma are still poorly understood. Twist protein is a regulator of epithelial-mesenchymal transition during development and also may play a key role in tumor progression by inhibiting differentiation and the p53 pathway. Although Twist is normally not expressed in lymphoid cells, we and others have detected a gain in chromosome copy number in region 7p21, containing the Twist gene, in MF/SS patients (pts). To study the oncogenic role of Twist, we first evaluated Twist protein expression in a panel of MF skin lesions using immunohistochemistry and mRNA expression by QT-PCR from CTCL cell lines (HH, Hut78, and MJ) and lymphocytes from SS pts. Skin lesions were taken from 17 MF pts with stage Ia (n=2), Ib (n=7), IIb (n=1), III (n=1), IVb (n=6), and from two suspicious lesions and 3 lymphomatoid papulosis lesions. Staining was absent to weak in < 5% of dermal lymphocytes in normal control skin. Weak to moderate staining was seen in a small portion of dermal lymphs in early (14.2%) or mid (12.7%) stage MF lesions. Weak to strong staining was present in one third of dermal infiltrates (32.7%) in late stage MF lesions. Strong immunostaining was present on dermal atypical lymphocytes more frequently in late stage MF lesions (3/8, 37.5%) than in early (1/7, 14.3%) and mid stage MF lesions (1/7, 14.3%). Twist mRNA was undetectable in CD4+ T cells from 5 of 6 healthy donors but was detected in all CD4+ T cells from MF/SS pts (n=5) and three CTCL cell lines. Levels of Twist mRNA in pts' CD4+ T cells varied (0.02-13.31) with highest levels found in pts with the highest percentages of Sézary cells. In conclusion, Twist expression is increased in advanced MF lesions and in Sézary cells. Overexpression of Twist could help promote tumor progression and further studies defining the oncogenic effects on MF/SS cells are in progress.

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The vitamin D receptor, hedgehog signaling and epidermal carcinogenesis

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Over 1 million skin cancers occur annually in the United States, making it by far the most common cancer. Extensive epidemiologic data support the concept that the incidence of a number of epithelial malignancies is reduced by increasing 25OHD levels. 1,25(OH)2D3, produced by the kidney from 25OHD or locally produced, reduces proliferation and enhances differentiation and thus has been investigated for a role in preventing or treating cancer. However, UVB exposure, which increases 1,25(OH)2D3 production in the keratinocyte, causes skin cancer. Conceivably, the 1,25(OH)2D3 produced by the skin provides at least partial protection from UVB induced skin cancer such that lack of 1,25(OH)2D3 or its receptor would make the skin even more sensitive to UVB induced malignancy. Mice deficient for the vitamin D receptor (VDRKO mice), for which 1,25(OH)2D3 is a ligand, have a marked hyperproliferation in the hair follicle and epidermis and decreased epidermal differentiation. When exposed to UVB, they display increased epidermal thickness coupled with hyperproliferation and delayed DNA damage repair, compared to wild-type mice. Unlike their wild-type littermates, VDRKO mice exposed to UVB develop multiple types of skin tumors, including some characteristic of overexpression of the Hedgehog (HH) signaling pathway in keratinocytes. We found that the epidermis and hair follicles of the VDRKO animals, as well as the UVB induced tumors in VDRKO mice, overexpress elements of the HH signaling pathway [Sonic Hedgehog (Shh), Patched 1, Smoothened, Gli 1, and Gli 2]. Moreover, 1,25(OH)2D3 treatment decreased the expression of HH signaling pathway members in epidermal sheets from wild-type mice but not from VDRKO. These results suggest that increased expression of Shh in the keratinocytes of the VDR null animal activates the HH signaling pathway, predisposing the skin to the development of tumors.

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MicroRNA-211 regulates melanoma metastasis by inhibiting migration and invasion abilities of melanoma cells

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Melanoma is the most aggressive skin cancer, with a propensity to metastasize, and is resistant to most of the current therapeutic regimens. MicroRNAs (miRNAs) are endogenous ≈22-nt noncoding small RNAs, which negatively regulate gene expression in a sequence-specific manner. Increasing evidence shows that miRNA gene expression is deregulated in human cancers. To identify important novel miRNAs involved in melanoma metastasis, we performed microarray-based miRNA profiling of comparing primary skin melanomas and metastatic melanomas collected from 6 patients. By comparing these paired samples from both metastatic and primary melanomas of the same patients, we may reduce the individuals' variations in miRNA profiling and identify important miRNAs related to the melanoma metastasis. We found that 5 miRNAs (miR-211, miR-107, miR-141, miR-187 and miR-514) were down-regulated in metastatic melanomas. Supportively, quantitative PCR study in human and mouse melanoma cell lines also revealed that miR-211 had reciprocal correlation with the migration/invasion ability. In addition, over-expression of miR-211 significantly reduced the migration/invasion abilities of A375 and B16F10 cell lines; in contrast, antisense oligonucleotide (antagomir) treatment of miR-211 increased the migration/invasion abilities of MeWo and B16F0 cell lines. *In vivo* animal model of melanoma metastasis also revealed A375 cells expressing miR-211 had a reduced metastasis rate as compared to the vector control A375 cells. The above results suggested that miR-211 regulates melanoma metastasis by inhibiting migration/invasion abilities of melanoma cells.

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TAT-mediated delivery of a DNA repair enzyme to skin cells rapidly catalyzes repair of UV-induced DNA damage

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Ultraviolet (UV) light causes DNA damage in skin cells, leading to more than one million cases of non-melanoma skin cancer diagnosed annually in the United States. Although human cells possess a mechanism (Nucleotide Excision Repair, NER) to repair UV-induced DNA damage, this mechanism is not sufficient to prevent UV-induced mutagenesis. While human cells have all enzymes necessary to complete another repair mode, Base Excision Repair (BER), they lack DNA glycosylases needed to initiate BER of dipyrimidine photoproducts. Certain prokaryotes and viruses produce pyrimidine dimer-specific DNA glycosylases (pdgs) that initiate BER of cyclobutane pyrimidine dimers (CPDs), the predominant UV-induced lesions. Such a pdg was identified in Chlorella virus and termed Cv-pdg. The Cv-pdg protein was engineered to contain a nuclear localization sequence (NLS) and a membrane permeabilization peptide (TAT). Here we demonstrate that the Cv-pdg-NLS-TAT protein can be delivered to repair-proficient keratinocytes and fibroblasts, and to keratinocytes within a human skin model. Cv-pdg-NLS-TAT rapidly catalyzed removal of UV-induced CPDs. Keratinocytes, but not fibroblasts, given Cv-pdg-NLS-TAT before UV exposure exhibited decreased heterochromatin protein 1γ aggregation compared to cells without the enzyme, suggesting completion of DNA repair. Enhanced DNA repair via exogenous delivery of Cv-pdg to skin can potentially help prevent skin cancer.

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Merkel cell polyomavirus is present in Merkel cell cancer (MCC) but absent in gastrointestinal neuroendocrine carcinomas and trichodysplasia spinulosa

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Merkel cell polyomavirus (MCPyV), a recently discovered human polyomavirus, has been found to be integrated into the genome of the eponymous carcinoma (MCC) and implicated in the development of this rare neuroendocrine carcinoma. Like Merkel cells, the neuroendocrine cells of the gastrointestinal (GI) tract can rarely give rise to cancers, most commonly carcinoid tumors. Moreover, MCPyV has also been detected in normal human appendix and small intestinal tissue. In addition, trichodysplasia spinulosa is a rare dermatosis of immunosuppressed patients characterized by alopecia, folliculocentric spiny papules, and, notably, intracellular viral particles whose ultrastructural size and shape are consistent with a polyomavirus. Based on these facts, we sought to determine if MCPyV was present in neuroendocrine tumors of the GI tract, adjacent normal GI tissue, or a sample of trichodysplasia spinulosa. DNA was extracted from formalin-fixed, paraffin-embedded tumors (30 MCCs, 10 neuroendocrine tumors with adjacent normal GI mucosa, and a biopsy sample of trichodysplasia spinulosa). Using primers based on the published sequence of MCPyV, PCR was used to assess the presence of the virus. MCPyV was detected in 16 of 24 (67%) MCC samples; 0 of 10 (0%) neuroendocrine tumors; 1 of 8 (12.5%) samples of normal GI mucosa; and 0 of 1 sample of trichodysplasia spinulosa. Although our study was somewhat limited in scope, there was no evidence that MCPyV integration plays a role in the pathogenesis of GI neuroendocrine tumors or trichodysplasia spinulosa. Larger studies may be necessary to definitively rule out a role for MCPyV in the pathogenesis of GI neuroendocrine tumors and trichodysplasia spinulosa.

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Remarkable tumor disruption induced by anti-angiogenic receptor tyrosine kinase inhibitor in a novel experimental model of human angiosarcoma

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Angiosarcoma is rare, but it is one of the most life-threatening human neoplasms. It shows strong resistance to conventional chemotherapy and radiotherapy; consequently, new therapeutic agents are urgently required. One factor in delaying the development of effective therapies is the limitation of experimental models of human angiosarcoma. Recently, we succeeded in establishing a novel experimental model of human angiosarcoma. Its cell line, HAMON, expresses endothelial-cell-characterized surface molecules (e.g., CD31, VEGFR2, Tie2) and develops angiosarcoma tumors in immunodeficient mice. HAMON is observed to form irregular vessel-like structures in tube formation assay, and its cell growth is strongly regulated by VEGF. Additionally, human angiosarcoma tissue has been serially passaged in immunodeficient mice for more than 2 years. In recent years, several VEGF-targeted agents have been shown to benefit patients with advanced-stage malignancies. We investigated the anti-angiosarcoma effect of sunitinib malate, a small-molecule receptor tyrosine kinase inhibitor against VEGFRs, *in vitro* and *in vivo*. Sunitinib was found to dose-dependently suppress HAMON proliferation. Next, mice implanted with angiosarcoma tissue were administered a daily oral dose of 40mg/kg or 120mg/kg sunitinib for 2 weeks when the implanted tumor had reached approximately 200mm3. Isolated angiosarcoma tumors treated with sunitinib showed significantly more intratumor necrosis than tumors treated with a vehicle ($p<0.05$). Our results clearly indicate that sunitinib is a promising therapeutic agent against human angiosarcomas.

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Balanced 9p22q translocation in a patient with melanoma, deafness and DNA repair deficiency disrupts p14arf and down-regulates TBX1

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A balanced reciprocal translocation 46, XY, t (9; 22)(p21;q11.2) was detected in a 12 yr male (DD129BE) with multiple melanomas, congenital deafness and DiGeorge syndrome-like (DGS) features. The translocation was investigated by fluorescent *in-situ* hybridization, and array comparative genome hybridization on flow-sorted chromosomes. The 9p21 breakpoint interrupts p14arf. The 22q11 breakpoint was in a translocation hotspot in an unclonable gap in a DGS critical region. The rearrangement involved a 6 bp palindromic sequence with a 71 bp-deletion in an AT rich repeat region of CDKN2A melanoma gene on 9p21 and a 62-bp deletion in a palindromic AT rich repeat (PATRR22) on 22q11.2. Gene expression was analyzed by microarray or qRT-PCR. p14arf was strongly underexpressed. A DNA repair defect was detected in fibroblasts that was improved by transfection with p14arf cDNA. Sequencing of p14arf from his melanoma cells harvested by laser capture microdissection revealed two UV-type missense mutations. Thus this melanoma might have arisen according to the Knudson model in which one copy of p14arf is disrupted by the translocation followed by a somatic mutation in the other copy. Audiologic assessment revealed no measureable hearing bilaterally and absent vestibular reactivity. CT and MRI showed bilaterally symmetric cochlear hypoplasia and vestibular dysplasia with absence of the cochlear nerve. No mutations were found in TBX1 on 22q11.2 but expression of TBX1 was greatly reduced. TBX1 is associated with development of the auditory apparatus in mice. We propose that the rearrangement on chromosome 22 resulted in down-regulation of TBX1 that contributes to the deafness and other DGS features.

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mtDNA changes in fibroblasts alter intracellular ROS levels resulting in enhanced cellular proliferation, migration and invasion

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Reactive oxygen species (ROS) have been shown to be involved in tumor promotion while antioxidants may inhibit malignant phenotypes. We modeled mitochondrial dysfunction in fibroblasts by creating identical murine fibroblast cybrid lines that differ only in their mtDNA haplotype. The mtBALB cybrids differ from the mtB6 in that it shows a relative respiratory dysfunction of decreased CI activity, lowered ATP production and increased ROS levels causing readily observable increased cellular proliferation. This haplotype was also associated with striking changes in motility such as increased migration through transwell inserts and increased invasion through matrigel matrix. We theorized that the hyperproliferative activity of the mtBALB cybrids compared to the mtB6 could be explained by an increased level of ROS. Cybrids were treated with the antioxidants N-acetyl-L-cysteine (NAC) or vitamin E (vitE) to determine if scavenging ROS would diminish their proliferative capabilities. We found that NAC reduced the proliferative capacity of the mtBALB cybrids while vitE caused reduction in proliferation of both cybrids. The ability of NAC to selectively inhibit the proliferation of mtBALB cybrids (effect not seen by vitE) may reflect the differences in ROS scavenging activity of the two compounds for specific radicals. Both antioxidants were also able to diminish the migratory capacity of the cybrids in transwell assays. NAC treatment resulted in the significant reduction in migration of the mtBALB cells. VitE significantly decreased migration of both cybrids. A similar reduction of migration seen in mtBALB cells treated with NAC or vitE suggests an effect potentially mediated through hydroxyl radical that is scavenged by both compounds. These data support a role of ROS as second messengers in signaling cascades involved in proliferation and migration. Modulation of ROS or mitochondrial function may serve as a therapeutic target to stimulate fibroblast migration in wound healing.

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Aberrant cell proliferation by enhanced mitochondrial biogenesis through mtTFA in arsenical skin cancers

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Arsenic-induced Bowen's disease (As-BD), a cutaneous carcinoma *in situ*, is thought to arise from mutation and uncontrolled proliferation. Arsenic-induced mutation requires appropriate mitochondrial function. However, how mitochondria regulate the arsenic-induced cell proliferation remains uncertain. The study aimed to clarify if arsenic interfered with mitochondrial biogenesis and function, leading to cell proliferation in As-BD. Skin from As-BD patients and controls were stained with cytochrome c oxidase (Complex IV), assayed for mitochondrial DNA (mtDNA) copy number, and expression of mitochondrial biogenesis genes. Mitochondrial functions were determined by oxygen consumption and intracellular ATP production. The results showed enhanced cytochrome c oxidase expression in As-BD. Expression of mtTFA, NRF-1 and PGC-1α were upregulated in As-BD. Treatment with arsenic at concentrations lower than 1 μM for 72 hours induced cellular proliferation *in vitro*, along with enhanced mitochondrial biogenesis and its associated factors. Besides, oxygen consumption rate and intracellular ATP level were both enhanced in arsenic-treated keratinocytes. Notably, blocking of mitochondrial function or mtDNA replication with oligomycin A or interference RNA against mtTFA, respectively, abolished arsenic-induced cell proliferation. We concluded that the increase in the number and function of mitochondria mediated arsenic-induced cell proliferation. Aberrant mtTFA upregulation and augmented mitochondrial biogenesis resulted in arsenic-induced keratinocyte proliferation in arsenical skin cancers. Targeting mitochondrial biogenesis may help treat arsenical cancers in the stage of cell proliferation.

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Human Numb regulates spindle pole maturation through localization of Plk1 in melanoma cells

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Mammalian Numb is an evolutionarily conserved protein that plays critical roles in progenitor cell fate determination, as well as functions in normal cell maintenance including a tumor suppressor role via regulating p53 stability. One of the most critical regulators of cell division is Polo-like kinase 1. Plk1 has been shown to play an essential role in the G2/M transition, bi-polar spindle formation, and regulation of the anaphase promoting complex for proper mitotic exit. Recently we have shown that Plk1 is over-expressed in melanoma and that Plk1 inhibition causes a mono-polar spindle phenotype surrounded by DNA rather than a bi-polar arrangement on either side of the metaphase plate. This severe mitotic phenotype results in a G2/M cell cycle arrest and mitotic catastrophe. Milder inhibition of Plk1 has been shown to result in errors in bi-polar spindle formation including multi-polar phenotypes or incomplete microtubule attachment and division. Some of these less pronounced phenotypes may not prevent cell division and may contribute to cellular aneuploidy and cancer. Therefore, in this study we aimed to define Numb's role in Plk1 regulation during mitosis. Employing melanoma cell lines A375 and HS294T, we demonstrate that Numb and Plk1 interact as shown by co-immunoprecipitation and immunofluorescent assays. We found that both Plk1 and Numb co-localize to the spindle poles in metaphase. Further, knockdown of Plk1 or Numb dysregulates the localization of the other. Most striking was the pronounced mislocalization of Plk1 from the spindle poles in the presence of Numb knockdown. Although, the majority of metaphase cells were still bi-polar, there was a marked increase in mislocalized γ-tubulin. Additionally, cells with two defined spindle poles demonstrated a more diffuse γ-tubulin staining pattern suggesting that the dysregulated Plk1 localization slows or prevents spindle pole maturation and γ-tubulin recruitment. These data suggested that Numb may possess tumor suppressor functions by regulating proper Plk1 localization and proper spindle pole maturation.

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Promoter methylation profiling of cancer/testis genes in human malignant peripheral nerve sheath tumor associated with neurofibromatosis type 1

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DNA methylation of a CpG island in the promoter region of a gene suppresses its transcription. Some cancer/testis genes, suppressed in most normal somatic tissues by methylation in their promoter regions, but activated in germ line cells and a wide range of cancer types by demethylation, encode antigens that are immunogenic in cancer patients. Malignant peripheral nerve sheath tumor often arises in patients with neurofibromatosis type 1 and is associated with poor prognosis. Until now, limited information about molecular target for the therapy has been available. To identify candidates of molecular target, we profiled promoter methylation statuses of cancer/testis genes, MAGEA1, MAGEA2, MAGEA3, MAGEB2 and SSX4, whose transcription are regulated by promoter methylation statuses, in the six human malignant peripheral nerve sheath tumor cell lines. Methylation-specific PCR revealed that MAGEA1, MAGEA2, MAGEA3 and MAGEB2 are demethylated in at least one of the six cell lines. These results indicate that the products of the genes may be candidates of molecular targets for the therapy.

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Upregulation of inflammatory cytokines and STAT3 characterizes the tumor microenvironment leading to tumor formation in a murine model of cutaneous lymphoma

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Previously, we showed that injection of MBL2 (a Moloney MuLV-induced T cell lymphoma line) cells into ear skin of wildtype C57BL/6 and SCID-beige mice only resulted in tumors (with dramatic ear thickening) in the latter group of mice. However, induction of local inflammation by one topical application of DNFB (2, 4-dinitro-1-fluorobenzene) following inoculation in WT mice resulted in progressive high grade lymphoma. Herein, we show that the development of skin lymphoma following DNFB application can be blocked by local immunosuppression with topical clobetasol dipropionate (0.05%) treatment. To assess early events leading to tumor formation, we examined biochemical markers of proliferation and apoptosis two days after DNFB application in MBL2-inoculated ears (before significant ear thickening occurred). By Western blot, Ki67 staining was increased, and, by flow cytometry, Annexin V staining was decreased by 10-fold in DNFB-treated ears. Flow cytometry analysis of dermal cells revealed that GR1+ granulocytes and F4/80+ macrophages constituted the majority (>99%) of the infiltrating leukocyte (CD45+) population in DNFB-treated ear skin. We used PCR arrays to assess the transcriptional cytokine and chemokine profile of the tumor microenvironment. Compared to control ears, MBL2-implanted ears that were treated with DNFB showed robust changes in the expression of genes regulating proliferation, apoptosis, and tumorigenesis, e.g. LIF, IL1β, IL11, CXCR2 and GDF5. Furthermore, enhanced phosphorylation of oncogenic signal transducer and activator of transcription-3 (STAT3) was observed by Western blot in DNFB-treated ears. Our data suggest that dysregulation of cytokine and chemokine expression may result in activation of known oncogenic pathways, providing a molecular basis for interaction between an inflammatory microenvironment and lymphomagenesis. Inhibition of inflammatory signals (e.g., via corticosteroids) or STAT3 may represent viable therapeutic targets for the treatment of cutaneous T cell lymphomas.

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Hypovitaminosis D and non-melanoma skin cancer

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The inverse relationship between vitamin D deficiency and malignancies of various types is currently under investigation. It is most consistently documented for colorectal cancer and has also been suggested in other cancers including breast and prostate. The relationship between 25-OH vitamin D3 and skin cancer is still uncertain. The skin produces vitamin D. Its main function is to maintain calcium homeostasis. 1:25 dihydroxyvitamin D3 (1:25 di-OH vit D3) is an antiproliferative factor for cells that have a vitamin D receptor. There are receptors for 1:25 di-OH vit D3 in the epidermis, and 1:25 di-OH vit D3 inhibits the proliferation of cultured keratinocytes and induces them to terminally differentiate. The epidermis thus serves as a target for vitamin D and as a source for its production. The relationship between ultraviolet exposure and non-melanoma skin cancer is well-documented. The effect of vitamin D on the development of non-melanoma skin cancer is not clear, however. Our objective was to establish the prevalence of vitamin D deficiency among basal cell (BCC) and squamous cell carcinoma (SCC) patients ages 18-65 in comparison to age-matched, cancer-free controls. We measured 25-hydroxyvitamin D3 and parathyroid levels in patients with a diagnosis of BCC or SCC within the previous 180 days. Patients completed a questionnaire assessing risk factors for skin cancer. 25-OH vitamin D3 levels were measured by isotope dilution tandem mass spectrometry performed on the API-4000 (Applied Biosystems) and PTH was quantified using the Siemens immunolite immunoassay. Samples were obtained in September and March to represent the 25-OH vitamin D3 peak and nadir. In patients with BCC or SCC (n=50) 32% had vitamin D deficiency with levels <30 ng/mL. In cancer-free controls (n=14), 7.14% had 25-OH vitamin D3 levels below 30 ng/mL. We are currently developing an assay for 1:25-di-OH vit D3 which will be used to measure the latter in the future.

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Inhibition of human papilloma virus-31 in human keratinocytes by the phosphatidylcholine specific phospholipase C-inhibitor LMV-601

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The purpose of our study was to determine the effect of LMV-601 on HPV-31 transformed human keratinocytes (CIN 612 9E HPV-31 infected cervical epithelial cells). Expression of early genes and episomal DNA replication of HPV depends on an active AP1 complex. Activation of AP1 was shown to be precluded by inhibition of phosphatidylcholine specific phospholipase C (PC-PLC). LMV-601 is (-)-*exo,exo*-O-Tricyclo-[5.2.1.0(2,6)]-dec-9-yl-dithiocarbonate potassium salt. A)*In vitro*, after 72 h exposure, LMV-601 inhibited cell growth (IC_{50} 16 μg/mL), HPV-31 specific RNA expression (IC_{50} 10.69 μg/mL) and reduced DNA content [62.5% at 32 μg/mL (max. conc. tested)]. B)*In vitro*, after each of 9 passages, viral RNA and DNA levels, and cell morphology were assessed. At 3.3 μg LMV-601/mL the number of passages required to reduce the amount of HPV-31 specific RNA by 50% (T_{50}RNA) was 2.23, T_{50}DNA was 3.28. At 10 μg LMV-601/mL both T_{50}RNA and T_{50}DNA were <1. After 6 passages the growth rate of the cells was reduced and the morphology changed from the spindle form to a normal phenotype. After passage 9, cells were enlarged, became senescent (identified by expression of the marker β-gal) and ceased to grow. In non-HPV immortalized HaCaT cells, LMV-601 at the same concentrations neither inhibited the growth rate nor induced senescence. C)*In vivo*: Cells were transplanted into the skin of nu/nu mice. When tumors were visible, they were treated topically. Primary endpoint: CIN 612 9E cell tumor size after 28 day bid treatment. LMV-601 3.5%, 1.0% and 0.3% creams inhibited tumor growth *vs* vehicle by 67% ($p<10^{-4}$, Student's t-test), 23% (p=0.052) and 7% (p=0.442), resp. In summary, LMV-601 inhibited HPV-31 specific RNA expression and DNA replication. As a consequence, CIN 612 9E cells acquired a "normal" phenotype. Furthermore, *in vivo* local treatment with LMV-601 inhibited the growth of CIN 612 9E cells. It can be concluded that eukariotic, host cell coded PC-PLC is a suitable target for the treatment of HPV infection.

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Mapping toll-like receptor activity in different stages of cutaneous T-cell lymphoma

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Cutaneous T-cell lymphoma (CTCL) includes a variety of lymphoproliferative disorders marked by neoplastic T lymphocytes which predominately infiltrate the skin. The most common clinical variants include mycosis fungoides(MF) and sezary syndrome. It is known that human keratinocytes express Toll-like receptors(TLR) and their activation elicits a cytotoxic Th1 antitumor environment. However, published reports conflict regarding TLR expression in CTCL patient keratinocytes. In our study we attempt to more clearly define the pattern of expression and detect any differences of TLR 1-9 expression in epidermal keratinocytes, dendritic cells(DC), tumor infiltrate(TI) and endothelial cell(EC) types through the stages of MF using immunohistochemical stains. The institutional review board at Wayne State University approved our study. Fixed and paraffin embedded sections of CTCL in patch, plaque and nodular stage were stained by immunohistochemistry. Our results showed increased epidermal staining of TLR 2,3,4,5,6,and 8 over normal control skin. Dermal blood vessels showed increased staining of TLR 4 and 6. TI staining was strongest with TLR 5 and 7. Patient biopsies were compared over a 2 year period to assess for changes in TLR expression over time. Individual cases with disease progression showed increased intensity of TLR 4,5 and 6 staining in the epidermis, TI and EC cells. In conclusion, we attempted to quantify TLR expression in MF and detect changes in TLR expression throughout the stages of MF by immunohistochemistry. In our limited sample size a trend was identified showing increased epidermal expression of TLR and increased EC staining as compared to controls. TLR expression may be driven by antigenic stimulation and may play a role in activation of neoplastic T-cells in the skin. Further definition of TLR patterns may help to refine the use of TLR modifiers for treatment. We plan to validate our immunohistochemistry results by measuring TLR mRNA and downstream activation targets of TLRs with real time PCR.

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CXCR3 ligand CXCL11 promotes proliferation of primary basal cell carcinoma cells *in vitro*

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Basal cell carcinoma (BCC) is the most common malignancy found in the Caucasian population. Previously we identified that CXCL9, 10, 11 and their receptor CXCR3 were significantly upregulated in K17+ BCC keratinocytes. Cell culture of primary basal cell carcinoma cells has proven difficult. We hypothesized that CXC chemokines are involved in BCC development and may promote cell growth *in vitro*. Cells were isolated from human nodular BCC tissues (n=6 patients) and seeded in multiwell-plates coated with collagen I and complete keratinocyte growth medium. CXCL11 peptide was added at 0nM, 5nM, 10nM or 20nM in triplicate wells. Cell samples were counted and cytospin preparations produced on days, 0, 14, and 21 of culture. Cell numbers did not increase in the absence of CXCL11 and failed to adhere to the substrate. 5nM and 20nM CXCL11 promoted statistically insignificant cell proliferation. 10nM was identified as the optimal concentration with a statistically significant increase in cell numbers by day 21. Dual-label immunohistochemistry revealed that on Day 0, K17-/CXCR3+ cells predominated (58.9%). By day 14, K17+/CXCR3+ cells were more predominant (43.66%). On day 21, both K17+/CXCR3+ and K17+/CXCR3- cell groups were at significantly higher numbers (65.47% and 33.09% respectively) than the K17-/CXCR3+ group (1.44%). The expression of CXCR3 and its ligands in human BCC keratinocytes, and the keratinocyte cell proliferation enhanced by CXCL11, suggest that CXCR3 and its ligands may be important mediators for BCC growth via autocrine and/or paracrine signaling. These chemokines may be novel targets for the development of new treatments.

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Aldehyde dehydrogenase ativity contributes to tumorigenesis of human melanoma

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Increasing evidence indicates that solid tumors are heterogeneous and originate from a distinct subpopulation of cancer initiating cells (CICs). Aldehyde dehydrogenase (ALDH) activity is suggested as a marker to define hematopoietic and neural stem cells and CICs in several tumor types, but its role in melanoma is still unknown. Here, we demonstrated that tumors from human melanoma patients had a distinct subpopulation with high ALDH activity, accounting for 0.5% to 2.5% of entire population. The patient melanoma tissues, maintained in immunocompromised mice using direct *in vivo* xenografting, displayed a similar distinct subpopulation. Interestingly, the percentage of ALDH+ subpopulation in the metastatic tissue was higher than that in the primary tissue obtained from the same patient. Likewise, immunohistochemical analysis showed a stronger and increased expression of ALDH1A1 in the metastatic melanoma tissue compared with the primary counterpart, suggesting that ALDH is correlated with melanoma progression and stages. Tumorigenic analysis using non-obese diabetic severe combined immunodeficiency (NOD/SCID) mice demonstrated that ALDH+ subpopulation from human melanoma tumors was more tumorigenic than ALDH- and parental subpopulations and that ALDH+ subpopulation generated a tumor in mice from as few as 10 cells. The xenografted tumor from ALDH+ subpopulation recapitulated the hierarchical organization of the parental tumor, implying its capability of differentiation. Microarray analysis showed differentially expressed genes associated with cell growth, apoptosis, cell cycles, tumor progression and several signaling pathways in ALDH+ subpopulation. Taken together, our study indicates that ALDH+ subpopulation is an enriched source of melanoma initiating cells and offers a new marker for the study of melanoma initiating cells and a rational target for effective therapy in melanoma.

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Induction of Fyn via the Ras/PI3K pathway is necessary and sufficient for enhanced migration and invasion

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Over-expression and activation of Src family kinases, including Fyn, is observed in human cutaneous squamous cell carcinomas (SCCs) and other epithelial cancers. Fyn is oncogenic and promotes keratinocyte migration and SCC invasion, making it a potential molecular target for cancer therapeutics; however the mechanism of Fyn over-expression in SCCs is not clear. Since activation of Ras oncogenes is a very common oncogenic event in SCCs, we explored if an active Ras could induce Fyn expression. Retroviral transduction of the immortalized human keratinocyte cell line HaCaT with oncogenic H-Ras dramatically up-regulated Fyn mRNA, protein, and kinase activity (>100 fold mRNA induction, $p<0.001$). Src levels and kinase activity were not affected by H-Ras transduction. Activation of Akt, but not MAPK or EGFR, was necessary and sufficient for induction of Fyn by H-Ras. The enhanced migration and invasion induced by H-Ras could be significantly blocked (70% reduction, $p<0.0001$) by Fyn specific siRNA knockdown or inhibition by PP2 (10 μM). In addition, expression of active Fyn in HaCaT cells was sufficient for increased migration and invasion. Focal adhesion kinase (FAK) activation has been strongly linked with the Ras-mediated increased migratory phenotype of tumor cells. We found that Fyn was necessary and sufficient for Ras-induced activation of FAK. In summary, we show that Fyn induction by oncogenic Ras/PI3K/Akt pathway can account for the elevated Src family kinase activity in SCCs, and that Fyn is a critical mediator of the Ras-stimulated invasive cell phenotype. These results have strong implications for the development of therapeutic strategies targeting Akt/Fyn pathway to block migration and invasion of tumor cells.

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PTEN regulation of cutaneous SCC

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Inhibition of tumor suppressor genes is one of the crucial mechanisms in UV-induced skin carcinogenesis. PTEN functions as a highly effective tumor suppressor in a wide variety of tissues by negatively regulating the PI3K/AKT pathway. Somatic mutations, deletions, or promoter hypermethylation of PTEN, however, are not detected in human squamous cell carcinoma (SCC), and the relevance of PTEN to human skin cancer has remained unclear. We evaluated nearly 94 human skin specimens for PTEN expression, including non-lesional non-sun-exposed normal skin (n = 10), actinic keratosis (n = 25), keratocanthoma (n = 15), SCC *in situ* (n = 13), and invasive SCC (n = 31). PTEN expression in hair follicles and non-sun-exposed non-lesional epidermis was used as the comparison control for that in tumors (T). As compared with non-tumor normal skin, PTEN levels were significantly down-regulated in skin tumors. When we analyzed PTEN levels in skin tumors as related to the pathologic diagnosis, PTEN was down-regulated in all four diagnosis groups of SCC. Such PTEN down-regulation was significantly associated with increasing actinic damage, as measured by degrees of solar elastosis. Male gender has been shown to be a risk factor for SCC in humans and in mice. When we compared PTEN expression in skin lesions from male and female patients, however, we found no significant differences, implying that PTEN down-regulation is a shared molecular defect in SCCs from both males and females. In epidermal keratinocytes, PTEN loss disrupted genomic integrity in response to UV damage. Our findings suggest that PTEN loss plays an active role in the pathogenesis of skin cancer.

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SOD3 expression is up-regulated by PKC-δ and inhibits cell proliferation in melanoma via the Stat1-p21 pathways

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The physiological role of IFN-γ has been implicated in the modulation of tumor cell proliferation, apoptosis as well as metastasis of tumor cells. It has been shown that IFN-γ induces PKC-δ induced inhibition of tumor cell proliferation and up-regulates SOD3 expression. These IFN-γ induced growth arrests and apoptosis in a variety of cell lines are accomplished by activation of the Stat1-p21 pathway. However, the details of the mechanism have not been well elucidated. In this study we investigated how PKC-δ and SOD3 interact with each other to inhibit tumor cell proliferation. To determine the relationship between PKC-δ and SOD3, we monitored SOD3 expression in the PKC-δ over-expressed human melanoma and explored the signaling pathways of SOD3. We observed a rapid increase in SOD3 expression in PKC-δ transfected cells while the PKC-δ-induced up-regulation of SOD3 was attenuated by pretreatment with PKC specific inhibitors. Our result also showed that treatment with PMA sufficiently induced SOD3 expression in A375 melanoma cells, confirming that PKC-δ up-regulates the production of SOD3. Additionally, we demonstrated that increased SOD3 drastically inhibited cell proliferation and suppressed lung metastasis in an SOD3 transgenic mouse via the Stat1-p21 expression. Finally, we confirmed that the transfection of SOD3 expression vector enhanced IFN-γ-induced apoptosis. These results indicate that IFN-γ directly induced SOD3 expression via activation of PKC-δ pathways in human melanoma. Taken together, our findings suggest that PKC-δ-induced over-expression of SOD3 inhibits melanoma cell growth via activation of the Stat1-p21 pathway.

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Carcinoma *in-situ* of the penis and β-HPV types

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High risk (HR) genital human papillomavirus (HPV) infection is strongly associated with cervical and anal cancers but the association with penile cancers is dependent on histological type. Low risk and HR genital HPV DNA has been found in 70 to 90% of penis *in-situ* cancer samples, and predominantly it is HPV-16. β-HPV types (associated classically with epidermodysplasia verruciformis) have been detected in 36 to100% of such patients. However, small numbers of cases have been studied. Almost always β-HPVs are found in low copy numbers as a mixed infection with HR genital HPV. The purpose of this study was to examine the prevalence of β-HPV in penis *in-situ* cancer (and correlate this with HR genital types). We investigated 21 penis biopsies from 21 patients with invasive cancer (n=5) or *in-situ* cancer (n=16). Host and viral DNA was extracted from formalin-fixed paraffin embedded archival tissue using laser capture microscopy to select cancerous/pre-cancerous zones. DNA derived from lesions was analysed with two HPV reverse hybridisation assays; one for genital types (SPF10-LiPA25, version 1) and one for β-HPV (PM-PCR RHA method). HPV was detected in 13/16 (82%) of in-situ cancer samples; the relative contributions were HPV-16 in 12/13 (92%) and HPV-68 /73 in 1/13 (8%). HPV-18 was detected in one of the five (20%) penis cancer samples. There were no mixed infections (with more than one HPV type). No β-types were detected. Regarding genital HPV types, this is in line with previous work but is discrepant with the high prevalence of β-types that have been reported by others (as mixed infections). This may be accounted for by our use of old archival tissue, the exclusion of surface contamination with microdissection and/or stringent laboratory standards.

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Epidermal expression of a mutant CYLD promotes skin carcinogenesis and metastasis

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CYLD, a deubiquitinase, has been recognized as a tumor suppressor due to its genetic linkage to skin appendage tumors. The molecular mechanisms of CYLD action and its role in other types of skin cancer are not well-understood. Here, we generated a transgenic animal model with K14-driven expression of a patient relevant and catalytically deficient CYLD mutant (CYLDm). We found that the transgenic mice displayed increased skin tumor development and malignant transformation in response to chemically induced carcinogenesis. Most surprisingly, over 50% of the transgenic mice developed lymph node metastasis following DMBA/TPA treatment. The tumor tissues and cells expressing CYLDm displayed increased activation of JNK and its downstream c-Jun and c-Fos, AP-1 proteins. Consistently, topical JNK inhibition significantly reduced tumor development and abolished metastasis in the transgenic mice. In addition, a majority of human squamous cell carcinomas (SCC) samples expressed decreased levels of CYLD and concomitantly increased levels of JNK/AP-1 activation. Moreover, expression of CYLDm or shRNA-mediated gene silencing of CYLD increased cell growth, migration and subcutaneous tumor growth of human A431 SCC cells, whereas expression of the wild type CYLD inhibited cell growth and tumorigenesis. These data indicate that CYLD loss-of-function promotes epidermal malignancy and metastasis in a JNK/AP1-dependent manner.

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Akt inhibition suppresses the growth of basal cell carcinomas (BCCs) in Ptc1+/-/SKH-1 mice by blocking Akt and sonic hedgehog (Shh)-dependent pathways

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Dysregulated PI3K/Akt is implicated in the pathogenesis of human sporadic cancers and in hereditary cancer syndromes. This pathway is essential for Shh signaling during embryonic development and for the growth of Hh-dependent tumors. Inhibition of PTEN or PI3K/Akt activation are critical for the growth of squamous cell carcinomas (SCCs), but little is known about their role in BCCs. We show that UVB-induced BCCs, generated in our Ptc1+/-/SKH-1 (PtSKH) mice, as well as sporadic human BCCs manifest increased Akt1 phosphorylation. Detailed analysis of tissue and tumor sections from these specimens showed that only a subpopulation of BCC cells manifest Akt1 phosphorylation, indicating heterogeneous cell populations in these tumors. Treating PtSKH mice with the smoothened (SMO) inhibitor cyclopamine and the Akt inhibitor LY294002, alone or in combination, reduced both the number and size of microscopic BCCs, and was associated with reduced p-Akt1-positive cells. In ASZ001 BCC cells derived from Ptc1+/-/C57Bl/6 mice, cyclopamine and Akt inhibitors (LY294002 or wortmannin) independently reduced BrdU incorporation by 10-20%, but when used in combination resulted in synergistic reduction of 50-60%. Cyclopamine treatment or SMO knockdown did not substantially alter the level of Akt1 phosphorylation in ASZ001 cells, suggesting that Akt1 activation is not a downstream event in the Shh pathway. However, Akt inhibition decreased Gli-1, and the introduction of constitutively active myristoylated Akt1 enhanced Shh pathway activation, leading to overexpression of Gli-1 and cyclin D1. Furthermore, the Akt1-expressing BCC cells had increased levels of cyclin D1, which decreased substantially following treatment with either cyclopamine or the Akt inhibitors. These results indicate that Akt1 might independently regulate the survival of subpopulations of tumor cells with high proliferative potential in BCCs.

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p38 MAPK regulates oxidative stress in UVB-induced squamous cell carcinoma by blocking NADPH NOX2

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Solar ultraviolet B (UVB) radiation generates reactive oxygen species (ROS), which can drive development of aggressive malignant phenotypes of cancer cells. p38α mitogen-activated protein kinase (MAPK), which coordinates cellular responses to many stressful stimuli, can function as an ROS sensor. p38α MAPK inhibits H-RasV12-induced ROS accumulation by triggering apoptosis in mouse embryonic fibroblasts, while p38α MAPK deficiency in these cells leads to malignant transformation. In normal keratinocytes, UVB-induced apoptosis entails the ROS-mediated activation of p38 MAPK, which likely is p53-dependent. However, the importance of p38 MAPK signaling in the pathogenesis of skin cancer in the absence of wt p53 is currently unknown. We found that the pharmacological inhibition of p38 MAPK activity with SB203580 markedly accelerated the rate of UVB-induced skin tumor growth in p53-/-/SKH-1 mice. In human SCC cells harboring mutant p53, genetic and pharmacological inhibition of p38 MAPK increased intracellular ROS via the NADPH oxidase NOX2. This was associated with increased levels of cyclin D1, cdc25B/C, and BrdU incorporation. Furthermore, p38 MAPK inhibition increased the phosphorylation and activation of the c-Jun N-terminal kinase/stress-activated protein kinase, and resulted in a concomitant increase in c-Jun phosphorylation and the DNA-binding activity of AP-1. ROS levels in UVB-induced SCCs harvested from p53-/-/SKH-1 mice were higher than those in UVB-irradiated epidermis, and were undetectable in non-irradiated control skin. NOX2 expression was higher in SCCs harvested from SB203580-treated/UVB-irradiated p53-/-/SKH-1 mice as compared to SCCs harvested from non-treated/UVB-induced SCCs. These data suggest that p38α MAPK regulates oxidative stress in SCCs by blocking NOX2 in the absence of functional p53, and that the loss of p38α MAPK contributes to an increased capacity for cell proliferation.

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Merkel cell polyomavirus impairs repair of UV radiation-induced DNA damage

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Merkel cell carcinomas typically occur on sun-exposed skin and are associated with a high degree of morbidity and mortality. Recently, these cancers have been strongly associated with a novel member of the polyomavirus family. The Merkel cell carcinoma polyomavirus (MCV) expresses a large T antigen (LTAg) that is homologous to that of other polyomaviruses in which it is well-known to inhibit p53 activity. We hypothesized that expression of MCV suppresses the DNA damage response to ultraviolet radiation (UVR)-induced DNA lesions via LTAg, possibly by inhibition of p53. In contrast to a Merkel cell carcinoma cell line (UISO) that does not harbor virus, a virus-infected cell line (MKL-1) exhibited both dose-dependent reductions in survival following UVR, and drastically impaired global genomic nucleotide excision repair of UVR-induced cyclobutane pyrimidine dimers and pyrimidine(6-4)pyrimidone photoproducts as measured using an immunoassay. In a specific test of the relationship between MCV LTAg and repair, LTAg from a Merkel cell tumor was ectopically expressed in UISO cells, and resulted in reduced expression of a downstream p53 target, p21, as well as deficits in global repair of cyclobutane pyrimidine dimers similar to those seen in MKL-1 cells. These results indicate that expression of LTAg by MCV can inhibit p53's transactivating functions and lead to loss of global genomic nucleotide excision repair, and these events may be an important mechanism of genomic instability involved in Merkel cell photocarcinogenesis.

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XPC silencing in normal human keratinocytes induces AKT activation and triggers metabolic alterations that drive the formation of squamous cell carcinomas

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Genomic mutations, the Warburg effect, and alterations in the levels of reactive oxygen species (ROS) are consistently observed in a variety of cancers. However, the inter-relationships among these factors and their impact on the neoplastic process remain poorly understood. We took advantage of the intrinsic genomic instability arising in the nucleotide excision repair disease xeroderma pigmentosum C (XPC) to look at underlying molecular mechanisms. Here we show that shRNA-mediated knockdown of XPC in normal keratinocytes (KC) leads to the activation of the serine/threonine kinase Akt1 by DNA-dependent protein kinase (DNA-PK). Akt activation in XPC-deficient KC resulted in 1) increased cell proliferation as determined by BrdU labeling, 2) metabolic alternation through reduction of mitochondrial function but increases glycolysis, as defined by decreases in the NADH dehydrogenase subunit 1 and cytochrome c oxidase subunit III; and increases in proteins involved in glucose uptake and lactate generation. We also detected high levels of basal cell layer markers Keratin 14, α6 and β1 integrin in epidermis reconstructed with XPC-deficient KC, which manifested epithelial hyperplasia with large and frequent extension of rete pegs, compared to control epidermis. These effects were also dependent on Akt activation. Furthermore, XPC-deficient KC formed SCCs in immunodeficient mice, but Akt1 inhibition blocked this effect. Our results indicate that Akt1 activation in cells lacking XPC contributes to high susceptibility to carcinogenesis and this may involve Akt1-mediated alteration of mitochondrial function.

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Elucidation of hair follicle differentiation patterns in human basal cell carcinoma

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Basal cell carcinoma (BCC) is a common epithelial cancer, with defined clinical and histological variants. The hair follicle origin of BCC, as suspected clinically because of their predilection for hair bearing skin, is supported by transgenic mouse studies. But unlike the elegant differentiation programme exhibited by the hair follicle, with both root sheath and hair shaft formation, by conventional microscopy differentiation appears absent in BCC. We therefore hypothesised that all BCC tumour cells represent a single hair follicle differentiation lineage. To test this hypothesis we carried out immunofluorescence microscopy using well characterised antibodies to hair follicle differentiation markers and transcription factors in 10 BCC samples, using normal hair bearing skin as both positive and negative control. All BCC samples expressed the outer root sheath (ORS) keratins, K6 and K17, consistent with over expression of the sonic hedgehog signalling pathway and nuclear translocation of the transcriptional regulator Gli1. In seven samples tested, there was also expression of the ORS and ORS companion layer keratins K16 and K75 respectively, in a sub-population of cells within the tumour mass; coinciding with nuclear translocation of C/EBP-beta. Those BCC samples expressing K16 and K75, also expressed the inner root sheath keratin K27 but not K28 in a small sub-population of tumour cells. In contrast, none of the BCC samples demonstrated expression of the early hair shaft keratins K31, K32 and K81. In conclusion, BCC exhibit multiple normal hair follicle differentiation patterns, demonstrating a hierarchical organisation albeit restricted to the hair follicle root sheath. These findings are consistent with the cancer stem cell model of tumour growth and support the potential existence of tumour initiating cells (cancer stem cells) within BCC.

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Specific T cells prevent oncogene-driven malignant transformation

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Oncogenic T antigen (Tag) under the control of the rat-insulin-promotor (RIP) leads to the development of beta-cell tumors in transgenic RIP1-Tag2 mice. Previous studies revealed that interferon-gamma (IFN-gamma)-producing, Tag-specific Th1 cells strongly inhibit tumor development in the absence of significant beta-cell destruction. Here we show that Tag-specific Th1 cells reduce the proliferation of oncogene-expressing islet cells in RIP1-Tag2 animals in a strictly IFN-gamma-dependent manner. To uncover the underlying mechanisms, we analyzed the time-dependent malignant transformation by staining tissues and isolated tumor cells for the beta-cell differentiation markers synaptophysin (early marker), insulin (mid-term marker) and the glucose transporter 2 (Glut2; late marker). The functionality of the beta-cells was characterized by a glucose-dependent mitochondrial activity assay *in vitro*. During oncogenesis islet cells lost their differentiation markers in an ordered fashion: first Glut2 then followed by insulin, whereas the Tag-expressing tumor cells remained positive for the early differentiation marker synaptophysin. The observed dedifferentiation of the tumor cells was reflected by the inability of insulinoma cells to respond to high glucose levels. Treatment of RIP1-Tag2 mice with Tag-specific Th1 cells prevented the malignant transformation of the beta-cells. The islet cells in the treated animals did not only survive without loosing their differentiation markers and functionality to respond to high glucose. Importantly, and in contrast to the sham-treated mice, islets from Th1-treated mice acquired neither the capacity to grow autonomously *in vitro* nor to form metastases upon adoptive transfer. Thus, our data provide the first experimental evidence that tumor-specific immune cells, rather than killing their targets, arrest malignant transformation of oncogen-driven tumors by keeping the cells in a pre-malignant, differentiated state.

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mTOR-dependent Sirtuin 1 phosphorylation regulates DNA damage-induced cancer cell survival in UVB-induced skin carcinogenesis

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Premature senescence (PS) is an irreversible stress condition in which damaged cells are unable to proliferate, but remain viable. Cancer cells are driven to enter PS by DNA-damaging chemotherapeutic drugs and this phenomenon is potentially a novel approach for controlling tumor growth. However, because cancer cells in PS are inherently less sensitive to DNA-damaging chemotherapeutic drugs, a major drawback with this approach is that PS tumor cells can re-enter the cell cycle and ultimately lead to tumor recurrence. Here, we identified a novel mechanism that regulates the survival of DNA damage-induced PS squamous cell carcinoma (SCC) cells. Treatment of human SCC A431 cells with the anthracycline antibiotic doxorubicin led to the accumulation of apoptosis-resistant G1-arrested cell populations. These apoptosis-resistant cells exhibit PS-like characteristics. Doxorubicin-induced PS involves the nuclear translocation of mTORC1, followed by the mTOR-dependent inhibitory phosphorylation of SIRT1 at S47. This phosphorylation-dependent inhibition of SIRT1 deacetylase activity leads to the concomitant upregulation of an anti-apoptotic Bcl-2 family gene, Bfl-1/A1, which involves increased acetylation of p65/RelA NF-κB. SIRT1 S47 phosphorylation requires physical interaction between the mTOR complex and SIRT1. This novel SIRT1 phosphorylation occurs predominantly in recurrent/metastatic SCCs, thus suggesting a role in tumor progression. Doxorubicin-induced PS A431 cells eventually re-enter the cell cycle, but rapamycin-mediated mTOR inhibition can subsequently block the re-growth of PS A431 cells. Furthermore, rapamycin treatment sensitizes UVB-induced doxorubicin-resistant SCCs for apoptosis in mice. Our data indicate that the mTOR inhibition of SIRT1 fosters the survival of DNA damage-induced PS SCC cells, and that targeting mTOR/SIRT1 signaling may be effective in treating apoptosis-resistant cancer cells.

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Comparison of the squamous cell carcinoma transcriptome with normal skin by ultra-high-throughput sequencing (RNAseq): detailed maps of changes associated with malignancy

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Our understanding of tumorigenesis is still incomplete. Several high-throughput approaches have been implemented to obtain a broader picture of the molecular alterations associated with cancer. Recent advances in sequencing technologies (RNAseq) have allowed for the sequencing of entire transcriptomes. This novel technology is offering new insights into the contribution of processes such as alternative splicing, antisense transcription, alternative 3′and 5′UTR usage, mutations, and expression changes to tumorigenesis at the single nucleotide level. Here, we describe the first transcriptome comparison between a squamous cell carcinoma of the skin and normal skin. We have obtained a detailed map of the genes expressed in the skin and skin cancer and their expression levels. We obtained more than 250 Mbp of sequencing information from this initial experiment and identified about 6000 at least two-fold differentially expressed genes. Using Gene Ontology (GO) terms to categorize the differentially expressed genes, the cancer tissue showed enrichment for genes associated for example with cell growth/mitosis, response to DNA damage, immune response, organelle organization, catabolic processes and establishment of RNA localization. Cell-cell and cell-substrate adhesion and cell motility genes were underrepresented in the tumor transcriptome. The challenges and pitfalls of whole transcriptome sequencing using the Illumina platform are complex but the prospects of having this wealth and detail of information provides new insights into the changes accompanying tumorigenesis.

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The role of cancer stem cells in the initiation and propagation of human cutaneous squamous cell carcinoma in an *in vivo* model

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Primary human cutaneous squamous cell carcinoma (SCC) is the second most common cancer in the US, and is a common cause of morbidity and mortality in high risk patients including renal transplants and recessive dystrophic epidermolysis bullosa. To identify SCC cancer stem cells, we have developed an SCC xenograft *in vivo* model that can accurately recapitulate the original SCC histology and growth rate. In this model, SCC cells are implanted into a humanized inflammatory bed that was created with either pre-implantation of a glass disc or a foam dressing together with human fibroblasts. With this *in vivo* model, SCC xenograft growth from unsorted SCC cells was dose dependent, but was not observed with <10*5 engrafted SCC cells. However we found that the CD133+CD45- subset representing approximately 0.9% of total SCC cells, could recreate growth with as few as 10*2 cells. Histologically, the CD133+CD45- cells are located on the outer edge of SCC tumor projections as rare clusters. In the xenografted SCC tumors, the CD133+CD45- persisted as a tiny subset and could be isolated for serial transfer of the SCC xenograft tumors, demonstrating the stem cell properties of self-renewal. The number of CD133+CD45- cancer stem cells did not vary significantly in different histological grades of SCC. Additionally, the resultant xenograft tumor size following engraftment was dependent upon the number of CD133+CD45- cells implanted, irrespective of the tumor histological grade or origin. In summary, the CD133+CD45- subset of SCC function as cancer stem cells and retain the intrinsic histology and growth properties of the original SCC tumor.

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Chronic exposure to nanosized titanium dioxide negatively effects DNA replication

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Titanium dioxide (TiO_2) is one of the most widely used nanomaterials in skin care products. Nanosized TiO_2 has unique properties making it effective at absorbing high-energy ultraviolet radiation (UVR). Limited evidence suggests nanoTiO_2 particles do enter the body. Most laboratory research has focused on short-term, high concentration exposures to nanoTiO_2. Regular use of products containing nanoTiO_2 indicates chronic, long-term exposure to lower concentrations is more relevant. We conducted a long-term exposure study of nanoTiO_2 using the epithelial Chinese hamster ovary (Cho) cell line. Cells were maintained under exponential growth conditions for 60 days and were continuously exposed to either 10, 20, or 40 μg/ml TiO_2 (<25 nm) nanoparticles. Effects on the production of reactive oxygen species (ROS), DNA content, cell cycle, light scattering, and mutagenicity have been measured. In addition, electron microscopy is being used to document particle size and uptake, and ICP-MS is being used to estimate cellular associated levels of TiO_2. No significant effects on viability or proliferation were apparent using the XTT assay. ROS levels increased in a time- and concentration-dependent manner using the ROS reactive dye, dihydrorhodamine 123. Variation in DNA content and the number of cells in the G2+M phase of the cell cycle increased in a concentration-dependent manner suggesting negative effects on DNA replication and cytokinesis. Light scatter increased with increasing levels of TiO_2 supporting a close, quantitative association between nanoTiO_2 and Cho cells (i.e., internalized or externally bound). The mutagenicity assay is currently being finalized along with the EM and ICP-MS analyses. This study suggests that chronic, continuous exposure to low levels of nanoTiO_2 increases oxidative stress, and impacts the cell cycle and the genetic material in a sub-toxic and sub-lethal manner. Such non-toxic, non-lethal impacts may increase adverse health risks in chronically exposed individuals.

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Lack of novel merkel cell polyomavirus in mycosis fungoides tumors

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The etiology of cutaneous T-cell lymphoma (CTCL) is unknown, but results from some studies support a hypothesis of an infectious cause. Numerous studies have evaluated the roles of various infectious agents in CTCL, including retroviruses, Staphylococcus aureus, and herpesviruses, but none have revealed a consistent association of any pathogen with CTCL. However, recent spectroscopy analysis of T-cell receptor (TCR) repertoire in CTCL demonstrated a loss of TCR complexity similar to that observed in patients with HIV. These findings reinforce the possibility that a new, as yet unidentified viral pathogen may play a role in CTCL. Recently, a novel polyomavirus (Merkel cell polyomavirus, or MCV) was discovered as a probable carcinogenic agent in Merkel Cell Carcinoma (MCC). Presence of MCV is not limited to MCC cells, having also been detected in normal skin, non-MCC skin tumors, and various other tissues. The association between MCV and CTCL has not been evaluated; we sought to determine whether MCV is present in CTCL tumors. Patients under our direct care with established diagnoses of Mycosis Fungoides (MF) were enrolled in the study after informed consent. Antigen retrieval was performed on formalin-fixed, paraffin embedded tumor biopsies, which were then stained immunohistochemically with CM2B4, a monoclonal antibody specific for the MCV T antigen. Known MCV-positive and MCV-negative MCCs were utilized as positive and negative controls, respectively. None of the tumors evaluated in this study demonstrated presence of MCV by immunohistochemistry. Findings from this small cohort of patients suggest a lack of association between MCV and CTCL. Larger scale studies using more sensitive methods for viral detection may be useful for completely ruling out the etiologic relationship.

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A novel approach to gene expression profiling in Sezary Syndrome

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The purpose of this study was to minimize the heterogeneity of information derived from whole-genome expression analysis by comparing highly purified malignant and nonmalignant (control) T cells from the same Sezary Syndrome (SS) patient. Previous microarray studies have compared T lymphocytes of SS patients to those of normal controls; a major limitation of this design is that intra-patient genetic variability produces differences in gene expression unrelated to disease state. Mononuclear cells were obtained from a patient with histologically confirmed SS. The malignant cells were separated from non-malignant cells by multiparameter flow cytometry. Malignant cells expressed the dominant T-cell receptor-Vβ (TCR-Vβ); controls lacked the dominant TCR-Vβ but were otherwise phenotypically identical (CD3+/CD4+/CD45RO+). These cell populations were compared using the Sentrix Human-6 expression BeadChip from Illumina, Inc. Analysis of the patient's transcriptome using the J5 test showed differential expression of 44 genes between the malignant and nonmalignant subsets. Promyelocytic leukemia zinc finger protein (ZBTB16) was the most profoundly upregulated gene in the malignant cell population, while interferon regulatory factor-3 (IRF3) and interferon-induced protein-35 (IFI35), which are important elements of the cellular response to viral infection, were significantly downregulated. A pathway-level impact analysis showed the leukocyte transendothelial migration and cell adhesion molecules pathways to be significantly impacted. The results of this study suggest the feasibility of this novel comparative approach to genomic profiling in SS. Using this approach, we identified several genes and pathways not previously described in SS. While these findings require validation in larger studies, they may have important implications for SS pathogenesis.

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Dysregulated ΔNp63α modulates p16 expression, blocks senescence, and promotes malignant conversion of keratinocytes

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p63 is critical for squamous epithelial development, and elevated levels of the ΔNp63 isoform are seen in squamous cell cancers of various organ sites. We have previously shown that adenoviral-mediated overexpression of ΔNp63 in primary mouse epidermal keratinocytes blocks differentiation-associated growth arrest and induction of differentiation-specific gene expression. However, long-term biological effects of sustained ΔNp63α dysregulation and its contribution to squamous cancer pathogenesis remain unknown. Lentiviruses were developed to drive long-term overexpression of ΔNp63α. *In vitro*, keratinocytes expressing lenti-GFP, but not lenti-ΔNp63α, underwent both spontaneous replicative senescence and oncogenic v-rasHa-induced senescence, as evidenced by the expression of SA-β-gal or the presence of nuclear foci of heterochromatin protein 1γ (HP1γ). Both replicative and oncogene-induced senescence were accompanied by an upregulation of p16, and decreased nuclear levels of E2F1. p16 upregulation is delayed and attenuated in lenti-ΔNp63α cells. The relationship between ΔNp63α and p16 was further confirmed following transient adenoviral-mediated expression of ΔNp63α, which delayed p16 induction until ΔNp63α decreased to endogenous levels. Keratinocytes expressing lenti-GFP- or lenti-ΔNp63α alone consistently formed normal skin following grafting to nude mice. In contrast, lenti-GFP/v-rasHa keratinocytes developed well-differentiated papillomas, whereas lenti- ΔNp63α/v-rasHa keratinocytes formed undifferentiated carcinomas. The average volume of lenti-ΔNp63α/v-rasHa tumors was higher than those in the lenti-GFP/v-rasHa group, consistent with increased BrdU incorporation detected by immunohistochemistry. Taken together, our findings suggest that long-term overexpression of ΔNp63α, as seen in human squamous cell cancers, supports keratinocyte proliferation and inhibits senescence, thereby facilitating keratinocyte transformation.

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Therapeutic potential of dermal rejuvenation for treating non-melanoma skin cancer: Restoring an appropriate UVB response in geriatric skin

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The majority of non-melanoma skin cancers occur in people over the age of 60. As dermal fibroblasts age, their capacity to produce IGF-1 is severely diminished; therefore, keratinocytes in aged skin are provided with a reduced supply of IGF-1 and a concomitant reduction in IGF-1R activation. Our recent data have shown that in the absence of IGF-1R activation, keratinocytes which survive UVB-induced apoptosis retain the capacity to proliferate despite DNA damage, potentially giving rise to initiated carcinogenic keratinocytes. Previously we have shown that geriatric skin *in vivo* responds to UVB via this inappropriate manner and that this response can be corrected by treatment with exogenous IGF-1. Therefore, therapies that can increase fibroblast IGF-1 expression in geriatric skin may prophylactically prevent the occurrence of non-melanoma skin cancer. We examined whether dermal rejuvenation therapies (dermabrasion and fractionated laser resurfacing) could alter dermal IGF-1 levels. Small areas of sun-protected skin on geriatric volunteers (>65 yo) were treated via dermabrasion or fractionated laser resurfacing and the skin was allowed to heal for three months. At that time, comparison of untreated and treated skin demonstrated that dermal rejuvenation therapies restored geriatric skin IGF-1 and pro-collagen levels to that seen in young adult skin. Moreover, dermal rejuvenation restored the appropriate UVB response to geriatric skin, preventing keratinocytes from proliferating with UVB induced DNA damage. Therefore, the use of dermal rejuvenation therapies may potentially be used to prevent the initiation of non-melanoma skin cancer.

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Restoring FAS expression by reversing FAS promoter methylation: A novel approach to CTCL therapy

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The FAS (CD95) pathway plays a major role in T-cell apoptosis. Using in-situ immunohistologic analysis of lesional skin, we showed previously that many cases of CTCL express low levels of FAS protein despite generally intact FAS gene coding and promoter regions. In order to explore other mechanisms of FAS down-regulation in CTCL, we used pyrosequencing to determine the extent of CpG island methylation within the FAS promoter in CTCL lines MyLa, Hut-78, HH and SZ4. SeAx could not be studied because it lacks FAS gene detectable by cytogenetics or FISH. We found that the FAS-high lines MyLa and Hut-78 had minimal FAS promoter methylation and marked sensitivity to FAS-ligand induced apoptosis. In contrast, the FAS-low lines HH and SZ4 had high levels of FAS promoter methylation at several CpG islands and were resistant to FAS-ligand mediated apoptosis. Following treatment with the demethylating agent, 5-azacytidine, FAS-low CTCL lines exhibited demethylation of the FAS promoter and significant up-regulation of FAS protein expression. This correlated with a major increase in apoptosis when these cells were exposed to aggregated FAS-ligand. Consistent with the known enhancer effect of NFkB on FAS expression, chromatin immunoprecipitation (ChIP) studies of FAS-low CTCL lines showed that demethylation-associated FAS up-regulation was accompanied by a 2-4 fold increase in NFkB1/RelA binding to the FAS promoter. In aggregate, these findings demonstrate a strong correlation among epigenetic FAS promoter methylation, FAS protein expression and sensitivity to FAS mediated apoptosis in CTCL. Furthermore, we show that demethylating agents can enhance NFkB binding to the FAS promoter, restore FAS expression and increase sensitivity to apoptosis. There are several agents suitable for clinical use that can demethylate DNA and others that can promote FAS pathway apoptosis. Therefore, our findings open a novel approach to CTCL therapy.

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Protein Kinase D protects keratinocytes from UVB-induced apoptosis

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Protein kinase D (PKD) is a serine/threonine kinase that has been implicated in numerous cellular signaling events. Previous studies performed in our laboratory show that PKD levels are upregulated in human basal cell carcinomas and in a neoplastic mouse keratinocyte line, supporting a possible tumorigenic role for PKD in epidermis. These data, along with the recent evidence that PKD localizes not only within the cytoplasm and nucleus, but also to the mitochondria, a major ultraviolet (UV)-responsive organelle, led us to investigate the ability of UVB to activate PKD. Our hypothesis is that irradiation of primary mouse keratinocytes with UVB leads to activation of PKD, and PKD can relay signals by responding to reactive oxygen species (ROS) generated within the cell with the net effect being survival and/or proliferation. Our data suggest that PKD is indeed activated in a time- and dose- dependent manner in primary mouse keratinocytes following UVB exposure. Pre-treatment of keratinocytes with anti-oxidants reduced PKD activation following UVB irradiation, suggesting a possible link between ROS generation and PKD activation. Using inhibitors against protein kinase C (PKC) and Src family tyrosine kinases, we have also shown that UVB-induced PKD activation is primarily mediated by an upstream Src family tyrosine kinase pathway rather than a PKC-dependent process. Also, UVB irradiation of primary mouse keratinocytes resulted in a dose-dependent increase in apoptosis, and this cell death could be rescued by adenovirus-mediated overexpression of wild-type PKD and not by mutant versions of the enzyme. Together, our data suggest that irradiation of primary mouse keratinocytes with UVB leads to activation of PKD in a time- and dose-dependent manner, and activated PKD can relay signals by responding to ROS generated within keratinocytes. Thus, PKD plays an important role in protecting cells from UVB-induced apoptosis, supporting the idea that this enzyme is a pro-survival signal in keratinocytes and linking basal cell carcinoma, sun exposure and PKD.

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Deregulated hedgehog signaling in the pathogenesis of Barrett's disease and gastric adenocarcinoma

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In skin, physiologic Hedgehog (Hh) signaling plays a central role in driving proliferation of hair follicle epithelium, and when abnormally activated, Hh signaling leads to basaloid skin cancer development. The Hh pathway has also been linked to the development of Barrett's esophagus and gastric adenocarcinoma, the second leading cause of cancer mortality worldwide, but a causal role for Hh signaling in the pathogenesis of this tumor has not been established, and little is known about Hh-responsive cell types in normal stomach. We show that mice engineered to mimic oncogenic Hh signaling in stomach, using the transcription factor GLI2*, develop invasive gastric adenocarcinomas which frequently arises near the squamocolumnar junction (SCJ). To identify Hh-responsive potential tumor progenitor cells in this region, we mapped Hh pathway activity using Gli1-, Gli2-, and Ptch1-lacZ reporter mice. Remarkably, endogenous Hh signaling in gastric epithelial cells is detected only near the SCJ in a small population of basal cells in the forestomach, a stratified squamous epithelium. To study the consequences of uncontrolled Hh pathway activation in these cells, we generated bitransgenic mice carrying a Cre-inducible GLI2* allele and either Keratin 5 promoter-targeted or Gli1 promoter-targeted Cre alleles. Both mouse models developed a dysplastic, Barrett's-like epithelium near the SCJ. While most of the abnormal cells expressed glandular keratins K8 and K19, a subset also expressed Keratins 5 or 14, supporting the idea that these lesions are derived from Hh-responsive basal cells in the forestomach. A similar keratin expression pattern was also seen in early gastric adenocarcinomas arising near the SCJ. Our data point to a causal role for deregulated Hh signaling in the pathogenesis of Barrett's disease and gastric adenocarcinoma, and identify Hh-responsive forestomach basal cells near the SCJ as the likely progenitors which are reprogrammed to give rise to these glandular lesions.

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Srcasm biology-a potential link between Notch 1 and senescence

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Srcasm functions as an anti-oncogene by inhibiting the formation of squamous cell carcinomas in K14 Fyn Y528F transgenic mice. In this model, Fyn lowers p53 and Notch 1 levels. Srcasm inhibits tumor formation by downregulating Fyn levels and normalizing p53 and Notch 1. The current study further defines a relationship between Notch 1, Srcasm, and neoplasia. Human keratinocytes treated with DAPT to lower NICD levels demonstrate dose-dependent decreases in Srcasm. In addition, human keratinocytes preincubated with DAPT to lower NICD and Srcasm levels demonstrate a rapid, time-dependent increase in the levels of both molecules upon DAPT removal. Such data demonstrate a tight correlation between NICD and Srcasm levels. In silico analyses of the murine and human Srcasm loci, demonstrate a novel Srcasm transcript initiating in intron 7 and containing exons 8-16. Quantitative RT-PCR analysis of murine skin from C57BL/6 and FVB mice confirmed the presence of both full-length Srcasm transcript (FL) (exons 1-16) and intronic Srcasm transcript (IN) at a ratio of approximately 4:1. qRT-PCR analysis of skin RNA from K14-Fyn Y528F and SMA-DNMAML mice which have impaired Notch 1 signaling demonstrated a ratio of FL:IN::1 to 0.1:1. Analysis of SCC tumor RNA from K14-Fyn Y528F and SMA-DNMAML mice demonstrated a ratio of FL:IN::0.025 to 0.016:1. Therefore, impaired Notch 1 signaling is associated with a marked decrease in full length Srcasm transcript and a relative increase in intronic Srcasm transcript. Notch promotes senescence in various cell types; therefore, we examined the effect of Srcasm levels on senescence in HCT116 cells. Stably transfected clones expressing elevated Srcasm demonstrated increased numbers of enlarged, multi-nucleated cells senescent cells. Stable transfectants expressing Srcasm demonstrated increased sensitivity to tyrosine kinase inhibitor dasatinib that was dose-dependent. These data suggest that Srcasm is regulated by Notch and promotes cell senescence.

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Hairless gene confers resistance to nonmelanoma skin cancer development

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Hairless gene (hr) is known to be important in regulating hair growth. Mutations in this gene cause alopecia leading to permanent hair loss. It is known that hairless mice which carrying homozygous mutations in hr gene develop more squamous cell carcinomas (SCCs) than their haired littermates. This has led to a hypothesis that hr may act as tumor suppressor for cutaneous SCCs. However, the role of hr in the pathogenesis of BCCs remains elusive. BCCs are considered to be a neoplasm originating from skin hair follicles. In this study, we show that hr is involved in the pathogenesis of both BCCs and SCCs. For this, we have bred ptch+/-/C57BL6 mice with SKH-1 hairless mice and then allowed brother-sister crossing to get F2 littermates carrying hr-/- or hr+/+. These animals were irradiated with UVB (240mJ/cm2) of for 44 weeks. We observed that hr-/- mice developed first tumor at week 22 whereas shaved hr+/+ mice developed first tumor following 32 weeks of UVB. At week 32, 100% of hairless mice developed >15 whereas only 20% of haired mice developed <3 tumors/mouse at 44 weeks of irradiation. The tumor spectrum in hr-/- and hr+/+ animals was also different. Hr+/+ mice developed some aggressive spindle cell carcinoma which were absent in hr-/- mice. Hr-/- animals developed more SCCs and some sarcomas. Similarly, the incidence of BCCs was significantly higher in hr-/- animals ($p<0.001$). We also observed that UVB-induced murine skin tumors in hr-/- are more aggressive than those developed in hr+/+ littermates. The molecular typing of these tumors showed that loss of hr is associated with a decreased expression of E-cadherin and an enhanced expression of mesenchymal proteins, twist, snail, slug, fibronectin and vimetin. These data suggest that the loss of hr may enhance epithelial-mesenchymal transition. The mechanism by which these alterations are mediated is not understood. We are exploring various mechanisms to explain these results.

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Epigenetic mechanisms regulate the T-Plastin (PLS3) gene, a gene highly expressed in Sezary T cells

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Sezary T cells have been found to express T-plastin, PLS3, at high levels. PLS3 is an actin bundling protein ubiquitously expressed in non-lymphoid cells, but silenced in lymphoid tissues. We have found that PLS3 can be used to monitor disease progression with Sezary syndrome. Analysis of the PLS3 gene reveals numerous CpG dinucleotides from the proximal promoter into the first intron, which may be targets for epigenetic silencing by DNA methylation. Using lymphoid (Jurkat and FSCCL) cells where PLS3 is silent and non-lymphoid (HT-1080 and DU145) cell lines where PLS3 is highly expressed, we study the role of CpG methylation in regulating PLS3 expression. By methylation-sensitive restriction enzyme analysis of the PLS3 gene, we identified differences in the methylation of CpG in the PLS3 gene between lymphoid cell lines and non-lymphoid cells. In transfection studies, we show that methylation of PLS3 promoter-reporter plasmids using the CpG-specific methyltransferase SSSI suppresses gene expression. Culturing Jurkat T cells in the presence of demethylation-drug, 5-Azacytidine, leads to increases in PLS3 transcriptional activation, supporting CpG methylation plays a substantial role in silencing of this gene. We show further that the PLS3 gene, in cells that express it, is in an accessible state to micrococcal nuclease (MNase) by chromatin accessibility assay. To further define the role of CpG methylation in PLS3 gene expression we analyze fresh Sezary and normal CD4 T cells. We show that normal CD4 cells have differential CpG methylation compared to Sezary T cells. These results provide evidence that epigenetic changes in DNA methylation play an important role in the aberrant gene expression observed in Sezary syndrome. Better understanding of the mechanism activating the PLS3 gene may provide insight into the pathogenesis of Sezary syndrome.

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High-resolution characterization of the cutaneous T-cell lymphoma genome

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A better understanding of the genetic mutations in human malignancies holds promise for improving clinical cancer care through the molecular classification and identification of novel therapeutic targets. Employing the latest microarray technology, we performed one of the highest-resolution genomic characterizations of cutaneous T-cell lymphoma (CTCL) to date. Malignant T cells were isolated from the peripheral blood of 23 CTCL patients from which we generated DNA copy number data (1.8 million probes) and mRNA expression data (764,885 probes). Using statistical algorithms that discern both genetic commonalities and outliers, we identified significantly altered regions in the CTCL genome harboring novel oncogenes and tumor suppressors. The most significant regions of copy number change were amplifications on 8q and 17q, and deletions on 17p and 10p. Moreover, in combining our data with seven other low-resolution genomic data sets, we determined a consensus of 13 regions of DNA copy gain and 13 regions of DNA copy loss. We believe this common set of copy number mutations in CTCL represents one of the most comprehensive views of the CTCL genome at present. We also assessed the clinical status of each patient's skin disease before and after treatment with either photopheresis alone or in combination with systemic therapy to identify differentially expressed genes associated with response to therapy. Interestingly, a mitotic checkpoint kinase NEK2, which has been previously found to promote aneuploidy, emerged as one of the most upregulated genes in treatment non-responders and may represent a novel therapeutic target in CTCL. Overall, our analytic approaches identified patterns in the CTCL genome with potential implications for pathogenesis. This may help focus future therapeutic developments and facilitate the genetic classification of CTCL.

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Novel ATM/ATR pathway inhibitors sensitize p53-deficient cells to DNA-damaging agents *in vitro* and *in vivo*

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ATM and ATR are two major kinases that sense DNA damage and activate DNA damage checkpoints to slow cell cycle progression and promote DNA repair. Inhibition of ATM/ATR pathways in the presence of DNA damage abrogates DNA damage checkpoints, leading to death of DNA-damaged cells. Because many cancers are resistant to cell death owing to p53 functional loss, sensitizing such cells to DNA-damaging agents is particularly important in cancer therapy and can be achieved by inhibition of ATM/ATR pathways. To discover novel small-molecule inhibitors of ATM/ATR pathways, we performed a microscopy-based screen of 9,195 compounds by examining the inhibitory effects of compounds on replication stress-induced phosphorylation of Chk1, a downstream target of ATR. We found 4 novel compounds that inhibited ATM/ATR signaling pathways over 200 times more potently than caffeine (a known, non-selective ATM/ATR inhibitor). The novel ATM/ATR pathway inhibitors showed synergistic effects on killing cancer cells in combination with cisplatin. These effects were greater in p53-deficient cancer cells than in p53 wild-type cells. Importantly, at the same doses, the compounds were not toxic to normal cells (primary human keratinocytes). One of the compounds was tested in a p53-deficient xenograft model in which it augmented the efficacy of cisplatin. Inhibition of the ATR pathway has been found to be beneficial in the elimination of UV-damaged cells and the prevention of UV-induced skin cancers. The novel ATM/ATR pathway inhibitors augmented UV-induced cell death in primary human keratinocytes, suggesting that these compounds may prevent UV-damaged cells from progressing into cancers. Identifying the cellular targets of these DNA damage response pathway inhibitors may lead to novel mechanisms for preventing UV-induced skin cancers and for augmenting the efficacy of DNA-damaging agents in cancer therapy.

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Identification of rare skin cancer stem cells using a syngeneic mouse model

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The concept of cancer stem cells (CSCs), first posited over fifty years ago, has in the last decade been buoyed by studies that identified tumor initiating cells in both hematapoetic and solid tumors. Using a mouse skin cancer model, we have uncovered evidence that squamous cell carcinomas (SCCs) are hierarchically organized and arise from CSCs. We are able to isolate these multipotent "side population" (SP) cells based on their relative ability to efflux Hoechst dye. We are able to show that as few as 25 of these SP cells can recapitulate the original tumor when grafted onto a nude mouse. However, recent studies have demonstrated that severely immunocompromised microenvironments are permissive to tumor formation by nearly any tumor cell. This may imply that an immunologically normal graft recipient is able to identify and eliminate most tumor cells while ignoring CSCs. Thus, immune evasion may be a property that distinguishes CSCs from non-CSCs. To address this possibility, we separately grafted SP cells and non-SP cells into immunologically normal, syngeneic hosts and found that tumor formation is solely a capability of SP cells. To further characterize these skin SCCs, we also investigated the importance of Wnt/ β-catenin signaling. It has been suggested that Wnt/ β-catenin signaling is essential to skin cancer stem cell maintenance even though β-catenin is not required for normal skin development. We find that the SP cells are in fact devoid of activated β-catenin, as are the SCCs from which they are derived. Further characterization of the SP population and identification of better markers of CSCs in these tumors are the next steps toward an understanding of the molecular differences that will allow more effective and specific skin cancer therapies.

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PAF receptor knockout mice exhibit increased chemical carcinogenesis and an increase in chronic PMA-induced inflammation

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While previous studies have suggested that the platelet activating factor receptor (PAF-R) may be involved in regulating tumor angiogenesis as well as the invasiveness or metastasis of established tumors, there is no definitive data implicating the PAF-R in *de novo* tumorigenesis. However, there is overwhelming evidence that PAF-R activation has potent pro-inflammatory activity, particularly in type I hypersensitivity reactions. In this study, we show that mice with germline deletion of the PAF-R (KO mice) exhibit an approximately two-fold increase in tumor multiplicity following a DMBA/PMA two-stage chemical carcinogenesis protocol. In addition, tumor progression to squamous cell carcinoma (SCC) was also augmented: 32% of tumors were classified as SCC in KO mice compared with <1% of tumors in wildtype (WT) mice. Surprisingly, while loss of the PAF-R was not associated with significant changes in PMA-induced sustained hyperplasia, tumor proliferation, or DMBA-induced apoptosis, the skin of KO mice did exhibit an unexpected increase in PMA-induced chronic inflammation. Compared with WT mice, KO mice treated twice weekly with PMA for 3 weeks showed a significant increase in skin thickness, increased myeloperoxidase expression and activity, and increased expression of a number of inflammatory cytokines and chemokines. The above data indicates that the PAF-R plays an important role in suppressing cutaneous carcinogenesis. Possibly of more importance, our data showing that PAF-R KO mice exhibit a paradoxical increase in chronic PMA-induced inflammation indicates that the PAF-R may possess more complex immunomodulatory functions than previously recognized.

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Further characterization of C-KIT and PDGFR-alpha expression and mutational status in Merkel cell carcinoma: Implications for treatment with imatinib mesylate

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Merkel cell carcinoma (MCC) is an aggressive malignancy with a poor response to conventional chemotherapy. Therefore, novel chemotherapeutic approaches are needed. Gastrointestinal stromal cells tumor (GIST) and MCC express the protein kinases KIT and PDGFRA. Activating mutations in either the C-KIT or PDGFRA genes are responsible for oncogenesis in GISTs and confer responsiveness to imatinib mesylate. We propose that MCCs may demonstrate mutations in C-KIT and/or PDGFRA and may therefore demonstrate a treatment response to imatinib mesylate. Paraffin embedded tissue was obtained from 25 MCCs. The diagnosis was confirmed by histopathologic appearance and expression of CK 20 with negative TTF-1 staining. Eighteen of 25 MCCs (72%) demonstrated KIT expression; 21 of 25 MCCs (84%) demonstrated PDGFRA expression. Genomic DNA was obtained from 18 of 25 MCCs by laser capture microscopic dissection of paraffin-embedded tissue. Preliminary sequencing data from PCR amplification of C-KIT exon 11 demonstrated a heterozygous missense mutation in 13 of 18 (75%) samples. The mutant allele resulted in a glutamic acid (GAG) to lysine (AAG) substitution. Sequencing of non-tumor tissue (adjacent epidermis) failed to reveal this base change. This heterozygous single nucleotide change is novel and was not recognized in the UCSC database. Sequencing from PCR amplification of PDGFRA exon 12 failed to reveal any mutations. Based on this data, the amino acid substitution resulting from a guanine to adenine substitution at base pair 55288438 in C-KIT exon 11 is likely to result is dramatic change in KIT as the substitution results in a change from an acidic to a more basic amino acid. While we have not shown that this substitution leads to aberrant ligand independent tyrosine kinase activity and imatinib mesylate responsiveness, C-KIT exon 11 is the most common site of activating mutations in GISTs lending support to the importance of this mutation in the pathogenesis of MCC.

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Polo like kinase 1 (Plk1) is expressed by Cutaneous T-cell lymphoma (CTCL) and its down-regulation promotes cell cycle arrest and apoptosis

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Plk1, the most well studied member of the mammalian polo family of serine-threonine kinases, is crucial for mitosis and DNA integrity. Plk1 is up-regulated in many cancers and is highly expressed in dividing cells with peak expression during mitosis. This makes Plk1 an attractive anti-cancer target. Unlike the effects of current antimitotic agents (taxanes, vinca alkaloids), only dividing cells should be affected by Plk1 blockade. There are several Plk1 inhibitors in clinical trials and some have shown significant activity including complete and partial responses in patients with relapsed or refractory non-Hodgkin lymphomas. Therefore, we were interested in Plk1 expression by CTCL. There was a wide range of Plk1 expression detected by immunohistology in CTCL skin lesions with approximately <1%-40% (median 10%) of lymphoid cells showing nuclear or nuclear/ cytoplasmic staining. The proportion of positive cells tended to parallel but be less than the proportion of cells expressing Mib-1 which is present during all phases of the cell cycle in actively dividing cells. Plk1 expression was confirmed in CTCL lines (HH, Hut 78, MyLa, SeAx, and SZ4) by quantitative RT/PCR and Western blotting. Protein levels were similar to the A-375 human melanoma line which is regarded as relatively high level expression. Knock-down of Plk1 using lentiviral mediated shRNA resulted in cell cycle arrest, reduced cell growth and viability, and increased apoptosis as assessed by Annexin V/PI flow cytometry. Given our findings, clinical trials of Plk1 inhibitors in CTCL may be warranted.

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Defining composite cancer vulnerabilities by genetic engineering of human melanoma

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Malignant melanoma accounts for the vast majority of skin cancer lethalities and is likely due to its early metastatic behavior and absence of effective systemic therapy in the advanced stage. Despite public health-care campaigns aimed at ensuring early diagnosis and surgical resection of suspect lesions there is still an increase in incidence of melanoma. Melanoma originates from dedicated pigment-producing cells, the melanocytes, and it is generally accepted that BRAF(V600E), found in the vast majority of melanoma specimens, drives the growth and/or survival of melanoma making it a candidate for targeted cancer therapy. However, despite successful targeted inhibition of single oncogenes, such as BCR-ABL in CML using Imatinib, it is probable that tumor cell vulnerabilities originate from combinatorial effects of not one but multiple genetic mutations acting in concert. As multiple mutational lesions in cancer do contribute to the transformed phenotype, cancer cell vulnerabilities are possibly a result of cooperation among the activated oncogenes. Consequently, targeted exploitation of such composite cancer cell vulnerabilities would define opportunities for effective cancer therapy. To this end, we have embarked on a systematic identification of composite cancer cell vulnerabilities using engineered human melanoma cells, which harbor specific combinations of genuine and representative oncogenes. Using chemical compound screening, we illustrate how particular combinations of oncogenes result in unique cellular vulnerabilities. In extension, we seek compounds that can sensitize cells towards pharmacological BRAF(V600E)-inhibition, as resistance to BRAF(V600E)-targeted therapy may become a clinical dilemma as learned from Imatinib treatment of CML.

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New cancer models assessed via quantitative transcriptome analysis

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Improved cancer models are required to advance cancer therapeutics, however, it is unclear how faithfully any cancer model represents clinical disease, an ambiguity which frustrates clinical translation. To complement human squamous cell carcinoma (SCC) xenograft models, we generated inducibly invasive 3-D organotypic tissue neoplasias (OTNs) via clinically-relevant oncopathways in human keratinocytes within an entirely human tissue environment, which included fibroblast-populated stroma and intact basement membrane. Engineered OTNs recapitulate natural features of tumor progression, including invasion. To assess the faithfulness of this new model in comparison to other models, including those based on human cancer cell lines, we undertook quantitative transcriptome analysis. 565 genes were differentially expressed in OTNs compared to normal control (n=7/each, >2-fold change with FDR < 0.03), the relative expression of which correlated highly with a series of clinical SCCs (p= 1.7 x 10-14). To determine how accurately OTN reflects transcriptional changes in actual cancer, we iteratively calculated Pearson correlation coefficients between differentially expressed genes in each of 22 spontaneous SCCs to 5 SCC models including: 1) OTN 2) 2-D culture of epithelial cells used to generate OTN C) human SCC xenografts D) 2-D culture of a series of 10 human SCC cell lines and E) a 2nd independent set of human SCC tumors. The highest correlation was between the two SCC series, however, OTN was also highly correlated to a degree nearly identical to the *in vivo* grafting model. In contrast, 2-D culture models displayed Pearson coefficients of 0.0 with natural SCC tumors, indicating that 2-D culture adaptation may abolish gene expression patterns of human SCC tumors *in vivo*. These data establish a quantitative approach to assessing cancer model fidelity to patient cancers based on comparative global transcriptome correlation and demonstrate that 3-D organotypic neoplasia represents a faithful new model platform.

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Rho-associated Kinase (ROCK) promotes primary and v-ras transformed mouse keratinocyte differentiation

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ROCK or Rho-associated kinase, a serine/threonine kinase, is an effector of Rho-dependent signaling and is involved in actin-cytoskeleton assembly as well as cell motility and contraction. To evaluate the role of ROCK activity in mouse keratinocyte differentiation, the effect of Y-27632, a ROCK-specific inhibitor, was tested. Primary mouse keratinocytes cultured in medium containing 0.05 mM calcium maintain the basal cell phenotype and are proliferative. When the calcium concentration of the medium is increased to 0.12 mM, cultured primary mouse keratinocytes cease proliferation and start to express terminal differentiation markers keratin 1 (K1), keratin 10 (K10), filaggrin, and loricrin. Inhibition of ROCK by Y-27632 induced K1 and K10 expression in primary mouse keratinocytes cultured in 0.05 mM calcium medium and enhanced K1, K10, filaggrin and loricrin expression in cells grown in 0.12 mM calcium. Oncogenic transformation by v-H-ras blocks calcium-induced K1 and K10 expression in primary mouse keratinocytes. Addition of Y-27632 to v-H-ras transformed keratinocytes restored calcium-induced K1 and K10 expression. Enhanced ROCK activity was detected in v-H-ras transformed mouse keratinocytes, that may contribute to resistance to differentiation in v-ras transformed keratinocytes. Our results suggest that ROCK plays an important role in maintaining keratinocyte basal cell phenotype and also contributes to v-ras mediated transformation.

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Low expression of desmoglein 3 protein, revealed with quantitative digital morphometry, in basal cell carcinomas: an impact on pemphigus vulgaris pathogenesis?

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Basal cell carcinoma (BCC) is the most common type of skin cancer. Reasons to undertake this study of desmoglein 3 protein (DSG3) expression in BCC were twofold: (i) life-threatening, DSG3-linked pemphigus vulgaris (PV) can occasionally coexist with malignancy, (ii) hair follicles, which physiologically express DSG3, apparently are involved in both PV pathogenesis (mature follicular cells) and BCC pathogenesis (stem cells of the bulge region of hair follicle). DSG3 expression was evaluated with immunoperoxidase staining using monoclonal murine anti-DSG3 antibody (immunogen was human DSG3 extracellular domain) on frozen sections in BCC-affected areas (BCC tumor) and non-BCC-affected epidermis (control 1) in 22 patients with BCC and in more benign epidermal tumors than BCC (seborrheic keratosis and keratosis senilis) in 22 patients (control 2). The digital microscopic image analysis with quantitative morphometric software was then used to evaluate the intensity of DSG3 expression in the area of interest (tumor-affected, tumor-free). There was significantly lower expression of DSG3 in BCC tumor compared to both control 1 and control 2. There was no significant difference of DSG3 expression in control 1 and control 2. Thus, low expression of DSG3 in BCC might reflect locally invasive behavior of that tumor. However, diminution/lack of DSG3-mediated adhesion is not enough for BCC cells to separate fully as tightly packed cancer cells showing a palisade arrangement on the periphery of the tumor are characteristic for BCC. One might even speculate then that the total loss of adhesion (acantholysis) seen in PV requires more powerful stimuli than those provided by distorted function of DSG3. Conceivably, factors independent from DSG3 are capable to keep BCC cells in clusters, but are incapable to do the same as far as keratinocytes in PV are concerned.

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Heart autoantibodies in El Bagre endemic pemphigus foliaceus

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Several patients affected by a new variant of endemic pemphigus foliaceus in El Bagre, Colombia, (El Bagre-EPF) died of a sudden death syndrome including people in their forties. El Bagre-EPF patients share several autoantigens with patients with paraneoplastic pemphigus (PNP), such as reactivity to plakins. Further, PNP patients have autoantibodies to the heart. Based on these factors, we tested for autoreactivity of El Bagre-EPF serum to rat, mouse, lamb, pork, bovine, chicken and human cardiac tissue. We tested 15 El Bagre-EPF patients, and 15 controls from the endemic area for autoreactivity to heart using varios immunological methods. We found that El Bagre-patients have a polyclonal immune response to several cell junctions of the heart, often colocalizing with known markers. These colocalizing markers included markers for the area composita, gap junctions, and for adherens junctions. The strongest patient serum autoreactivity was observed against the transverse T tubule system of the heart. We conclude that El Bagre-EPF patients display autoreactivity to multiple cell junctions; and further, that the cardiac pathophysiology of this disorder warrants further evaluation.

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Keratinocyte cell surface heparan sulfate proteoglycans are required for uptake of double-stranded RNA prior to toll-like receptor 3 activation

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During infection and skin injury, keratinocytes respond to exogenous double-stranded RNA (dsRNA) with the production of IL-6 and other proinflammatory cytokines in a toll-like receptor 3 (TLR3)-dependent manner. The mechanism used by keratinocytes for uptake of dsRNA prior to endosomal TLR3 activation has not yet been elucidated. We previously demonstrated that nontransfected Poly(I:C), a synthetic dsRNA, inhibited HSV-1 infection of keratinocytes through direct competition for a cell surface receptor rather than through activation of a cellular immune response via TLR3. Since heparan sulfate proteoglycans (HSPGs) on the cell surface can be bound by HSV-1, we hypothesized that HSPGs serve as the receptor for both dsRNA and HSV-1, and are thus required for keratinocyte binding and uptake of dsRNA prior to TLR3 activation. To test this, cultured keratinocytes were treated with 10μg/ml Poly(I:C) in the presence of increasing doses of soluble heparin, a glycosaminoglycan structurally similar to heparan sulfate, and IL-6 release was evaluated by ELISA. Heparin treatment inhibited Poly(I:C) uptake in a dose-dependent manner. Poly (I:C)-induced IL-6 secretion was suppressed by heparin concentrations as low as 0.1μg/ml, (21% suppression, $p<0.001$) and treatment with 100μg/ml heparin inhibited IL-6 secretion to levels detected in unstimulated cells ($p<0.001$). In contrast, treatment with chondroitin sulfate, an alternative glycosaminoglycan component of cell membrane proteoglycans, had no effect on dsRNA uptake and TLR3-induced IL-6 secretion. These results suggest that HSPGs on the surface of keratinocytes are required for dsRNA uptake and subsequent TLR3 activation and suggest the important role of these molecules in inflammation and injury.

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A C-Xylopyranoside derivative is a potent stimulus for increased synthesis of chondroitin sulfate by keratinocytes

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Glycosaminogylcans (GAGs) are essential components of the extracellular matrix. Previously we have reported that chondroitin sulfate B (dermatan sulfate, DS) is a potent initiator of the action of fibroblast growth factor (FGF) - 2, 7 and 10, and is vital for wound repair. Here we investigated the capacity of a new C-xylopyranoside derivative (C-Xyloside), to serve as an initiator of GAG synthesis. Keratinocytes exposed to C-Xyloside in culture media increased sulfated GAG secretion from undetectable (less than 0.2 μg/ml) to 8.00 μg/ml. However, fibroblasts similarly treated showed minimal GAG increase. Chondroitinase ABC digestion of the induced GAG showed 80.6% was CS. HPLC and mass spec analysis of monosaccharide composition revealed that secreted GAG had 70.4% GalNH2, 20.9% GlcA and 8.69% IdoA, while disaccharide composition showed D0a4 was 62.4%, D0a6 was 27.3%, and D2a4 was 6.6%. These results indicated most of the secreted CS was as DS. The involvement of xylosyl transferase in synthesizing GAG was examined by using mutant CHO745 cells lacking xylosyl transferase and thus unable to initiate GAG synthesis on proteoglycans (PG). C-Xyloside increased GAG in media of CHO745 from undetectable to 7.39 μg/ml, which was similar to wild type CHOK1 cells. Thus, C-Xyloside stimulates GAG synthesis without a core protein. Furthermore, C-Xyloside did not induce CS synthesis enzymes. Functionally, keratinocytes treated with C-Xyloside showed enhanced migration in response to FGF-10 in an *in vitro* scratch assay. Furthermore, culture media of C-Xyloside treated keratinocytes promoted FGF-10 dependent cell proliferation in a BaF3 assay. These observations show C-Xyloside serves as an initiator of DS synthesis by keratinocytes and enables enhanced FGF-10 responsiveness. Such an effect suggests that C-Xyloside might be a novel therapeutic agent to enhance GAG-dependent processes in the skin such as wound healing.

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UVA irradiation following treatment with 8-methoxypsoralen suppressive the sclerosis in a mouse model for scleroderma through the decrease of gene expression of collagen

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Recent studies have demonstrated that PUVA therapy is effective against the sclerotic skin lesions in scleroderma. However, the mechanisms underlying the improvement of the skin sclerosis by PUVA therapy in scleroderma remain unknown. We investigated the effects of UVA irradiation following treatment with 8-methoxypsoralen (8-MOP) on dermal sclerosis by evaluating the histologic changes, amount of collagen deposition and the expression of genes encoding extracellular-matrix-related proteins of sclerotic skin in a mouse model of bleomycin (BLM)-induced scleroderma. Methods: One ug of BLM / PBS or PBS was injected subcutaneously into the shaved back of BALB/C mice daily for 28 days. Before the start of the injections, the back skin was UVA-irradiated following local application of 0.3% of 8-MOP. The back skin was removed on the day after that of the final injection. The dermal thickness was measured from photographs obtained under a light microscope of hematoxylin & eosin-stained sections. Collagen deposition was estimated by determining the content of hydroxyproline in the skin extracts by HPLC. The expressions of the genes encoding type I and type III collagen and TGFβ1 in the skin samples were measured by real-time RT-PCR. Results: Marked reduction in the thickness of the dermis was observed in the mice treated with injections of BLM and UVA irradiation following treatment with 8-MOP. Reductions in the hydroxyproline contents in the skin samples were also observed. The gene expression levels of type I and III collagen were reduced, whereas that of TGFβ1was not altered. Conclusion: These results suggest that the beneficial effects of PUVA therapy against the sclerotic skin lesions in scleroderma were induced by reduction of the collagen gene expression. It is also suggested that the mechanism of the reduction of collagen gene expression is not due to the enhanced collagen transcription by TGFβ1.

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Transcriptional regulation of the human type I collagen alpha1(I) chain gene: Function analysis of the promoter gene and analysis of DNA binding factors in the transcriptional enhancement region

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Type 1 collagen synthesis by fibroblasts is increased at the transcriptional level in scleroderma, whose principal lesion is fibrosis. Thus, analyzing the transcriptional regulatory mechanism of type I collagen is important from the standpoint of considering the treatment of fibrosis, including of scleroderma. In this study we conducted a function analysis of the human type I collagen alpha1(I) chain gene (COL1A1) promoter and analyzed the DNA binding factors for the transcriptional enhancement region. Methods: Using human dermal fibroblasts and DNA in which from -2.3 kilobase pairs to +42 base pairs of the human COL1A1 promoter were fused to the luciferase gene as the base, a luciferase assay was carried out using DNA in which the COL1A1 promoter had gradually been deleted starting at the upstream side. A gel shift assay was performed by 32P radiolabeling DNA fragments from -401 to -332 and using fibroblast nuclear proteins. Results: The luciferase assay showed almost the same level of activity in the deletions down to -402, but activity decreased markedly in the deletions down to -332, and the transcription regulatory region appeared to lie between them. When a gel shift assay was performed using DNA fragments from -402 to -332, a band that suggested binding of a protein was observed. As a result of a competition assay with DNA in which substitution mutations had been introduced, a protein that bound to the -386 to -371 region was found. In the luciferase assay, a decrease in activity was seen with the DNA in which a substitution mutation had been introduced in the same region. Conclusion: It is suggested that the region is involved in the transcriptional enhancement of the human COL1A1.

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Platelets interact with B16 melanoma cells to augment integrin-mediated tumor cell adhesion and promote lung metastasis formation

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Fundamental aspects determining the molecular basis for the reciprocal relationship between metastasizing tumor cells and soluble components of the coagulation cascade have recently become understood. However, the involvement of platelets, the cellular component of thrombus formation, in the pathogenesis of cancer metastasis is still poorly comprehended. We demonstrate that integrin-mediated interactions between platelets and tumor cells are an important contributing factor in the early phase of B16 melanoma (B16M) lung metastasis *in vivo*. As determined by *in vivo* and *in vitro* bioluminescence analysis the depletion of platelets after i.v. inoculation of luciferase-transduced murine B16M (B16M-luc) into syngeneic C57BL/6 mice resulted in a more than 30 % decrease in micrometastasis to the lung. While we found no significant platelet mediated effect on the growth of subcutaneously implanted B16M tumors *in vivo* nor B16M rolling (parallel-plate flow chamber), chemotaxis (wound assay) and migration (transwell chamber) *in vitro*, we determined an up to 50fold augmentation in the adhesion of melanoma cells on immobilized platelets under static condition and an up to 6fold increase in platelet-mediated B16M adhesion to endothelial cells under shear stress conditions. Blocking mAb to alphaV integrin, an adhesion molecule highly expressed on 97,8% of B16M and blocking mAb to GPIIbIIIa, the most abundant platelet adhesion receptor, both significantly abrogated the platelet-mediated increase in *in vitro* B16M adhesion. Furthermore, in a murine model of B16M lung metastasis systemic platelet depletion and an integrin $\alpha v\beta 3$ antagonist synergistically decreased the lung tumor burden up to 90%. We conclude that platelet-tumor interactions are critically involved in the early formation of metastasis of melanoma cells to the lung and suggest that the specific targeting of adhesion molecules involved in this process may represent a promising strategy for therapeutic intervention.

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Novel role for human desmoglein 3 in regulating E-cadherin mediated adherens junctions through Src family kinase activation

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Desmoglein 3 (Dsg3), a member of desmosomal cadherins, plays a role in regulating keratinocyte differentiation and is a characterized autoantigen in pemphigus vulgaris. Currently, the underlying mechanism of this disease remains not completely clear. In this study, we investigated the function of Dsg3 in epithelial cells by utilizing both the ectopic retroviral expression of a myc tagged human Dsg3 and loss-of-function approach mediated by RNAi. We found that Dsg3 directly regulates E-cadherin mediated adherens junctions and its downstream Src signaling, upon calcium induced cell-cell contacts. We showed that endogenous Dsg3 expression and its association with E-cadherin also occurred in a calcium dependent manner. Knockdown of Dsg3 impaired E-cadherin junction assembly induced by calcium. Furthermore, we demonstrated that over-expression of Dsg3 augmented Src activity locally in the cadherin-catenin complex whereas knockdown of Dsg3 lessened this effect. However, both the gain- and loss-of-function of Dsg3 resulted in reduction and instability of junctional proteins. Over-expression of Dsg3 in A431 elicited a phenotype resembling that of the Src activated cells (i.e. cadherin dissolution in both junctions, elevated tyrosine phosphorylation of the E-cadherin-catenin complexes and augmented cell motility). On the other hand, knockdown of Dsg3 led to reduction and instability of desmosomal proteins at steady state. Interestingly, alterations in Src signaling and disruption of E-cadherin junctions were detected readily in the oral mucosa of PV patients, implying that this pathway might be involved in the pathogenesis of this disease. Together, our findings support the notion that tightly regulated Src signalling at adherens junctions is critical for normal function and maintenance of intercellular junctions and that this regulation involves the desmosomal cadherin, Dsg3.

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Defective laminin-332 expression, secretion, and processing by squamous carcinoma cells and premalignant keratinocytes

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High level expression of the $\alpha 3$, $\beta 3$, and $\gamma 2$ polypeptides that comprise the basement membrane protein Laminin-332 (Lam332) occurs *in vivo* in three settings: in keratinocytes at the edge of healing wounds, in squamous carcinoma cells (SCC) at the stromal interface, and in premalignant dysplastic keratinocytes. Keratinocytes in culture that engage a form of Lam332 in which the $\gamma 2$ chain remains unprocessed (Lam332′) display sustained directional hypermotility, modeling wound and invasive migration. Using immunofluorescence and Western blotting, we asked whether premalignant human keratinocytes and SCC cells display abnormal regulation of Lam332 expression or processing in culture, which could account for their invasive potential *in vivo*. We find that synthesis of the laminin $\gamma 2$ chain by normal keratinocytes is strongly repressed as cultures approach confluence, similar to the status of normal epithelium *in vivo*. TGFβ treatment of dense keratinocyte cultures induced high levels of $\gamma 2$ synthesis, accompanied by secretion of Lam332′ trimer and some $\beta 3\gamma 2'$ dimer and $\gamma 2'$ monomer. Six of eight SCC lines we examined failed to repress $\gamma 2$ synthesis at confluence, much of the secreted trimer was the Lam332′ form, and two SCC lines secreted much $\beta 3\gamma 2'$ dimer and $\gamma 2'$ monomer. A TGFBRI kinase inhibitor repressed Lam332 synthesis in SCC cells, indicating that Lam332 synthesis remains dependent upon TGFβ signaling as it does in normal cells. The SCC lines expressed elevated levels of EGFR and their $\gamma 2$ expression at high density was reduced by the EGFR kinase inhibitor AG174, suggesting a role for EGFR signaling in Lam332 regulation. POE9n, a premalignant oral keratinocyte line, and normal keratinocytes transduced to express HPV16E6E7 also failed to repress $\gamma 2$ synthesis at high density. These results identify dysregulation of Lam332 synthesis and incomplete Lam332 assembly and processing as frequent features of SCC, defects which may begin early during neoplastic progression.

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The desmosomal armadillo protein plakoglobin regulates keratinocyte motility through extracellular matrix modification

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The junctional armadillo protein plakoglobin (PG) is emerging as an important regulator of motility. We previously showed that PG suppresses motility not only of keratinocytes in contact, but also, unexpectedly, cells not in contact with their neighbors. Here, based on observed disruption in cortical actin organization and focal adhesions that correlate with increased single cell motility in murine keratinocytes lacking PG, we hypothesized that PG inhibits motility by regulating cell-substrate interactions. Interestingly, extracellular matrix (ECM) deposited by cells expressing PG inhibited individual cell motility of PG-null cells, indicating that the effects of PG on motility are at least in part mediated through its modulation of ECM. In order to test whether PG regulates the expression and deposition of individual ECM components and their membrane receptors we used a novel approach to mass spectrometric analysis of samples enriched in cell adhesion related molecules. Among over 70 ECM and adhesion related molecules differentially regulated by PG, a 50-fold change in fibronectin (FN) expression stood out, especially since it was reversed by PG expression in PG-null cells. One of the major motility pathways controlled by FN is signaling through Src family kinases (SFK). Therefore, we examined the role of PG-dependent changes in ECM expression on SFK activity. PG-deficient cells exhibited an increase in activated SFK, a phenotype reversed by plating PG null cells on ECM deposited by control keratinocytes. In summary, we propose a novel, cell-cell adhesion independent role for PG in regulating the cell-ECM interactions and its downstream SFK signaling through direct modulation of ECM.

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Dynamic relationship of focal contacts and hemidesmosome protein complexes in live cells

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Epidermal cells adhere to the basement membrane zone via cell-matrix junctions termed hemidesmosomes. During wound healing hemidesmosomes are disassembled to allow keratinocytes to move over wound sites. Such movement is mediated by both hemidesmosome protein complexes (HPCs) and focal contacts (FCs). In this study, we investigated the interaction between HPCs and FCs in live HaCat cells expressing YFP-tagged $\beta 4$ integrin and CFP tagged α-actinin as markers of HPCs and FCs, respectively. In HaCat cells migrating to repopulate wounds, FC proteins cluster rapidly in the direction of the wound. HPC assembly then follows and the newly formed HPCs occupy sites vacated by the disassembled FCs. HPC dynamics are dramatically reduced and HaCat cells cease migration upon treatment with reagents that impact FC integrity/function. Upon treatment with reagents that destabilize HPCs, the dynamics of FCs in HaCat cells at the edges of wounds are enhanced although FC assembly is irregular and the migration of the cells is aberrant. We also demonstrate that the complex interaction between HDs and FCs in keratinocytes is myosin dependent and requires energy. In summary, we suggest that HPCs and FCs dynamics are tightly co-regulated in keratinocytes undergoing migration during wound healing.

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Cotinus coggygria extracts enhance the elastic network and reduce pigment deposition

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Skin aging is associated with wrinkles, sagging and uneven pigmentation. Upon chronological aging, skin elasticity is reduced as the elastic fiber network is being degraded by elastases, and elastin synthesis is declined. Photodamage in sun-exposed areas further enhances the damage to the elastin fiber network, and also contributes to the creation of uneven skin tone and hyperpigmentary lesions. Cotinus coggygria (smoke tree) extracts were found to protect the elastic network from degradation, and to induce new elastin synthesis. Cotinus extracts induced elastin promoter activity and elastin gene expression, and inhibited the activity of numerous elastases in a dose dependent manner. Additionally, Cotinus extracts inhibited trypsin enzymatic activity and reduced pigment deposition in pigmented epidermal equivalents, possibly due to inhibition of melanosome transfer via affecting the protease-activated receptor pathway. Human skin explants topically treated with Cotinus extracts showed reduced pigment deposition and enhanced elastic fiber network. The induction of tropoelastin and fibrillin-1 synthesis in the cultured explants documents the enhanced synthesis and assembly of elastin fibers upon Cotinus extracts treatments. These results suggest that extracts from Cotinus coggygria could even skin tone and restore skin elasticity, and could provide an effective, natural, anti-aging skin care treatment.

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The role of the receptor tyrosine kinase Axl in cell-cell adhesion and signaling in cancer

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Axl is a receptor tyrosine kinase that is upregulated in a variety of tumors including cutaneous squamous cell carcinoma (SCC) and breast cancer. As Axl expression correlates with the metastatic potential of some tumors, we hypothesized that Axl might play a role in epithelial-mesenchymal transition (EMT), a crucial step in cancer progression causing disruption of cell-cell adhesion to allow invasion and metastasis. To investigate this hypothesis, we used short hairpin RNA interference in combination with a retroviral delivery system to achieve stable knock-down of Axl in two cutaneous SCC cell lines and one breast cancer cell line. Subsequently, we studied colony morphology, expression of junctional proteins and related signaling pathways such as the Wnt pathway. We found that stable knock-down of Axl resulted in formation of tight colonies *in vitro*, increased strength of cell-cell adhesion, as well as downregulation of some EMT markers. Western blotting and immunofluorescence staining of monolayers and/or 3-D organotypic cultures demonstrated that Axl knock-down led to changes in expression of total plakoglobin (PG), nuclear beta-catenin, tight junction proteins such as occludin and zonula occludens-1 (ZO-1), as well as altered distribution of alpha-catenin, p120-catenin, desmoplakin, PG and ZO-1 with decreased phosphorylation of glycogen synthase kinase-3. Flow cytometry sorting of CD44-high cells, the putative cancer stem cells, with subsequent immunoblotting for Axl demonstrated that Axl expression correlated with the expression of CD44 in cutaneous SCC, indicating a possible role for Axl in the chemotherapy-resistant cancer stem cell population. In conclusion, Axl depletion leads to increased intercellular adhesion and loss of EMT markers confirming Axl as a possible small molecule kinase inhibitor target in cutaneous SCCs and other Axl-overexpressing cancers.

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Optimization of human skin explant cultures as an *in vitro* alternative to animal testing

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In vitro model systems are a vital component of basic and applied research. The development of such model systems for the skin is of increasing priority, following the increased awareness of the 3Rs concepts and the European Community ban on animal testing for cosmetics. Cultured human skin explants have been studied for more than 50 years, mainly for epidermal biology and for cancer research. However, these model systems have poor dermal metabolic activity and their tissue architecture is compromised. The goal of our studies was to develop an *in vitro* model system that best represents the physiological complexity, the metabolic activity, and the structural integrity of the epidermis, dermis, and subcutaneous fat layers. Using full-thickness human skin biopsies obtained from healthy donors undergoing abdominal surgeries with informed consent, we developed a system for studying skin biology and for evaluating dermatological agents for biological activities via both systemic and topical treatments. Various culture parameters were optimized for the maintenance of metabolic activity of all three compartments. We have identified biomarkers for epidermal, dermal and subcutaneous adipose functions that enable a quick but thorough evaluation of the effects of agents on pigmentation, skin aging, and lipid metabolism. The skin explants system was validated using known cosmetic, skin care actives such as soy and retinol. In summary, a skin explant system was established, that is viable and metabolically active, for better understanding of skin biology, for mechanistic understanding and for predicting efficacy of agents.

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Retinol induces dermal elastin synthesis and elastin fiber formation

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Elastic fibers are an essential element of the dermal connective tissue, which are produced by fibroblasts and are involved in skin elasticity. The dermal elastin network decrease with age by reduced synthesis of elastin and its accessory proteins, and by increased elastin fiber degradation, resulting in skin sagging and reduced skin elasticity. The anti-aging effects of retinol (ROL) on the skin are extensively documented, including enhancing keratinocyte proliferation, promoting collagen synthesis and reducing pigmentation. Here we show that ROL is also enhancing elastin fiber formation. ROL induced elastin gene expression and elastin fiber formation in 2D and 3D cultured human dermal fibroblasts, and enhanced the elastic fiber network in ROL-treated human skin explants, as compared to untreated controls. ROL induced tropoelastin and fibrillin-1 gene expression and protein synthesis in the treated skin explants, as documented by QPCR and immunohistochemistry staining. These data point to a novel mechanism of action of ROL, and demonstrate that ROL exerts its anti-aging benefits via an increase in elastin production and assembly, additional to its induction of epidermal proliferation and collagen production.

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Elemental bi-mineral complex increases extracellular matrix production in human skin explants

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Bio-electric fields generated in skin during wounding are known to affect wound healing by promoting directional migration of keratinocytes and melanocytes toward the cathode. The effect of such bio-electrical fields on dermal cells and on the dermal extracellular matrix (ECM) is not well studied. A novel elemental bi-mineral complex that produces biomimetic electricity was, therefore, evaluated for its possible effects on dermal ECM. Topical treatment of human skin explants with elemental bi-mineral complex, once daily for 7-9 days, induced elastin fibers formation and collagen synthesis, as compared to untreated controls. QPCR analysis documented an increase in elastin and collagen expression in the elemental bi-mineral complex - treated skin explants. Our *in vitro* results demonstrate that elemental bi-mineral complex is effective in restoring and enhancing the integrity and functionality of dermal ECM, and in particular of the elastic fiber network, suggesting its cosmetic anti-aging use.

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GM6001, a broad spectrum metalloproteinase inhibitor, has multiple efficacies against skin aging

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There is a pressing need for ingredients effective against signs of skin aging that result from extracellular matrix (ECM) degradation, inflammation and hyper-pigmentation. Matrix-associated Metallo Proteinases (MMP) are a group of more than twenty Zn-requiring enzymes that digest ECM components, mediate inflammatory responses and may also facilitate melanosome transfer during pigmentation. GM6001 is a hydroxamate compound that inhibits a broad spectrum of MMPs and our work demonstrates that in cell culture and in reconstructed skin models, it is effective at inhibiting processes related to skin damage triggered by inflammatory stimuli, and at inhibiting UV-induced pigmentation. To evaluate the ability of GM6001 to inhibit MMPs secreted by dermal fibroblasts, cells were treated with an inducing cocktail containing IL-1β, histamine and lipopolysaccharide, in combination with an increasing amount of GM6001. Fluorescein-labeled gelatin was added to the medium as a substrate, and digestion was measured by fluorescence over a 2 day period. GM6001 dose-dependently inhibited degradation of the substrate by ~45% at 5 μg/ml. We also tested the ability of GM6001 to prevent production of Tumor Necrosis-Alpha (TNF-α) by keratinocytes induced by the same cocktail. GM6001 dose-dependently inhibited TNF-α by ~80% at 4 μg/ml. In addition, we tested the ability of GM6001 to protect the ultra-structural integrity of full-thickness skin model tissues when challenged with the cocktail. GM6001 performed as well or better than hydrocortisone controls in preserving proper tissue organization and basement membrane integrity as indicated by dermal invasion. Lastly, the whitening effect of GM6001 was evaluated in a commercial epidermal skin model and was found to be highly effective at preventing UV-induced pigmentation as compared to kojic acid. Taken together, GM6001 exhibited multifaceted efficacy in preventing age-related alterations in cell/ tissue physiology.

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Genomic study of fibroblasts links mtDNA changes to alterations in MMP and collagen expression affecting migration and invasion

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Although mtDNA mutations have been observed frequently in human malignancies, their functional significance is not fully understood. We generated murine fibroblast cybrid cell lines that have identical nuclear genomes and differ only in their mtDNA. We observed increased cellular proliferation and resistance to apoptosis in the mtBALB compared to the mtB6 cybrids, phenotypes seen in malignant cells. Based on these observations we investigated whether these phenotypic differences could be caused by a unique spectrum of nuclear gene expression alterations induced by the mtDNA changes. Microarray analysis (Agilent, 44K mouse chip) was conducted in order to elucidate the expression profile of three independent clones of mtBALB and mtB6 cybrids. This experiment revealed that the specific mtDNA changes result in more than 1,000 differentially expressed genes between the two cell lines. From the list of potential targets we selected MMP-9 and Col1A1 for further studies. Real-time PCR confirmed up-regulation of MMP-9 and down-regulation of Col1A1 in mtBALB cybrids. Based on the reported role of MMP-9 in tumor cell invasion and migration we examined whether the mtBALB haplotype would be associated with higher level of cell migration and invasiveness compared to mtB6 haplotype. We conducted transwell migration and matrigel invasion assays. MtBALB cybrid cells demonstrated 3-fold higher migration through uncoated inserts and significantly increased (2.5-fold) invasion ability through matrigel. Treatment of cybrids with GM6001, a nonspecific MMP inhibitor, significantly inhibited the invasive and migratory phenotypes confirming a role for MMP in invasiveness of cultured fibroblasts and also suggesting a critical role for these proteins in cellular motility. This enhanced migration and invasion capabilities caused by up-regulated MMP-9 may contribute to the malignant phenotypic characteristics of mtBALB cybrids.

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Critical role of Paxillin in aging of human skin

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Skin aging is a complex process involving a series of cellular and extracellular matrix events. Recent studies indicate that both intrinsic aging and photodamage can lead to disruption of normal cellular behaviors, and important factors contributing to this process include degradation of collagen matrix and breakdown of focal adhesion complexes (Varani et al., 2004; 2006; Fisher et al, 2008). Paxillin is a key focal adhesion adaptor protein that mediates cell-matrix signaling and regulates cytoskeleton assembly, which is essential to cell attachment, spreading, and migration. Loss of Paxillin activity has been related to impaired cellular functions in various animal and cell experimental models, however, a direct link between Paxillin and human skin aging is yet to be established. To better understand the functions of Paxillin during skin aging, we performed a combination of molecular, cellular, and *in vivo* studies. The role of Paxillin in cellular functions was studied by targeted gene disruption and the effect of age on Paxillin expression levels in human dermal fibroblasts was investigated. Paxillin appears to play a critical role in skin by helping fibroblasts maintain proper interactions with the dermal matrix and a decline in Paxillin may contribute to the aging of human skin.

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The p65 subunit of NF-kB inhibits COL1A1 gene transcription in human dermal fibroblasts through protein interaction wit hc-KROX, SP1 and SP3

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Transcriptional mechanisms regulating type I collagen genes expression in physiopathological situations are not completely known. In this study, we have investigated the role of transcription factor NF-κB (Nuclear Factor-kappaB) on type I collagen expression in adult normal human (ANF) fibroblasts. We demonstrated that NF-κB, a master transcription factor playing a major role in immune response/apoptosis, down-regulates COL1A1 expression by a transcriptional control that involves the -112/-61-bp sequence. This 112-bp region mediates the action of two zinc-fingers, Sp1 (Specific Protein-1) and Sp3, acting as transactivators of type I collagen expression in ANF. Knock-down of each one of these trans factors by siRNA confirmed the transactivating effect of Sp1/Sp3 and the p65 subunit of NF-κB transinhibiting effect on COL1A1 expression. Despite no existing consensus sequence for NF-κB in the COL1A1 promoter, we found that Sp1/Sp3/c-Krox and NF-κB bind and/or are recruited on the proximal promoter in chromatin immunoprecipitation (ChIP) assays. Attempts to elucidate whether interactions between Sp1/Sp3/c-Krox and p65 are necessary to mediate the NF-κB inhibitory effect on COL1A1 in ANF, immunoprecipitation assays revealed that they interact each others and this was validated by re-ChIP. Finally, the knock-down of Sp1/Sp3/c-Krox prevents the p65 inhibitory effect on COL1A1 transcription in ANF. As a conclusion, our findings highlight a new mechanism for COL1A1 transcriptional regulation by NF-κB.

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Plakoglobin rescues adhesive defects induced by desmoglein 1 truncation: Implications for exfoliative toxin-mediated blistering

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Desmoglein 1 (Dsg1), a desmosomal cadherin expressed in differentiating keratinocytes, maintains epidermal integrity. In bullous impetigo (BI) and Staphylococcal scalded-skin syndrome (SSSS), cleavage of Dsg1 by a Staphylococcal protease, exfoliative toxin A (ETA), results in subcorneal blistering. ETA hydrolyzes a single peptide bond in the ectodomain of Dsg1, producing an adhesion-deficient, membrane-tethered cadherin. Beyond Dsg1 cleavage, other cellular consequences precipitating gross loss of adhesion in BI/SSSS are unknown. We investigated the mechanism of ETA-induced blistering by expressing an N-terminally truncated Dsg1 mimicking the toxin-cleaved cadherin (Δ381-Dsg1), which compromised desmosome organization in human keratinocytes and severely impaired monolayer integrity. Ectodomain-deleted Dsg1 remained bound to its catenin partner, plakoglobin (PG), and Δ381-Dsg1 reduced levels of endogenous desmosomal cadherins in a dose-dependent manner. Thus, we tested whether truncated Dsg1 might de-stabilize other desmosomal cadherins via PG sequestration. Three mutations in the catenin-binding segment of Δ381-Dsg1 were introduced to uncouple the truncated cadherin from PG; interestingly, this mutant form of Dsg1 did not compromise cell:cell adhesion, indicating that interaction with PG is essential to the pathogenic potential of truncated Dsg1. We further hypothesized that cadherin competition for PG could be alleviated by increasing PG levels. Accordingly, ectopic PG increased desmosomal cadherin expression, restored desmosome organization, and rescued adhesion in cells expressing Δ381-Dsg1. Finally, our preliminary results indicate that ectopic PG significantly reduced ETA-induced blistering of organotypic epidermis. In summary, these findings further our understanding of the molecular mechanism of exfoliative toxin-induced disease and suggest novel strategies to increase PG expression toward suppressing blistering in BI/SSSS.

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ENaC mediates keratinocyte galvanotaxis by promoting lamellipodial protrusion at the cathodal side

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Galvanotaxis, the directional migration of keratinocytes in an electric field (EF), is one of the mechanisms to facilitate wound healing. We found that the epithelial sodium channel (ENaC) is involved in keratinocyte galvanotaxis. In addition to its role in conducting a sodium flow, ENaC has been shown to mediate cell migration and to associate with F-actin. To investigate if ENaC could initiate the asymmetric migratory response needed for a directional galvanotaxis, we examined the lamellipodia of polarized, migrating wild type (WT) and keratinocytes from ENaC -/- mice (KO) in an EF and analyzed the protrusion and retraction by kymography. During the galvanotaxis of WT keratinocytes, within 10 min of exposure to the EF, both the protrusion speed and protrusion distance at the cathodal side of the cell are increased (24-29%) over than those at the anodal side. The distances of the lamellipodia retraction at both sides are similar and there is no difference of the switching frequencies between protrusion and retraction. The results indicate that the lamellipodia of WT keratinocytes extend further at the cathodal side in the EF, which may orient cells toward the cathode in galvanotaxis. However, in the ENaC KO keratinocytes the lamellipodia at both the cathodal and the anodal sites protrude a similar distances and retraction is likewise equal on both sides of the cell, suggesting that the ENaC KO cells are unable to establish an asymmetric motile response to the EF because they cannot establish dominant lamellipodia at the cathodal side of the cells as do WT cells. Additionally, the average distances of protrusion and retraction in ENaC KO keratinocytes are 24% and 44% longer than in the WT cells, but the ENaC KO cells migrate at a same speed of 1-2 μm/min as wild type cells. The result suggests that depleting ENaC generates non-stable lamellipodia, which does not increase the migration rate. Therefore, our results indicate that ENaC is required for directional migration by promoting stable lamellipodial protrusion at the cathodal side in WT keratinocytes, which then directs keratinocyte migration and mediates galvanotaxis.

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Therapeutic implications of EGFR transactivation in pemphigus acantholysis

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Pemphigus autoimmune bullous disorders are caused by autoantibody binding to desmoglein 1 and/or 3 (dsg1/dsg3). It is becoming evident that the desmosome functions not only in adhesion but also as a "molecular synapse." Accumulating data suggests that disruption of dsg3 adhesion by pemphigus vulgaris (PV) IgG triggers a complex series of intracellular events resulting in loss of cell-cell adhesion and blister formation. The role of the epidermal growth factor receptor (EGFR) in pemphigus has been a subject of debate. This study was undertaken to investigate the role of the EGFR in pemphigus IgG induced blistering in both tissue culture and mouse models. Human keratinocytes were incubated in the presence of PV or control normal human IgG. Using western blot of cell extracts and confocal immunofluorescent microscopy, keratinocytes were assayed for PV IgG induced EGFR phosphorylation, p38 mitogen activated protein kinase (p38MAPK) activation, endocytosis of dsg3 and EGFR, and keratin intermediate filament retraction. We show that the EGFR is transactivated following PV IgG treatment and that this transactivation is downstream of p38MAPK. EGFR inhibition blocked PV IgG induced biochemical changes associated with loss of cell-cell adhesion including dsg3 internalization and cytokeratin retraction. Significantly, inhibiting EGFR prevented PV IgG induced blistering *in vivo* in experiments utilizing the PV passive transfer mouse model. These data demonstrate (i) crosstalk between dsg3 and the EGFR, (ii) that this crosstalk is regulated by p38MAPK, and (iii) that the EGFR is a potential therapeutic target for pemphigus. Small molecule inhibitors and monoclonal antibodies directed against EGFR are currently used to treat several types of solid tumors. We suggest that EGFR inhibition may be a valuable tool for treating pemphigus. Additionally, the cross-talk between dsg3 and the EGFR raises the possibility that intracellular signaling cascades regulating desmosome cell-cell adhesion may modulate other processes including cell proliferation, motility and invasion.

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Discovery of novel ingredients which stimulate the expression of the focal adhesion protein, paxillin, in human skin and skin cells

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Paxillin is a key focal adhesion adaptor protein that regulates cytoskeleton assembly. It is a downstream mediator of integrin and growth factor signaling, transduces the messages from extracellular matrix into the cells, and activates the cytoskeleton system as a response. Recent work at Avon Global R&D has linked Paxilin to skin aging. We initiated a program to identify novel botanical and synthetic ingredients which would stimulate the expression of paxillin in skin. We have identified several that stimulate paxillin expression in skin cells and also show an increase in paxillin production in human biopsy studies. *In vitro* and *in vivo* screening results will be presented along with data regarding efficacy in human biopsy testing.

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p38 alpha MAPK is not required for the loss of intercellular adhesion in pemphigus vulgaris

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Pemphigus vulgaris (PV) is a potentially fatal blistering disease characterized by autoantibodies against desmoglein (Dsg) cell adhesion proteins. p38 MAPK is activated in PV lesions, and inhibitor studies have suggested that p38 is necessary for blister formation. To more specifically define the role of p38 in desmosomal cell adhesion and PV, we studied the effects of pathogenic PV monoclonal antibodies (mAbs) in normal and p38-silenced primary human keratinocytes, as well as mice with a K14-targeted deletion of p38 alpha in the epidermis. In primary keratinocyte culture, as in epidermis, Dsg3 and Dsg1 must both be inactivated to cause cell dissociation. Using a pathogenic anti-Dsg3 mAb plus exfoliative toxin (which inactivates Dsg1) to cause cell dissociation in culture, we show that Dsg3 is internalized and p38 is activated (determined by phospho-p38 immunoblot). The same pathogenic mAb, without exfoliative toxin, causes Dsg3 internalization but does not cause cell dissociation and does not activate p38, suggesting that p38 activation is secondary to the loss of intercellular adhesion. Furthermore, mice with epidermal p38 alpha deficiency show blistering similar to wild type mice after passive transfer of high and low doses of pathogenic PV mAbs, indicating that loss of cell adhesion is not dependent on p38 alpha. p38 may play a downstream role in regulating Dsg3, as p38 inhibition prevents internalization of pathogenic mAbs, and RNAi silencing of p38 prevents depletion of desmosomal Dsg3 by pathogenic mAbs. Additionally, exogenous activation of p38 by oxidative stress or anisomycin causes internalization of Dsg3, desmocollin 3 and desmoplakin, with delayed effects on E-cadherin. Our studies demonstrate that p38 alpha is not required for blistering in PV, but may function downstream to augment the pathogenic effects of PV mAbs via Dsg3 internalization. A better understanding of the role of p38 isoforms in modulating desmosomal cell adhesion may lead to novel adjunctive therapies for pemphigus.

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Gottron's papules exhibit accumulation of CD44 variant 7 (CD44v7) and its binding partner osteopontin: a unique molecular signature

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We previously reported an abundance of chondroitin sulfate (CS) in dermis from dermatomyositis (DM) lesions (SID 2008). Because the CD44v7 variant has CS side-chains (Invest Ophthmol Vis Sci 48:1164, 2007) and can mediate autoimmunity in the gut (J Immunol 161:1069, 1998), we examined its distribution in CS-rich regions of DM skin. Surprisingly, CD44v7 was abundant in Gottron's papules, a hallmark lesion of DM overlying interphalangeal (IP) joints, but not in lesional or non-lesional dermis in other regions. Staining density of CD44v7 in Gottron's dermis was double that of healthy IP dermis ($p=0.0045$). Moreover, healthy IP dermis showed far more CD44v7 than did healthy non-IP dermis from the same volunteer, indicating location-specific induction, independent of DM. We hypothesized a role for mechanical stretching, because it induces CD44v7/v8 in non-dermal cells (Invest Ophthmol Vis Sci 48:1164, 2007). We found that confluent dermal fibroblasts cultured on membranes and stretched constantly for 6 hours showed a 62%±3% increase in CD44v7 mRNA levels relative to unstretched cells ($p<0.0001$). Interaction of CD44v7 with the cytokine-like molecule osteopontin has been implicated in chronic inflammation (JCI 107:1055, 2001). Here, we found 2.8-fold increased density of osteopontin staining in the dermal matrix of all DM skin compared to healthy controls ($p=0.0055$), with no significant difference between Gottron's vs. non-Gottron's skin. IFN-γ stimulates osteopontin expression by monocytoid cells, and IFN-γ polymorphisms associate with myositis. Treatment of dermal fibroblasts with IFN-γ for 6 hours provoked a ~10-fold increase in OPN mRNA ($p<0.0001$). Overall, we propose that stretch-induced expression of CD44v7 over joints, in concert with IFNγ-induction of osteopontin, provides a unique molecular signature of Gottron's papules in DM and may contribute to their pathogenesis.

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The role of Dermatopontin in the human skin. Histological and ultrastructural studies

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Dermatopontin (DPT) is a non-collagenous extracellular matrix protein, present in various tissues, especially in skin, where it is mainly located around collagen fibers. It is known that cultured fibroblasts synthesize DPT in the cytoplasm and secrete it into the medium. It is also known that DPT plays putative roles in cell proliferation, attachment, and the spreading of dermal fibroblasts, and is central to collagen fibril assembly and stabilization, as has been shown in knockout animal models. In the present study, we focused on the effect of enhancing DPT synthesis in keratinocytes and human skin, using an active ingredient (IV09.002) specifically designed to target DPT synthesis. Our studies confirmed the effect of IV09.002 on upregulating DPT synthesis in human fibroblasts and human skin biopsies within 48h of application. This effect was associated with an enhancement of expression of key ECM proteins, including collagen I, collagen III, and fibronectin, as revealed by immunofluorescence staining on human fibroblasts and skin biopsies. More interestingly, we found that normal human keratinocytes also express DPT, and more intensively after IV09.002 treatment. In the epidermis, DPT immunostaining was mainly located at the level of the basal layer. Furthermore, electron microscopy studies showed that enhancing DPT level in the cells had beneficial effects on NHK, such as increasing mitochondria. Moreover, these cells exhibited extensive interdigitations between adjacent keratinocytes cultured on a Transwell membrane, compared to their respective controls. These observations correlate with improved histochemical and ultrastructural features observed in *ex vivo* skin treated with the active ingredient. Our results show that treatment with the active enhances interdigitations and hence cellular cohesion of cultured keratinocytes. In *ex vivo* skin, it induces increased immunoreactivity for DPT in the basal cell layers, and key ECM proteins in the dermis.

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Non-muscle myosin II isoform roles and regulation during *in vivo* wound closure in the mouse epidermis

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Cutaneous wounding is a dramatic event and keratinocytes which are normally programmed to differentiate to form the upper cornified layer of the epidermis are now required to undergo a complex series of events to facilitate epidermal migration across the wound bed. Myosin II is a force-producing protein that utilizes ATP to produce contractile forces important for cell migration and tissue morphogenetic changes. To understand the role of the three mammalian non-muscle myosin II isoforms (IIA, IIB, and IIC) during keratinocyte migration following a wounding event, we are studying myosin II expression and activation behavior during wound responses in cultured cell monolayers, in an organotypic epidermal equivalent model, and *in vivo* in mice. In cultured cells and in organotypic skin culture, myosin II is dramatically activated in response to a wound stimulus. Immunohistological approaches reveal distinct expression patterns for myosin IIA versus IIB in mouse skin and in human neonatal foreskin, with myosin IIA predominantly expressed in the basal cell layer and in the spinous and granular layer of the epidermis. In contrast, myosin IIB shows low expression in the basal and spinous layers, with strongly elevated expression in the upper granular layer of the epidermis. These expression patterns suggest the possibility that myosin II isoforms play distinct roles during skin development and maturation. Moreover, myosin IIA appears to be upregulated in the suprabasal region of the epidermal "tongue" during wound healing, suggesting roles for the IIA isoform during wound responses. We are initiating skin-restricted myosin II gene knockout studies in mice to test these possible roles.

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Polyclonal nature of pemphigus vulgaris IgG contributes to desmosomal disassembly by causing clustering and endocytosis of Dsg3

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The mechanism by which pemphigus vulgaris (PV) IgG induces blistering is not fully understood. We have previously shown that desmosomal disassembly in response to PV IgG occurs in distinct phases beginning with internalization of non-junctional desmoglein-3 (Dsg3) followed by depletion of desmosomal Dsg3. In the present study, we tested if the polyclonal nature of PV IgG contributes to pathogenesis. Pulse chase studies demonstrated that a pathogenic mouse monoclonal IgG (AK23) that bound to the keratinocyte cell surface remained at the plasma membrane for up to 6 h. However, addition of AK15 and AK19 caused clustering of AK23-Dsg3 complexes at cell-cell borders and subsequent loss of surface Dsg3. Similar results were obtained with PV patient IgG, suggesting that polyclonal IgG contributes to Dsg3 endocytosis and loss of adhesion. Consistent with this possibility, the addition of goat anti-mouse (GAM) IgG to drive clustering of cell surface AK23 mimicked the effects of polyclonal IgG. Furthermore, GAM IgG treatment enhanced the clustering, endocytosis, and pathogenic activity of a non-pathogenic Dsg3 antibody (AK15). To understand how clustering and loss of adhesion contribute to Dsg3 endocytosis, a chimeric protein comprising the IL2 receptor EC domain and the Dsg3 cytoplasmic tail (IL2R-Dsg3) was constructed. Interestingly, the addition of the Dsg3 tail dramatically slowed internalization of the receptor. Since IL2R-Dsg3 is non-adhesive, these data further suggest that clustering rather than loss of adhesion causes Dsg3 internalization. Moreover, a series of Dsg3 cytoplasmic domain deletion constructs demonstrated that the RUD domain of Dsg3 harbors sequences that prevent Dsg3 endocytosis. Together, these data suggest that the pathogenic activity of PV IgG can be attributed to both steric hindrance of adhesion by specific antibodies within PV patient sera and the polyclonal nature of patient IgG which causes aberrant clustering and endocytosis of Dsg3.

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Mariponics® SCF-1 has anti-aging activity by stimulating collagen production and promoting fibroblast proliferation

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Mariponics® SCF-1 is a seaweed-based active ingredient generated using a unique combination of controlled marine production technology, and yeast-based biotransformation. It is a proprietary blend of Chondrus crispus extract and fermented Sarcodiotheca gaudichaudii extract. The objective of this research is to study the anti-aging properties of Mariponics® SCF-1. In cultured human dermal fibroblasts, Mariponics® SCF-1 stimulated a 3.3-fold increase in collagen I in the cell culture medium. The activity of Mariponics® SCF-1 is extremely stable, in sharp contrast to some other anti-aging actives such as vitamin C. After extended storage at elevated temperatures, this ingredient retained or increased its collagen stimulating activity. In human fibroblasts, Mariponics® SCF-1 also caused 43% increase in MTT activity and 50% increase in total protein content suggesting a proliferative effect in these cells. Microarray analysis showed that at least seven proliferation-related genes are up-regulated in cultured fibroblasts after 72 hours treatment with Mariponics® SCF-1. Some of these genes are involved in moving cells through the cell cycle while others are required for the division process. In addition, genes related to barrier function and wound-healing were also upregulated two folds or more at 24hrs. Atomic spectroscopy demonstrated the presence of numerous minerals important to skin health and function while HPLC analysis showed that Mariponics® SCF-1 contains Mycosporine-like Amino Acids (MAA), especially palythine and shinorine. These MAAs are known to absorb UV radiation and shinorine has been reported to stimulate fibroblast proliferation. By stimulating collagen production, supporting fibroblast activity and supplying a unique blend of nutrients to the skin, Mariponics® SCF-1 has potential as an anti-aging ingredient for cosmetic use.

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Both Bullous Pemphigoid antigens regulate signaling to Rac and cofilin and thereby determine the motility behavior of keratinocytes

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α6β4 integrin, the two bullous pemphigoid antigens BPAG1e (BP230) and BPAG2 (BP180, collagen type XVII) and plectin are components of a complex termed the hemidesmosome that mediates keratinocyte adhesion to laminin-332 in the basement membrane of the skin. In addition, α6β4 integrin and BPAG1e dictate keratinocyte motile behavior via regulation of the activity levels of the small GTPase Rac1 and the actin-severing protein cofilin. Specifically, keratinocytes deficient in either β4 integrin or BPAG1e exhibit a reduction in Rac and cofilin activities, display a loss of polarity accompanied by decreased lamellipod stability and show aberrant motility behavior patterns. Normal motility and lamellipod stability as well as Rac and cofilin activities are restored in β4 integrin-deficient cells induced to express a truncated form of β4 integrin, lacking binding sites for both BPAG1e and plectin. This was a surprise since one might assume that direct interaction of BPAG1e and β4 integrin is necessary for proper keratinocyte migration. However, immunofluorescence observations indicate that in keratinocytes expressing the mutant β4 integrin, BPAG1e colocalizes robustly with the truncated subunit. Plectin does not. The likely candidate for mediating this indirect association of BPAG1e with the α6β4 integrin heterodimer, containing the mutated β4 integrin, is BPAG2 which possesses both BPAG1e and α6β4 integrin binding sites. To test this possibility, we induced a knockdown of BPAG2 expression in keratinocytes using shRNA. BPAG1e is mislocalized in BPAG2 deficient keratinocytes. Moreover, BPAG2 deficient cells display impaired migration directionality and reduced lamellipod stability. Together these data indicate that the BP antigens, but not plectin, act as a scaffold for signaling by β4 integrin. Such signaling regulates Rac and cofilin activities and, in turn, controls lamellipod stability and keratinocyte migratory behavior.

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A novel function for α6 integrin as a translational regulator of α3β1 integrin expression

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α3β1 and α6β4 integrins are key players in the process of wound healing. α3β1 integrin regulates the motile machinery of keratinocytes, in part, through its interaction with the actin cytoskeleton. α6β4 integrin regulates directed migration through signaling to Rac1 activity, such that cells lacking β4 integrin surface expression display an altered migration phenotype. However, signaling from α6β1 integrin may also contribute to this aberrant migration pattern, since the formation of this heterodimer is induced in β4 integrin deficient keratinocytes. To assess whether this is the case, we knocked down expression of both α6β4 and α6β1 integrins in human keratinocytes using α6A integrin shRNA. Keratinocytes lacking these integrins attached poorly to laminin-332 and, once attached, adhered less strongly to their matrix compared to normal cells. In addition, keratinocytes lacking α6β4 and α6β1 integrins failed to exhibit directed migration, and moved in circular paths at a slower rate than normal keratinocytes. Interestingly, knockdown of α6 integrin led to a decrease in surface expression of the α3 and β1 integrin subunits. mRNA levels of these integrin subunits in the knockdown cells were equivalent to normal keratinocytes, implying translational control of α3 and β1 integrin subunits by the α6 integrin. Consistent with this hypothesis, phosphorylation/inactivation of the translational repressor protein 4E-binding protein was decreased in α6 integrin knockdown cells. Re-expression of the α6 integrin subunit in the knockdown cells failed to bring back β4 integrin expression in most cells. However, it partially restored α3 integrin surface expression, increased cell migration rate but did not induce directed migration. Our data indicate a novel mechanism of integrin crosstalk where α6β4 integrin not only determines directed migration but also controls, via translational regulation, the expression of other integrin subunits required for keratinocyte motility.

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Role of protease-activated receptors-1 and -2 in murine skin fibrosis and human scleroderma

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The role of proteases in fibrosis and scleroderma (SSc)is still uncertain. Protease-activated receptor-1 and -2 (PAR-1, PAR-2) are G protein-coupled receptors involved in inflammation, vascular and immune responses. Because their role in fibrotic inflammatory processes is still uncertain, we investigated the impact of PARs in experimentally-induced skin fibrosis using PAR-1 and PAR-2 gene-deficient mice and human skin SSc tissues by immunohistochemistry. The *in vivo* effects on skin fibrosis and SSc were examined in a bleomycin-induced mouse model by histology, immunohistochemistry, electron microscopy and protein analysis in PAR-1 and PAR-2 gene-deficient (KO) mice. In both wild-type mice and human SSc, PAR-1 and PAR-2 immunoreactivity was enhanced in dermal cells such as myofibroblasts and mast cells of fibrotic mouse skin and human SSc as compared to KO animals and healthy human skin. In PAR-1- and more pronounced in PAR-2 KO mice, bleomycin-induced SSc was markedly diminished. PAR-2 gene-deficiency completely prevented mice from fibrosis-associated accumulation of matrix proteins. The production of TGF-b1 and soluble receptor for TNF-α was diminished in these mice. Cytokine arrays also demonstrate down-regulation of proinflammatory cytokines and chemokines in PAR KO animals. Murine and human dermal fibroblasts showed upregulation of PAR-2 expression when pretreated with bleomycin, which was negative in healthy human and murine skin fibroblasts. In sum, PAR-1 and especially PAR-2 are involved in fibrotic processes during experimentally-induced SSc. This corresponds with enhanced staining for PAR-1 and PAR-2 in dermal (myo)fibroblasts. Thus, PAR-1 and especially PAR-2 may contribute to SSc, and may serve as targets for future therapies of fibrotic skin diseases including SSc.

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Elucidation of the structural and functional abnormalities of type VII collagen RDEB mutants created by site-directed mutagenesis

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Type VII collagen (C7), the major component of anchoring fibrils, is composed of a central triple helical domain (TH) flanked by globular non-helical domains, NC1 and NC2. Mutations in the type VII collagen gene (COL7A1) cause dystrophic epidermolysis bullosa (DEB). In this study, we generated 35 substitution mutations associated with recessive DEB and purified the recombinant mutant proteins. Biochemical characterization of the purified mutant C7s and structural and functional comparisons of them with wild type C7 demonstrated: (1) Mutations within NC1 (G150R and R886P) and within the TH domain (G1703E, G1719R, R1772W, G1782V, M2415I, and G2671V) resulted in synthesis and secretion of a 290 kDa mutant C7 at levels significantly lower than wild type C7. (2) Y1250S, the mutation in the potential phosphorylation site within NC1, resulted in much higher levels of C7 production compared with wild type, indicating that tyrosine phosphorylation may play an important role in the stability of C7. (3) The G1347R, G1522R, G1604R, G1652R and G1673R mutations all produced mutant C7s with an increased sensitivity to proteases and a decreased ability to form trimers. (4) Mutations with different amino acid substitutions (G2674D and G2674R) produced mutant C7s with different structural and functional properties. (5) The G1522R, G1604R, G1652R, and G1673R mutations resulted in C7 mutants that failed to promote fibroblast, but not keratinocyte, migration. These data indicate that the domain(s) in C7 that drives keratinocyte or fibroblasts migration are distinct. (6) Two mutations, G1719R and R1772W, all generated mutated C7s with a significantly reduced ability to promote keratinocyte migration. (7) Mutations R2008C and G2009R interfered with protein folding and caused intracellular accumulation of mutant C7s. We conclude that mutations in the same locus of C7 sequences alter the same C7 function such as folding, molecular stability, cell attachment, or cell motility.

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Down-regulation of desmocollin-2 induces epithelial cell proliferation through activation of Akt/beta-catenin signaling

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The desmosomal cadherins desmocollin (Dsc) and desmoglein (Dsg) are the transmembrane proteins of desmosomes. In addition to their role as cell adhesive molecules, Dsc and Dsg have also been implicated in the regulation of cell proliferation, however the mechanisms are poorly understood. Here, we use a loss of function approach to investigate the mechanism by which Dsc2 regulates epithelial cell proliferation. siRNA mediated down-regulation of Dsc2 in a model epithelial cell line SK-CO15 induced cell proliferation, as measured by EdU incorporation and MTT (tetrazolium reduction) assays, without influencing apoptosis. The increase in cell proliferation correlated with activation of the pro-survival and pro-proliferative Akt and beta-catenin signaling pathways, as determined by immunoblot analysis for phospho-Akt and beta-catenin protein levels. Furthermore, loss of Dsc2 led to increased beta-catenin/TCF-dependent transcriptional activity. Inhibition of Akt using triciribine prevented the increase in beta-catenin nuclear activity and cell proliferation following Dsc2 knockdown. Our results demonstrate that Dsc2 regulates epithelial cell proliferation, by controlling the activation of the Akt/beta-catenin signaling pathway.

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Inactive extracellular superoxide dismutase disrupts the secretion and function of active extracellular superoxide dismutase

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Human extracellular superoxide dismutase (EC-SOD) is an antioxidant enzyme that is located in the extracellular matrix of tissues. Because of the location, EC-SOD prevents cell and tissue damages caused by extracellularly produced ROS. It has been known that EC-SOD is composed of tetrameric subunits and that the subunits exist in two distinct folding variants based on the different locations of the disulfide bonds. One variant is enzymatically active (aEC-SOD) and contains free cysteine 195 residue which is not involved in disulfide bonds. The other one is inactive (iEC-SOD) and contains free cysteine 45 residue. Even though only 2 folding variants have been discovered so far, theoretically, 5 folding variants are possible because there are 5 cysteine residues involved in intramolecular disulfide bonds. Therefore, we constructed 5 mutant expression vectors producing each one of 5 variant EC-SODs and a wild type expression vector producing all 5 different variants to determine whether the variant subunits are produced, secreted and functional. After transfection of the expression vectors to the HEK 293 cell, we observed that all variants were expressed. However, we found that variants containing free cysteine at 45, 107, 189, or 190 residues are degraded by endoplasmic reticulum-associated protein degradation pathways, and only the EC-SOD from wild type vector and the EC-SOD containing free cysteine at 195 residue can be secreted. Finally we demonstrated that co-transfection of the wild type EC-SOD vector and one of the mutant EC-SOD vectors expressing free cysteine at 47, 107, 189, or 190 results in reduced secretion of wild type EC-SOD, suggesting that the mutant EC-SOD may form a dimer with wild type EC-SOD and inhibit the secretion of EC-SOD. These results imply that malfunctions of the degradation of iEC-SOD may causes ROS-mediated diseases such as UV-induced skin inflammation.

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Loss of Ras suppressor 1 induces skin aging through increased generation of reactive oxygen species

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Cellular senescence is induced by a number of cellular stresses such as oncogene activation, oxidative stress, and DNA damage through elevated levels of reactive oxygen species (ROS). Oncogene-induced senescence is a mechanism of tumor suppression that restricts the progression of benign tumors. Ras suppressor protein 1 (RSU-1), which was bound to the IPP (integrin-linked kinase, PINCH-1/LIMS1, parvin) focal adhesion complex based on its interaction with the LIM 5 domain of PINCH 1, is tumor suppressor that have involved in the Ras signal transduction pathway, cell growth, attachment, migration and differentiation. To elucidate the precise mechanism of Ras induced senescence and the role of RSU-1 in skin aging, we investigated function of RSU-1 in HaCaT, NHFB and HDMECs using RNA-mediated interference. HaCaT, NHFB and HDMECs were transfected with 3 siRNAs against RSU-1 using RNAiMAX transfection reagent. Following incubation for 3days, cell proliferation was measured by the MTT assay, senescent cell was counted by SA-β-gal staining, H-Ras gene expression was analyzed by RT-PCR and ROS generation of cells was analyzed with H2-DCFDA by flow cytometry. RSU-1 knock-down HaCaT, NHFB and HDMECs showed decrease of cell proliferation and increase of SA-β-gal staining, H-Ras expression and intracellular ROS. These results indicate that RSU-1 regulates Ras signaling pathway, which subsequently could provoke ROS induced skin aging. In addition, changes in cell migration and attachment were detected in RSU-1 knock-down HaCaT cells. These findings suggest that RSU-1 links to focal adhesion complex and integrin signaling may be regulated by Ras activation. In conclusion, RSU-1 plays an important role in cellular senescence and these studies could provide the insights into the mechanism of skin aging associated with oncogene induced cell senescence.

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Wound healing delay in HIF-1alpha knockdown keratinocytes

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Considering skin mild hypoxic environment, previous work in our laboratory has suggested a potential role of the hypoxia-inducible factor (HIF) in skin physiology.. HIF-1alpha plays a central role in regulating the expression of over 200 genes involved in biological and physiological processes such as glycolysis, apoptosis, adhesion, and cell migration. We demonstrated a key role of HIF-1alpha in skin photoprotection, especially via ROS modulated apoptosis and DNA repair cell responses. In order to investigate in a more physiological environment HIF-1alpha function in skin, we examined whether HIF-1alpha-knockdown (HIF-1αKD) keratinocytes enable the reconstruction into a fully stratified epidermis (Prunieras model). To this end, we inhibited the expression of HIF-1alpha using a lentiviral vector expressing a shRNA directed against the HIF-1alpha mRNA and we seeded HIF-1αKD keratinocytes on dead dermis. HIF-1αKD cells were unable to form an epidermis. We speculated that downregulation of HIF-1alpha might promotes failure of keratinocyte adhesion. Therefore, we probed the effect of HIF-1alpha on expression of the genes involved in keratinocyte adhesion and spreading. Among different genes tested, we found that HIF-1αKD cells were deficient in mRNA/protein expression of all laminin-5(322) subunits, integrin α6 and β1 (its counterpart) as well as collagen IV. Immunostaining confirmed a decrease of focal adhesion plaques associated with an attenuation in the expression of vinculin and laminin-5. We next established that adhesion deficiency of HIF-1αKD cells triggered apoptotic death. The introduction of fibroblasts in the dead dermis could rescue the reconstructed epidermis deficiency. Consistent with these results, HIF-1alpha knockout targeted into mouse keratinocytes resulted in normal skin but provoked a significant delay of wound healing *in vivo*. HIF seems to allow skin to adapt to various stresses, including UV responses and wound healing.

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An *ex vivo* human skin model for the study of (photo)ageing repair ingredients

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In chronically photoaged skin, the fibrillin-rich microfibrils, key structural components of the dermal extracellular matrix, are extensively remodelled and the microfibrils that are found in close proximity to the dermal epidermal junction are markedly reduced. This has been proposed as an early marker for photoageing. Additionally there is a loss of mature dermal collagen, with an involvement of the MMP family of matrix degrading enzymes. The purpose of this study was to investigate whether or not a treatment that induces protein glycation can mimic the photoageing process. A protocol was set-up in which *ex vivo* skin was treated for 9 days with 500 μM of methylglyoxal as a stress inducing agent. The skin models were immunostained for fibrillin-1, collagen-1 and MMP-2. The treatment with methylglyoxal caused a statistically significant decrease of the fibrillin-rich microfibrils in the papillary dermis just underneath the dermal epidermal junction (p=0.0002), thus mimicking *in vivo* (photo)ageing of the skin. Under these experimental conditions, expression of collagen-1 and MMP-2 was not significantly altered by the exposure to methylglyoxal. Treatment of the *ex vivo* skin with 0.1μM of all-trans retinoic acid caused a marked increase in the fibrillin-1 containing microfibrils in the 'aged' conditions to a level exceeding the non-aged untreated control (p = 0.003). The photoageing repair activity of all-trans retinoic acid in this model was further demonstrated by an increase in collagen-1 expression under 'aged' skin conditions (p=0.0003), but not in the non-aged control. Expression of MMP-2 was not affected by all-trans retinoic acid. From these data it is concluded that treatment of *ex vivo* skin with a relatively low concentration of methylglyoxal mimics photoageing of the skin. The anti-ageing activity of all-trans retinoic acid was confirmed in this model.

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Expression of active Matrix Metalloproteinase-1 in human skin fibroblasts causes morphological and functional alterations that mimic aged human skin

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Aged human skin is fragile largely because of fragmentation and loss of type I collagen fibrils, which confer strength and resiliency. Active MMP-1, which is elevated in aged human skin, is capable of cleaving the triple helix structure of type I collagen fibrils, making them susceptible to further proteolysis. We have established a model system to better understand the molecular mechanisms by which fragmented collagen deleteriously alters dermal fibroblast function, in aged skin. Firstly, a series of MMP-1 mutants were constructed, and their enzymatic activities analyzed. Among five mutants, only MMP-1 V94G mutant efficiently hydrolyzed collagen lattices, releasing characteristic 3/4 and 1/4 length collagen fragments. Increased expression of MMP-1 V94G in dermal fibroblasts, using recombinant adenoviral delivery, markedly accelerated three-dimensional collagen lattice fragmentation and increased release of collagen (2.1-fold, n=3, p<0.01). Also, fibroblasts in MMP-1-fragmented collagen lattices had reduced cytoplasmic area, and a collapsed appearance, similar to fibroblasts in aged human skin *in vivo*. Reduction of MMP-1 activity by siRNA-mediated knockdown, or MMI270, a broad-spectrum MMP inhibitor, markedly delayed three-dimensional collagen lattice fragmentation. Furthermore, fibroblasts cultured in MMP-1-fragmented three-dimensional collagen lattices displayed reduced collagen mRNA (reduced 40%, n=3, p<0.01), and reduced expression of regulators of collagen production, including type II TGF-β receptor (reduced 30%), CTGF (reduced 80%), and CYR61 (reduced 83%) (all n=3, p<0.01). Importantly, these alterations mimic those observed in fibroblasts in aged human skin *in vivo*, and thereby support the concept that MMP-1-mediated fragmentation of dermal collagen impairs the structure and function of dermal fibroblasts. Our data provide the foundation for understanding specific mechanisms that link collagen fragmentation to decline of fibroblast function, and the development of novel approaches to improving skin health in the aged.

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Characterization of dermal interstitial proteoglycans in human skin.

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Proteoglycans (PG), which are composed of core protein and covalently linked glycosaminoglycan (GAG), are major structural components of dermal extracellular matrix. Dermal interstitial PGs play important roles in skin homeostasis by acting as ground substance and in the formation and organization of type I collagen fibrils. Dermal interstitial PGs are not well characterized, partly due to the heterogeneity of the PG family. The present study systematically quantified and characterized dermal interstitial PGs with respect to mRNA, protein, GAG chain and ultrastructure. Five of 12 known interstitial PG genes, decorin, biglycan, versican, lumican and fibromodulin, were found to be expressed in human skin, as determined by real-time RT-PCR. Decorin mRNA level is 10-fold more than biglycan, 30-fold more than versican and lumican, 100-fold more than fibromodulin. Decorin is predominantly made by dermal fibroblasts and secreted into extracellular matrix, as demonstrated by *in situ* hybridization and immunostaining. In contrast, expression of the other four PGs is found in both dermis and epidermis. Analysis of total sulfated GAG chain in human skin by SDS PAGE and Alcian blue staining demonstrated that decorin GAG chain is the predominant sulfated GAG chain in human dermis. Electron microscopy revealed that the majority of PG GAG chains in human dermis are bound to type I collagen fibrils. PG GAG chains form an orthogonal array, in a periodic pattern, on the surface of collagen fibrils. All of the collagen-binding PG GAG chains were removed by chondrointinase ABC treatment, consistent with decorin accounting for most (if not all) collagen-binding PG. In summary, decorin is the predominant PG in human dermis and binds with collagen fibrils. These findings are consistent with the critical role of decorin in maintaining appropriate organization of collagen fibrils and skin mechanical properties, as demonstrated by decorin deficient mice.

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Human facial and abdominal subcutaneous preadipocytes are distinguished by their differentiation potential and responsiveness to lipolytic agents

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The loss of subcutaneous fat under the eyes, along the jaw line, and under the cheekbones is associated with aging. In contrast, aging is also associated with an increase in abdominal subcutaneous fat. Understanding the molecular and cellular pathways that regulate the deposition and metabolism of subcutaneous fat in these anatomical areas could identify targets for therapies for these conditions. Mesenchymal progenitor cells (preadipocytes) were isolated from human facial and abdominal skins obtained with informed consent, and were compared for their capacity to differentiate, to synthesize lipids, and to produce lipid droplets. As expected, the combination of IBMX, dexamethasone, and insulin induced the production and accumulation of lipid droplets in both types of preadipocytes. The inclusion of rosiglitazone, a PPAR-gamma agonist, further increased the number of differentiated adipocytes and the size of lipid droplets. The expression of several lipogenic genes (FABP4, FAS, PPAR-gamma, and GLUT4) was similarly induced in facial and abdominal cells. However, a greater proportion of facial preadipocytes were induced to differentiate *in vitro*, and facial cells retained their ability to differentiate through a greater number of sub-passages. Finally, IPTL, a β2-adrenergic receptor agonist, differentially affected facial and abdominal cells, stimulating lipolysis in abdominal cells but not in facial cells. These results suggest that the loss of facial subcutaneous fat with aging is not due to the loss of preadipocytes. The observed differences may be due to tissue-specific mechanisms, possibly to the UV-exposure of facial but not abdominal skin.

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Retinoid suppresses CYR61/CCN1, a negative regulator of collagen homeostasis, in chronologically aged and photoaged human skin *in vivo* and reconstructed human skin.

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Alteration of dermal connective tissue collagen is a prominent feature of both chronologically aged and solar ultraviolet (UV) irradiation-induced premature skin aging (photoaging). Age-related dermal abnormalities are mediated in part by CCN family member, cysteine-rich protein 61 (CYR61/CCN1). CYR61 is elevated in the dermis of both chronologically aged and photoaged human skin *in vivo*, and is involved in aberrant collagen homeostasis by down-regulating production of type I collagen, the major structural protein in skin, and promoting collagen degradation. Retinoids (all-*trans* retinoic acid and its metabolic precursor retinol) have been shown to improve chronologically aged and photoaged skin by promoting deposition of new collagen. We investigated regulation of CYR61 by retinoids in chronologically aged and photoaged skin *in vivo* and three-dimensional reconstructed human skin. Topical treatment of chronologically aged (80+ years) or photoaged human skin *in vivo* with retinol (vitamin A, 0.4%) significantly reduced CYR61 mRNA (60-70%, n=7-10, $p<0.05$) and protein expression (50-65%, n=4, $p<0.05$), compared to vehicle-treated skin. In reconstructed human skin, human recombinant CYR61 reduced type I procollagen (50%, n=3, $p<0.05$) and increased MMP-1 (3-fold, n=4, $p<0.05$), indicating that CYR61 regulates collagen homeostasis. All-*trans* retinoic acid, significantly reduced CYR61 (60%, n=3, $p<0.05$) and MMP-1 expression (50%, n=3-9, $p<0.05$), and increased type I procollagen production (3-fold, n=4-10, $p<0.05$) in reconstructed human skin. These data suggest that suppression of CYR61 expression by retinoids represents a novel therapeutic mechanism to improve the health of chronologically aged and photoaged human skin.

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A conditional knockout of the α3 laminin subunit

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Three α3 containing laminins (-311, -321 and -332) in the skin basement membrane contribute to keratinocyte adhesion and migration. Functional analyses of α3 laminin in adult mice have been stymied because α3 laminin null mice die soon after birth. To circumvent this problem, we developed lama3flox/flox mice, in which exon 42 of the Lama3 gene is flanked by Cre lox sequences, to permit conditional disruption of the Lama3 gene. To test the efficacy of the system, keratinocytes derived from lama3flox/flox mice were infected with adenovirus encoding GFP Cre recombinase. Cells expressing GFP were selected and RT-PCR confirmed excision of exon 42. Excision results in a frame shift followed by a stop codon, disrupting the production of the Lama3 transcript. Cells exhibiting excision are rapidly lost in culture by overgrowth of the small number of cells in which excision does not occur, indicating that the knockout cells are at a survival/proliferation disadvantage. Thus, we cloned GFP Cre recombinase expressing lama3flox/flox cells. In early passage, the clonal α3 knockout cells adhere and proliferate only when maintained on laminin-332 coated substrates and in wild type keratinocyte conditioned medium. Within five passages, the α3 laminin knockout cells adapt to laminin loss, adhere to tissue culture dishes and proliferate in the absence of conditioned medium. Nonetheless, these cells exhibit adhesive deficits consistent with their inability to deposit a laminin matrix. They fail to attach to poly-L-lysine coated substrates even after 2 hours and spread poorly onto substrate. Adhesion and spreading is rescued by plating the knockout cells onto collagen or laminin-332 coated substrates. However, this rescue is incomplete since the α3 laminin knockout cells exhibit significantly weaker adhesion to laminin-332 coated surfaces than lama3flox/flox keratinocytes expressing the α3 laminin subunit. The lama3flox/flox mouse and its cells should prove invaluable for analyzing α3 laminin function in intact skin, cancer and in wound healing.

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<Development of a 3D reconstructed psoriatic tissue model.>

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Despite the progress made in therapeutic measures for psoriasis, development of an immunocompetent in vitro tissue model that mimics the in vivo psoriatic tissue is lagging. In this study, we report the development of a 3-dimensional psoriatic-like tissue model using normal keratinocytes, psoriatic fibroblasts, and plasmacytoid dendritic cells (pDC) using serum free culture medium. The reconstructed 3D psoriatic tissue model was characterized using histology, immunohistochemistry (cytokeratin expression and, HLA-DR staining for pDC), proliferation of basal cells (Ki67 staining), and gene expression (RT-PCR). Results showed that the reconstructed psoriatic 3-D tissue model has phenotypic and architectural similarity to its in vivo counterpart. Cytokeratin (CK)-16 and the proliferative cell marker, Ki67, were overexpressed by the psoriatic tissue model and the expression levels of involucrin were similar to that of explant psoriatic tissues. HLA DR+ pDC were mostly observed in the stratum granulosum and stratum spinosum layers of the epidermis. Released levels of IL-8 and IL-6 were measured following addition of IL-2 and T cells, IL-4, and IL-13 to the culture medium. While IL-2 and T cell exposure resulted in enhanced expression of IL-6 and IL-8, IL-4 treatment decreases IL-8 and inhibits IL-6 release by 24% and 84%, respectively, when compared to untreated controls. Furthermore, like psoriatic skin, the reconstructed tissue model showed over expression of the IL-8 receptor CXCR2 and the human beta defensins 1 and 2. In conclusion, the developed psoriatic tissue model mimics the in vivo counterpart in terms of tissue morphology, tissue structure, and gene expression. The model will likely serve as a valuable tool to study the biology of psoriasis and for preclinical assessment of therapeutic candidates.

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***In vitro* cleavage of desmoglein 1 by matrix metalloproteinases-2**

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Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that are best known for their ability to remodel the extracellular matrix (ECM) by degrading the ECM components. MMP-2 or gelatinase A is constitutively produced and secreted as a zymogen by many types of cells including keratinocytes. Like other members of MMPs, active MMP-2 is also capable of degrading non-matrix proteins such as signaling molecules and certain cell surface receptors. Our previous animal model study has suggested that MMP-2 may be involved in acantholysis and/or tissue injury of pemphigus foliaceus (PF) (JID, 129, Sup1, S38, Abst#227, 2009). PF, an autoimmune skin blistering disease, is mediated by IgG autoantibodies to desmoglein-1 (Dsg1). In the present study, we evaluated the capacity of MMP-2 to cleave Dsg1 *in vitro*. Purified active MMP-2 degraded the recombinant ectodomain of Dsg1 in a time and dose-dependent fashion. A major cleavage fragment of ~50 kDa was detected by antibody that recognized the N-terminal region of Dsg1 but not the anti-His antibody that recognized the C-terminal His-tag. The degradation is completely inhibited by EDTA or synthetic MMP-2 inhibitors. These results suggest a potential role for MMP-2 in Dsg1 shedding and degradation *in vivo*. Further studies are required to demonstrate the *in vivo* degradation of Dsg1 by activated MMP-2 in pathological conditions such as PF.

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Caveolin-1 interacts with desmogleins and modulates desmosome homeostasis

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Desmosomal cadherins are often aberrantly expressed in malignant skin carcinomas, though the mechanism by which these proteins or their binding partners could activate mitogenic signaling is not well understood. Using biochemical and immunological means, we demonstrate that desmogleins bound to and co-localized with caveolin 1 (Cav-1), the major protein of the specialized membrane microdomains caveolae. Cav-1 has been implicated to play significant roles in cell signaling and tumor development. Discontinuous sucrose-gradient ultracentrifugation showed localization of desmogleins to the lipid raft fractions. Disruption of caveolae shifted Cav-1 and desmogleins into non-raft fractions, perturbed desmosomes and compromised cell-cell adhesion. Focusing on Dsg2, sequence analysis revealed that the intracellular domain contains a putative Cav-1 binding motif. To further confirm a Dsg2-Cav-1 interaction, we generated a competing peptide resembling the Cav-1 scaffolding domain and showed that it bound to Dsg2. Finally we demonstrate that disruption of caveolae resulted in an accumulation of a truncated Dsg2 membrane-spanning fragment. A similar cleaved fragment was detected in skin tumors and malignant carcinoma cells resulting from proteolytic processing of Dsg2. The potential role of this short fragment in desmosome homeostasis, cell adhesion and signaling is discussed. In summary the data presented here provide tantalizing clues that the mechanism by which desmogleins mediate intracellular signaling may involve Cav-1.

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DSC3 in skin tumor development and progression

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Desmocollin 3 (DSC3) is a member of the cadherin superfamily of transmembrane glycoproteins and a component of the adhesive core of desmosomes. Desmosomes are cell-cell adhesion structures that are essential for maintaining epidermal integrity. Recently, we have shown that ablation of Dsc3 in mouse skin leads to intraepidermal blistering. Histologically, the lesions in our mice resemble the epidermal blisters observed in pemphigus vulgaris patients. This study demonstrated that DSC3 functions as a cell adhesion protein in the skin. It has been speculated that loss of cell adhesion could facilitate tumor development, in particular tumor metastasis. To test whether loss of Dsc3 function would contribute to tumor development and progression, we generated mice with inducible inactivation of the Dsc3 gene and simultaneously activation of a K-Ras oncogene (Kras LSL G12D) in the basal layer of stratified epithelia (Dsc3 fl/fl; Kras LSL G12D; K5.Cre*PR1 mice). We activated Cre in the back skin of mice by topical application of an inducer (RU486), which led to Dsc3 ablation and K-Ras oncogene activation. Mutant mice showed significantly higher tumor incidences and higher tumor loads than control mice (Dsc3 fl/+; Kras LSL G12D; K5.Cre*PR1 mice). Nevertheless, we did not observe increased malignancy of mutant tumors. We hypothesize that the increased tumor incidence in mutant mice is due to an increased inflammatory response, triggered by micro blisters in the skin, which drives tumor development. Surprisingly, we found that in control tumors, DSC3 expression is lost early during tumor progression beginning at the papilloma stage. In summary, our studies suggest that loss of Dsc3 expression is an early marker for tumor progression from papillomas to squamous cell carcinomas (SCC). Future studies will address whether loss of Dsc3 expression is required for tumor progression towards malignant tumor types, such as low grade SCC.

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Desmosome assembly and dynamics in migrating epithelial cells

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Desmosomes are prominent cell-cell adhesive junctions that anchor the intermediate filament cytoskeleton systems between adjacent cells and thereby provide skin the ability to withstand mechanical stress. The transmembrane core of the desmosome is comprised of cadherin family members, desmoglein and desmocollin. The cytoplasmic domain of the desmosomal cadherins associate with a number of desmosomal plaque proteins and in turn recruit the intermediate filament cytoskeleton to sites of cell-cell adhesion. Cell-cell adhesion in a tissue is a dynamic process which allows individual cells the ability to change their location relative to neighboring cells. Tumor cells often display increased motility, lose adhesion to their neighbors and invade the surrounding stroma. In this study we examined the dynamics of desmosome assembly and junction localization in migrating oral SCC cell lines. In order to image desmosomes in live cells, desmosomal components (desmocollin 2a and plakophilin-3) were fused to GFP and stably expressed in UM-SCC-1 cells. Scratch assays were used to initiate cell migration and desmosome dynamics were characterized. We observed initiation of new desmosome assembly near the leading edge of migrating cells and during cell migration the new desmosomal plaques are transported in a retrograde fashion. In addition, desmosomal plaques appear to accumulate some distance from the leading edge where their migration rate is decreased. Disruption of the actin cytoskeleton perturbed desmosome dynamics suggesting desmosome remodeling in migrating cells is actin dependent. Finally, live cell microscopy was used to observe desmosome assembly in an A431DE cell culture model. Induction of plakophilin-1 caused rapid assembly of Dsc2a/GFP at sites of cell-cell contact but not on the free cell surfaces. These studies begin to examine the complex process of coordinating desmosomal cell adhesion structures in migrating cells.

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A dermal equivalent developed from fibroblast culture alone: Effect of serum

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We have recently developed a new dermal equivalent without exogenous materials by culturing dermal fibroblasts alone with several supplements. In this study, we investigated the effects of serum on the formation of a dermal equivalent. After cultured dermal fibroblasts reached a confluence they were treated with several concentrations of serum. Macroscopically, in contrast to the culture with 10% serum the addition of higher concentration of serum produced a fibrous sheet. Histologically, serum alone in DMEM induced a three-dimensional tissue containing several layers of fibroblasts. It was composed of abundant extracellular matrix containing fibroblasts, suggesting a dermis-like tissue. It revealed collagen fibers by Masson-trichrome staining. Immunohistochemically, the components of dermal extracellular matrix such as type 1 collagen, elastin, and fibrillin-1 were diffusely expressed. Ultrastructurally, a large number of collagen fibrils with cross-striated patterns were found around the fibroblasts. These results showed that a dermal equivalent could be formed by culturing dermal fibroblasts alone with high concentration of serum. They suggest that serum plays an important role in the formation of a dermal equivalent.

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Treatment of the severe photodamage

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Psittacofulvins are a class of pigments found in parrots feathers that are not derived from food carotenoids but endogenously synthesized within the mature feather follicle. Octatrienoic acid is a new molecule, belong to psittacolfulvin family, sharing with carotenoids and retinoids some structural features. 20 subjects with severe facial photoaging were enrolled in this double-blind, randomized, parallel group study. Subjects were divided in two different groups: 10 subjects in the active cream group, 10 subjects in eccipient cream group. All the subjects applied the topic (active or placebo) once a day for 45 days.During the clinical trial no other topics were applied on the skin of the face but the cream and broad-spectrum sunscreens. At basal time (T0), after 45 days (T1) and after 75 days (T2) from the beginning of therapy we evaluated: redness, dryness, scales, and texture of the face skin in all the subjects. The dermatological evaluation was made to verify the presence of actinic keratoses or non melanoma skin cancer and melanoma at T0 and T2. At T0 and T2 we evaluated the photoaged skin (face, front area) by the reflectance confocal microscopy (RCM) to study the effect of octatrienoic acid on the skin. All the considered symptoms were significantly modified from T0 to T1 in the active group respect the placebo group (redness $p< 0.01$, dryness and scales $p < 0.05$).At T2 (1 month after the end of therapy) the same values were present. At the RCM the skin before the treatment showed an important hyperkeratosis, with scales, a significative activation of epidermal melanocytes expression. In the dermis we could see a significative enlargement of diameter of dermal capillaries (vasodilatation) and markers of inflammation. At T2 we noted and improvement of the epidermis physiological condition and a decrease of vasodilatation and inflammation in active group, while we did non see any significative improvement in placebo group. The clinical evidence and the instrumental evaluation provide a strong evidence that octatrienoic acid can improve photoaged skin.

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xCT expression within KS lesions correlates with histopathologic staging

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The Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma (KS), one of the most common malignancies arising in the setting of immune suppression. The amino acid membrane transport protein known as xCT replenishes intracellular glutathione in an environment of oxidative stress, and our lab has demonstrated that KSHV itself upregulates xCT thereby protecting cells from oxidative stress-induced cell death. Interestingly, xCT also serves as a fusion-entry receptor for KSHV. Interruption of xCT expression or function may, therefore, offer a novel therapeutic approach for the treatment or prevention of KS, but whether xCT is expressed by cells within KS lesions to support this hypothesis is unknown. We used immunohistochemistry to quantify xCT expression within KS skin lesions representing the full spectrum of histopathologic progression of KS. We found that stage I tumors (patches) and stage II tumors (plaques) exhibited either no or minimally discernable xCT expression, respectively. In contrast, stage III tumors (nodules) exhibited easily discernable membrane expression of xCT by the majority of cells in these lesions, including nearly all spindle-shaped cells. Moreover, we confirmed that stage III tumors contained significantly more KSHV-infected cells than stage I lesions. Collectively, these are the first clinical data supporting a putative role for xCT in promoting KSHV dissemination and the survival of KSHV-infected cells in the KS tumor microenvironment.

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Toll-like receptor signaling increases kallikrein in rosacea and affect skin barrier function

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The epidermis of patients with rosacea has increased and abnormally processed Cathelicidin antimicrobial peptides that are generated by serine proteases such as kallikrein 5 (KLK5). These peptides lead to an inflammatory and vascular reaction in mice that resembles the characteristic histopathological changes in rosacea. We sought to understand the mechanism of abnormal Cathelicidin and KLK5 expression in rosacea by evaluating genes known to influence cathelicidin expression. Quantitative RT-PCR and immunostaining showed higher TLR2 expression in rosacea skin (P=0.04, N=11). When overexpressed in cultured keratinocytes this reproduced findings in rosacea: TLR2 expressed by adenovirus (Ad-TLR2) in normal human keratinocytes resulted in increased KLK5 protein (Ad-TLR2:104.3±36.06 vs Ad only:0.07±0.02 ng/ml, P<0.01) and total protease activity (2-fold increase, P<0.001). Effects of TLR2 on KLK5 expression seemed to be post-transcriptional because 1) TLR1/2 ligand (Pam3CSK4) barely increased KLK5 mRNA, and 2) brefeldin A (vesicle transport inhibitor), but not cycloheximide (gene transcription inhibitor), decreased KLK5 protein release, and 3) Pam3CSK4 induce calcium flux and calcium ionophore Calcinomycin suppressed Pam3CSK4-dependent KLK5 increase in cultured media. Because KLK5 is also involved in skin barrier formation, we examined transepidermal water loss (TEWL) of *Tlr2*$^{-/-}$ mice and found that the mice showed delayed TEWL recovery after the skin barrier was disrupted. We also observed by electron microscopy that *Tlr2*$^{-/-}$ skin delayed lamellar granule release after skin barrier disruption. These data suggest that TLR2 signaling is important to multiple elements of skin innate immunity including protease activity, production of antimicrobial peptides, and formation of the physical barrier of the stratum corneum. Thus, increased TLR2 in rosacea may further explain aberrant action of cathelicidin and kallikrein in this disorder.

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Broad defects in epidermal cornification in atopic dermatitis (AD) identified through genomic analysis

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Background:Psoriasis and Atopic Dermatitis (AD) are common inflammatory skin diseases. Both diseases display immune infiltrates in lesions and epidermal differentiation alterations associated with defective barrier. An incomplete understanding of differences between these diseases makes it difficult to compare human disease pathology to animal disease models. Objective: To characterize differences between these diseases in expression of genes related to epidermal differentiation and inflammatory circuits. Methods:We performed genomic profiling of mRNA in chronic psoriasis (n=15) and AD (n=18) skin lesions, compared to normal human skin (n=15). Results: As expected, clear disease classifications could be constructed based on expected immune polarity (Th1, Th2, Th17) differences. However, even more striking differences were identified in epidermal differentiation programs that could be used for precise disease classifications. While both psoriasis and AD skin lesions displayed regenerative epidermal hyperplasia, which is a general alteration in epidermal growth, keratinocyte terminal differentiation was differentially polarized. In AD, we found selective defects in expression of multiple genes encoding the cornified envelope, with the largest alteration in loricrin (2% of normal skin). At the ultrastructural level, the cornified envelope in AD was broadly defective with highly decreased compaction of corneocytes, and reduced intercellular lipids. Hence, the entire keratinocyte terminal differentiation program (cytoplasmic compaction, cornification, and lipid release) is defective in AD potentially underlying the immune differences. Our study shows that although alterations in barrier responses exist in both diseases, epidermal differentiation is differentially polarized, with major implications for primary disease pathogenesis.

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Modulatory effect of platelet-rich plasma on human fibroblasts

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Recently usefulness of autologous platelet-rich plasma has attracted attention in the field of anti-aging medicine, and it has been applied successfully in clinical use for mesotherapy for skin rejuvenation. Promoting the biosynthetic capacity of fibroblasts which play an important role in tissue remodeling is a one of the goal of this technique. However, little has been reported regarding the effect of the platelet-rich plasma on Human fibroblast. The aim of this study is to investigate the effect of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) on fibroblast cell function *in vitro*, which will provide important data for clinical application, and identify the mechanism underlying this process. PRP and PPP were prepared using a double-spin method and activated with thrombin and calcium chloride. To measure the proliferative potential of activated PRP and PPP, Cell proliferation was measured by [3H]thymidine incorporation assay. To evaluate synthetic capacity, the level of procollagen type I carboxy-terminal peptide (PIP) was quantified using enzyme linked immunosorbent assay (ELISA). In addition, collagen and matrix metalloproteinases (MMP) production was studied through Western blotting and Reverse transcriptase-polymerase chain reaction (RT-PCR). PRP clot releasate stimulates cell proliferation and collagen deposition and enhances the gene expression of matrix-degrading enzymes by human fibroblast *in vitro*. This suggests that *in vivo* PRP application could lead to stimulates collagen synthesis, which may promote accelerated tissue remodeling.

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Antibacterial and antiviral activity of a new non-alcoholic hand sanitizer

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Encapsulation of personal care ingredients is a new strategy to circumvent adverse processing effects and to enhance the stability profile of otherwise volatile or thermally-sensitive ccnstituents. Starch encapsulation technology was applied to the development of a cosmetically-acceptable non-alcoholic moisturizing hand sanitizer. The active biocide, BC, benzalknoium chloride (0.1-0.13%)meets FDA's category III OTC drug guideline for antiseptics. Other inactive ingredients are: Hydrogenated Soybean Oil, Cocoa Nucifera, Hydrogenated Cottonseed Oil, Cornstarch, Distearyldimonium Chloride, Glycerin, Cyclopentasiloxane, Dimethicone, Citra Gradis (Grapefruit) Fruit Extract, and Fragrance. These ingredients contribute to the moisturizing emolliency and super-soft feel of the product.Low thermal and low shear processing create a micro-emulsion, that encapsulates oil droplets in a starch shell. Independent laboratory testing has confirmed that this hand sanitizer lotion rapidly (1, 2, and 5 minutes) kills greater than 5-logs of most common bacterial pathogens including: Escherichia coli, a methicillin-resistant strain of Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Clostridium difficille cells. It also kills greater than 8-logs of the H1N1 strain of the Swine Flu virus and of the Feline Calcivirus (Norwalk virus), that is responsible for viral entrogastritis. The results of bacterial agar transfer studies showed that BC activity was persistent on human skin for at least 4 hours. By substituting BC for alcohol as the active,a more skin friendly, non-irritating, non-combustible hand santizer is made possible. Other independent laboratory testing has established that this hand sanitizer formulation passes the highest oral toxicity safety rating (LD50=5g/Kg). Finally, it can be labelled "hypoallergenic" as there were no adverse effects in a 200 human subject skin irritation and dermal sensitization study. The new non-alcoholic hanbd sanitizer is now commercially available under the trade name, Soft and Shield(TM).

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Correlation of livedo racemosa, cutaneous inflammatory plaque, and antiphospholipid antibodies in patients with cutaneous polyarteritis nodosa

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We previously reported the presence of anti-phosphatidylserine-prothrombin complex (anti-PS/PT) antibodies (Abs) and/or lupus anticoagulant (LAC) in all 16 cutaneous polyarteritis nodosa (CPN) patients we investigated. We further suggested that serum IgM anti-PS/PT Ab levels could be closely related to pathogenic factors that trigger the development of livedo racemosa. We examined the prevalence of various cutaneous symptoms including livedo racemosa and inflammatory plaques, antiphospholipid Abs in patients in 50 patients with CPN. We retrospectively investigated the clinical and serological features, the direct immunofluorescence findings, and treatment methods used. Subcutaneous nodules were observed in all 50 of our CPN patients. Forty-four (88.0%) had livedo racemosa, 30 (60.0%) had skin ulcers, and 14 (28.0%) had inflammatory plaques. Serum IgM anti-PS/PT Abs were significantly higher in patients with livedo racemosa than in patients without livedo racemosa. Serum IgG anti-PS/PT Ab levels differed significantly between those with inflammatory plaques (12.86±13.16 U/ml) and those without inflammatory plaques (6.53±5.92 U/ml). Similar trends were seen with respect to IgG anti-cardiolipin (aCL) Ab positive findings. In contrast, levels of IgM anti-PS/PT Ab were significantly lower in patients with inflammatory plaques compared to patients without. Inflammatory plaques were significantly more prevalent in patients with skin ulcer. Warfarin and prednisolone were selected as the primary therapy at a significantly higher rate in CPN patients with inflammatory plaques and skin ulcer than in patients without. We suggest that a variety of antiphospholipid Abs could influence the cutaneous patterns of CPN. In particular, IgG anti-PS/PT Abs and/or IgG aCL Abs could reflect the presence of inflammatory plaques as an especial cutaneous manifestation of CPN.

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Non invasive study for the detection of advanced glycated end products within the skin and nails in patients with Diabetes mellitus

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Advanced glycation end products (AGEs) is a class of complex products resulted from chronic hyperglycemia which leads to an increased non enzymatic glycation of proteins with irreversible formation and deposit of these reactive end products. The major AGEs *in vivo* are 3-deoxyglucosone, glyoxal, and methylglyoxal; these products are used for assess metabolic control, such as glycated hemoglobin. Recently AGEs has been associated to endothelial dysfunction, ageing, chronic renal disease and neurodegenerative disease. Our aim was to detect AGEs within keratinous tissue (skin and nails) in patients with Diabetes mellitus (DM), with the purpose of developing a non invasive technique in order to asses the metabolic history and thus improve the therapeutic control of the patients with DM. One hundred and ten patients were included with or suspected diagnostic of Diabetes mellitus (41 male), mean age 51.8 years, measuring 3-deoxyglucosone, glyoxal and methylglyoxal with Raman spectrometer (Raman system 3000), in three anatomic places such as back of left ear lobe, inside of left arm, and over the left thumbnail at 6mm from the nail fold. We cross referenced the amount of AGEs with the concentration of glycated hemoglobin obtained from patient's serum. It's possible to detect AGEs within the skin and nails, however no relationship was found with glycated hemoglobin. Raman spectroscopy represents a noninvasive alternative to determining AGEs within the skin and nails. This technique might be used in monitoring patients with Diabetes mellitus. Since a linear relationship was found between patient age and the concentration of methyglyoxal, this might help in determining problems that appear in the hypergeneration of AGEs, such as endothelial dysfunction, ageing, chronic renal disease and neurodegenerative disease.

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An effusion of blood and phlegmon secondary to anakinra

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A 52 year-old male with psoriatic arthritis on anakinra for one month presented with two black ulcerations, surrounding erythema and induration on his right knee. He denied any fevers. The patient received an escharectomy and one dose of levoquin without much improvement. He was started on topical retapamulin but cultures later showed no growth. He was switched to methylprednisolone with some improvement in erythema and induration, but then returned to clinic one week later with an increase in swelling. Upon incision and drainage, a copious sanguinous purulence was removed (Fig 1). Cultures were again negative. MRI of the thighs bilaterally showed fluid or phlegmon within the subcutaneous tissue of the thighs bilaterally. Findings represented an abscess or phlegmonous inflammation. A week later, the patient developed a left thigh fluctuance which contained 11 cc of purulent material. Cultures were obtained and showed no growth (Fig 2). Anakinra is a recombinant methionyl human interleukin-1 (IL-1) receptor antagonist which competes for the IL-1 receptor with IL-1 and thus prevents IL-1 signaling. It is indicated for the treatment of rheumatoid arthritis. IL-1 has been implicated in the pathogenesis of psoriatic arthritis due to increased levels measured in synovial fluid of patients with psoriatic arthritis compared to osteoarthritis. This theory has led to the off-label use of anakinra in psoriatic arthritis. This is the first reported case of a severe reaction of blood and phlegmon secondary to anakinra.

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The dermatologic manifestation of hyperandrogenism: A retrospective chart review

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In order to evaluate the cutaneous features and metabolic findings in women with hyperandrogenism, a retrospective chart analysis compiled by three dermatologists in both academic and private settings was performed, including patients presenting with two or more manifestations of hyperandrogenism. Relevant dermatologic and associated manifestations, laboratory and imaging study findings were reviewed. Acne was the most common skin finding for hyperandrogenism (88%), the majority classified as moderate to severe (62%). Other common manifestations include hirsutism (58%), androgenetic alopecia (32%), seborrheic dermatitis (51%), acanthosis nigracans (53%) and skin tags (31%). Oligomenorrhea was the most common systemic presenting sign, found in 74% of women in the survey. Moderate to severe acne and skin tags were both correlated with low levels of sex hormone binding globulin (40%). Interestingly, acanthosis nigracans, skin tags and seborrhea were all correlated with elevated total testosterone and elevated insulin levels. The calculated free androgen index was a strong marker of hyperandrogenism. This study demonstrates that in patients presenting with two or more hyperandrogen symptoms, hormonal and metabolic abnormalities are prevalent. Acanthosis nigricans, seborrhea and tags are markers for diabetes.

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Results of a phase 2b clinical trial of valomaciclovir versus valacyclovir for treating herpes zoster

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Herpes zoster (HZ) and post herpetic neuralgia (PHN) cause significant morbidity. Early treatment of HZ decreases rash resolution time, morbidity, pain, PHN incidence, and improves quality of life. Valomaciclovir is a novel acyclic guanosine analog that is 400 times more active in vitro than acyclovir against varicella zoster virus, which reactivates and yields HZ. A 2b randomized, double-blind, active-controlled, multi-center, parallel-group dose ranging study evaluated the safety and efficacy of valomaciclovir vs. valacyclovir in immunocompetent patients with acute HZ. Forty-six centers randomized and treated 359 patients in 4 groups: 1000 mg (n= 119), 2000 mg (n=119), or 3000 mg (n=18, PK group) of valomaciclovir daily, or 1000 mg valacyclovir TID (n=116). Patients received 7 days of medication with evaluation through day 28, and further follow-up to 120 days in patients with persistent pain. Participants were 131 males and 228 females; mean age of 53.8 years. The primary endpoints in rash evaluation were times to complete crusting and crusting resolution. Secondary endpoints were times to rash resolution, cessation of new lesion formation, and pain cessation. The 2000 and 3000 mg valomaciclovir arms met all endpoints, being at least non-inferior to valacyclovir in these markers of treatment efficacy. The 3000 mg dose was highly significant (p=.007) in time to lesion crusting. Faster pain resolution and more patients with total pain resolution were in the 2000 and 3000 mg groups as compared to valacyclovir. In patients presenting between 48-72 hours, all valomaciclovir groups had faster rash resolution vs. valacyclovir, indicating that valomaciclovir is still effective when initiated 3 days after vesicle appearance. Valomaciclovir was well tolerated without serious adverse events. The most common adverse events were nausea (n=18), vomiting (n=9), and headache (n=6). Valomaciclovir is effective treatment for acute HZ with advantages of QD dosing and data supporting a wider treatment window.

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(-)-Epigallocatechin-3-gallate reactivates silenced tumor suppressor gene by modifying DNA methylation and histone acetylation patterns in human epidermoid carcinoma cells

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Epigenetics, the heritable processes that modulate gene activity without changes in the DNA sequence, have gained considerable interest in cancer development and molecular targets of cancer treatment. Dietary factors have been shown to alter cancer risk by modifications of epigenetic processes like DNA methylation and histone modification in cancer cells. Therefore, the present study was designed to investigate whether (-)-epigallocatechin-3-gallate (EGCG), a polyphenol from green tea, would alter epigenetic events to regulate methylation-silenced tumor suppressor genes in human skin cancer cells. DNA methylation, histone modification and gene expression profile were studied using human epidermoid carcinoma A431 cells as an,i>in vitro *model after EGCG treatment using RT-PCR, western blotting, cytostaining, dot-blot analysis, chromatin immunoprecipitation and fluorescence activated cell sorting analysis of apoptotic cell death. Our study shows that treatment of A431 cells with EGCG (5, 10 and 20 μg/ml) for 3 to 6 days resulted in DNA demethylation and reduction in the mRNA and protein levels of DNA methyltransferases (DNMTs) 1 and 3a and 3b and reduction in the activity of DNMTs in a dose- and time-dependent manner. EGCG also increased the levels of acetylated lysine 9 and lysine 14 on histone H3 (H3-Lys 9, H3-Lys 14), but decreased levels of methylated H3-Lys 9. EGCG re-expressed mRNA and protein levels of tumor suppressor genes, p16INK4a and p21WAF1/CIP1, in A431 cells. Re-expression of p16INK4a and p21 by EGCG treatment of the A431 cells leads to the inhibition of cell proliferation and induction of apoptosis of A431 cells. Together, our study demonstrates that EGCG can epigenetically modulate DNA and histones to activate methylation-silenced tumor suppressor genes. These epigenetic modifications by EGCG may contribute to skin cancer prevention/treatment strategies in humans.*

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Modification of skin discoloration by an anti-oxidant

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Skin hyperpigmentation, and the reactions that precipitate it, has been linked to free radicals by the fact that free radical scavengers or antioxidants can slow that hyperpigmentation. We have screened several hundred plant extracts for anti-oxidants and discovered one that is both a strong antioxidant and can reduce skin hyperpigmentation. Extracts of Dianella ensifolia contain 1-(2, 4-Dihydrophenyl)-3-(2, 4-dimethoxy-3-methylphenyl) propane (DP), which was found to inhibit the free radical 1,1 – diphenyl-2-picrylhydrazyhydrazl (DPPI) with an EC50 value of 78 uM. The DP was also found to inhibit UVC induced lipid oxidation with an EC50 of about 30 uM. We next investigated the effects of this antioxidant on skin hyperpigmentation. The reduction of discoloration by different topical treatments has been assessed in human volunteers using an *in vivo* assay for the rate of fading of UVB-induced tan. Two pharmaceutical formulas containing 4% hydroquinone (HQ) were used as positive controls, and we tested the ability of 1-(2, 4-Dihydrophenyl)-3-(2, 4-dimethoxy-3-methylphenyl) propane (DP), a plant-derived amphoteric antioxidant, to increase performance of non-HQ cosmetic formulations. We found that the cosmetic formula containing DP produced an increase in the rate of fading compared to the two pharmaceutical treatments containing HQ.

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Cutaneous T cell lymphoma utilizes distinct syndecan-4 moieties to bind DC-HIL avidly and to trap TGF-β: Novel mechanisms that may blunt host anti-tumor responses.

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We discovered that DC-HIL on antigen presenting cells attenuates T cell activation by binding heparan sulfate (HS) moieties of syndecan-4 (SD-4) on activated T cells. We also showed malignant lymphocytes in lesional skin and peripheral blood of patients with cutaneous T cell lymphoma (CTCL) to overexpress SD-4 that again was capable of inhibiting T cell activation via the TCR/CD3. We noted that SD-4 expression by CTCL lines (MJ and HuT-78) was similar to those of *in vitro*-activated normal T cells, but found CTCL cells to bind DC-HIL with much greater affinity than activated normal T cells. Positing this differential affinity to be due to structural heterogeneity among HS moieties of SD-4, we tested 3 mAb directed against distinct HS epitopes. CTCL cells expressed F58 and Hepss-1 epitopes highly, whereas activated normal T cells did so only at very low levels. Functionality of both epitopes was verified by the ability of epitope-specific mAb to abrogate binding of SD-4$^+$ CTCL to DC-HIL. Because malignancies are associated with the inhibitory cytokine TGF-β, we examined its expression and that of its receptors by CTCL. CTCL cells produced TGF-β1 and expressed some (not all) types of receptors. We questioned whether SD-4 on CTCL can bind TGF-β1 and found that it does (at least 3 days in culture). Pretreatment of CTCL cells with anti-SD-4 Ab did not block binding to TGF-β1, whereas addition of anti-F58 and anti-Hepss-1 mAb did. To examine the impact of CTCL-bound TGF-β, MJ cells pretreated (or not) with TGF-β1 were added to culture of normal T cells with anti-CD3 Ab. TGF-β1-treated MJ cells inhibited activation of normal T cells more efficiently than untreated controls. We conclude that CTCL is endowed with distinct SD-4 moieties that enable it to bind DC-HIL with greater affinity as well as trap/accumulate TGF-β1 on its surface. Either attribute may allow CTCL to blunt host immune responses mounted against it.

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A phase I/II trial of photoactivated tissue bonding ("nanosuturing") for excisional wound closure

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Inflammation initiated by epidermal sutures can stimulate fibrosis and accentuate scarring after closure of skin wounds. We have developed a novel light-activated technology that forms protein-protein covalent crosslinks ("nanosutures") between tissue surfaces to produce an immediate and tight seal. We investigated whether superficial closure of excisional wounds with this technology (photoactivated tissue bonding, PTB) produces less scarring than closure with standard interrupted sutures. A randomized, single-blind split-lesion design was used to evaluate healing of 31 skin excisions. Following deep closure of all excisional defects with coated Vicryl sutures, one-half of each wound was superficially closed with interrupted nylon sutures while the other half was stained with Rose Bengal dye (0.1% in PBS) and treated with green light (532 nm KTP laser, 100 J/cm2, 0.5 W/cm2, 200 seconds). The primary outcome measure was scar appearance at 6 months as evaluated by two blinded physicians, the patient and three independent dermatologists viewing scar photos. A secondary outcome was patient satisfaction. Mean values and standard deviations were compared using Students t-test. Both the on-site clinical observers and the post hoc panel of dermatologists rated the scar appearance with PTB closure to be significantly better than that with sutured closure ($p=0.002$ and $p=0.001$, respectively). The scar width was the major differentiating characteristic ($p = 0.002$). Patient satisfaction with scar appearance was also significantly greater for PTB ($p<0.001$) than sutures and patients had a slight preference for the PTB procedure ($p = 0.036$). There were no significant complications with the PTB closure. These results indicate that excisional wound closure with PTB is similarly effective and less scar forming than superficial interrupted sutures. This first-to-human trial shows that molecular crosslinking of tissue proteins is a viable alternative to standard closure methods.

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Infant skin is similar in New Jersey and Mumbai and markedly different in Beijing in winter

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Infant skin has been assumed to react similarly to external factors across the continents. In the present study we extended our previous studies on infant and adult skin carried out in New Jersey (NJ), USA to Beijing, China and Mumbai, India to test this assumption. Beijing has similar climatic conditions as NJ, and Mumbai has a tropical climate throughout the year. Measurements were conducted in winter and in summer at Beijing and NJ and in summer at Mumbai. Measurements included Transepidermal Water Loss (TEWL) and conductivity on the dorsal forearm, and facial imaging in visible, cross polarized and UV fluorescence modalities in Beijing (112 infants, 35 adults), in NJ (21 infants, 21 adults), and in Mumbai (145 infants, 40 adults). To avoid motion artifacts, an imaging station was specially developed to allow the acquisition of a rapid series (5-10) of images of the infants. The results show similarities in the summer data for NJ and Mumbai in both TEWL and conductivity as well as in imaging. In summer, conductivity values in Beijing show similarities with those of NJ; TEWL values were significantly higher in Beijing infants. The images obtained in the winter in Beijing show marked differences from those obtained in the other locations, as pronounced facial erythema on the cheeks and chin (but not on the nose and forehead) was observed on more than 75% of the subjects, and was accompanied by scale in some cases. These results show both the consistency of the data between Mumbai, NJ and summertime Beijing and the surprising differences in facial skin in the winter in Beijing, which may indicate the possible influence of environmental factors on skin health. The contributing factors may include the cold and dryness of the winter air of Beijing and possibly to some skin care regimens particular to this region and season. Further studies need to be conducted.

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A clinical study on herpes zoster meningoencephalitis

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Herpes zoster meningoencephalitis is a rare neurological complication and the known risk factors include immunocompromised patients, infiltration into trigeminal ganglion, disseminated herpes zoster, elderly patients, and relapsing herpes zoster. Complications including cerebral hemorrhage, disturbance of consciousness, hemiplegia, and seizure may develop; therefore, an early diagnosis is important. However, the diagnosis must be made very carefully because patients may not have the typical symptoms of meningoencephalitis such as neck stiffness, headache, and fever. Therefore, we have carried out this study to diagnose herpes zoster meningoencephalitis at an early stage. Medical records of 5114 herpes zoster patients (1996 ~ 2009) were examined. Among them, 18 patients diagnosed as herpes zoster meningoencephalitis by cerebrospinal fluid tests were subject to assess incidence rates, age distribution, ganglion distribution, clinical aspects, underlying diseases, and presence of complications. The cases that patients had headaches and accompanying nausea or vomiting symptoms, sensitivity(88.9%), and positive predictive value(69.6%) showed a statistically significant increase ($p<0.001$). Sensitivities to neck stiffness and fever, which are typical symptoms of meningoencephalitis, were low in patients with herpes zoster; 33.3% and 11.1%, respectively. Infiltration into the trigeminal ganglion and immunocompromising underlying diseases accounted for 50% and 16.7% of total patients, respectively. Old age and disseminated herpes zoster were not found to be associated with the occurrence of meningoencephalitis. In the case of disseminated herpes zoster patients, no meningoencephalitis developed. All patients were treated with acyclovir for 10~14 days, and cerebral hemorrhage occurred in 1 of them (5.5%) after treatment. In conclusion, when a patient has a headache and nausea or vomiting symptoms, herpes zoster meningoencephalitis should be considered even if neck stiffness and fever are not accompanied.

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Anti-tumor effects of sapacitabine in cutaneous T-cell lymphoma (CTCL) cells

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Sapacitabine (CYC682), an oral deoxycytidine analog, is in clinical trials for the treatment of solid and hematological malignancies including cutaneous T-cell lymphoma (CTCL). Sapacitabine has displayed potent anti-tumor effects in a variety of transformed tumor cell lines and in animal models. The purpose of this study was to address *in vitro* anti-tumor effects of sapacitabine and to explore whether sapacitabine improves sensitivity to vorinostat in vorinostat-sensitive (HH) and -resistant (HH/VOR) CTCL cell lines. Cells were treated with sapacitabine at 0, 50, and 500 nM for 48 and 72 hrs. Cell viability was determined by the MTS assay. Apoptosis and cell cycle arrest were measured by FACS analysis of annexin V/PI binding and cell cycle distribution. In the vorinostat-sensitive HH cell line, sapacitabine at 50 to 500 nM for 48 and 72 hrs increased annexin V binding by 13 to 50% and 42 to 55% respectively in a dose- and time-dependent manner. Under the same experimental conditions, sapacitabine also increased sub-G1 arrest at all concentrations but induced G2/M arrest only at 50 nM. In the vorinostat-resistant HH/VOR cell line, sapacitabine at 50 nM did not increase annexin V binding; however, sapacitabine at 500 nM for 72 hrs increased annexin V binding by 16%. Sapacitabine at 50 nM did not induce sub-G1 but induced G2/M arrest. Sapacitabine at 500 nM for 72 hrs induced not only G2/M but also sub-G1 arrest in resistant cells. Co-treatment with sapacitabine did not increase apoptosis induced by vorinostat in the vorinostat-resistant HH/VOR cells compared to baseline controls. In summary, our data suggest that sapacitabine at the clinically relevant concentration of 50 nM induces apoptosis and G2/M arrest in vorinostat-sensitive CTCL cells, but only induces G2/M arrest in vorinostat-resistant cells. The vorinostat-sensitive CTCL cells are more sensitive to sapacitabine than resistant CTCL cells. Sapacitabine could not reverse the resistance to vorinostat in the vorinostat-resistant CTCL cells. This could have implications for future clinical trials.

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Green tea extract protects normal human skin fibroblasts from hydrogen peroxide-induced necrosis *in vitro*

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Green Tea Extract (GTE) has previously been shown to demonstrate protective effects from oxidation, aging, cancer, and inflammation in skin. However, little is known about the effects of GTE on skin necrosis. Using an *in vitro* model, we studied the effects of several concentrations of GTE on hydrogen peroxide-induced necrosis in normal human skin fibroblasts. Cell numbers, viability, morphology and intracellular free radicals were assessed by fluorescence microscopy and flow cytometry. The results demonstrate GTE protects from hydrogen peroxide-induced necrosis in a dose dependent manner. The highest dose of GTE (0.01%) resulted in the greatest protection from necrosis, as shown by increased cell numbers, viability and improved cell morphology. The protective effects of GTE on hydrogen peroxide-induced necrosis appear to be mediated directly by decreasing reactive oxygen species. These results suggest an anti-inflammatory role of GTE mediated by decreased fibroblast necrosis.

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Caffeine protects normal human skin fibroblasts from hydrogen peroxide-induced necrosis *in vitro*

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Caffeine has previously demonstrated anti-oxidant, anti-carcinogenic and anti-apoptotic properties in several skin models. Little is known about the ability of caffeine to prevent necrosis. Using an *in vitro* model, we studied the ability of caffeine to protect against hydrogen peroxide-induced necrosis and intracellular reactive oxygen species free radicals. As demonstrated by an Annexin V (AV) and Propidium Iodide (PI) flow cytometry assay, four hour incubation of 0.01 mM Caffeine provided statistically significant (p value < 0.05) protection from 120 minute exposure of 1.2 mM hydrogen peroxide-induced necrosis in normal human skin fibroblasts AG13145 when compared to cells exposed to hydrogen peroxide under the same conditions with no caffeine treatment (90.1 ± 1.0% versus 61.8 ± 2.4% AV-PI- viable cells). These results are interesting because caffeine 0.01 mM treatment did not result in statistically significant reduction of hydrogen peroxide-upregulated intracellular reactive oxygen species free radicals when compared to the fibroblast cells exposed to hydrogen peroxide alone. Our findings implicate that the anti-necrotic mechanism demonstrated is not dependent on reduction of intracellular free radicals.

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Cure rate, duration required for complete cure and recurrence rate in onychomycosis according to clinical factors

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Despite great advances in the treatment of onychomycosis, many factors affect the cure rate (CR), duration required for complete cure (DC) and recurrence rate (RR). The purpose of this study was to evaluate CR, DC, and RR in onychomycosis according to various clinical factors. We reviewed retrospectively medical records of the 637 patients who were diagnosed as onychomycosis and started treatment between December 2000 and December 2005 in our research hospital. We obtained the following 6 clinical factors: age, sex, clinical types based on Zaias classification, treatment patterns (itraconazole, terbinafin, and only topically treated groups), presence of diabetes mellitus (DM), and extent of nail involvement. Cure was defined as normal clinical appearance and negative for potassium hydroxide preparation. DC and recurrence were designated as the weeks from the beginning of therapy to the complete recovery and the condition when patients experienced any new infection after having achieved a cure, respectively. From these data, we compared statistically significant differences in CR, DC, and RR depending on the above-mentioned 6 clinical factors. Total of 207 patients were finally evaluated except 328 patients (51.5%) who had not completed systemic and/or topical antifungal medications and 102 patients (16%) who had been lost before the complete recovery. Our study revealed as follows; CR was 78.3%, DC was 31.7 ± 18.4 weeks, and RR was 36.0% as a whole; extent of nail involvement (under vs. over 50% of the nail) affected CR, DC, RR; age (under vs. over 60 years old) affected CR, DC; existence of DM affected DC, RR. On the other hand, there were no significant differences according to gender, clinical types and treatment patterns. In conclusion, we found the greater extent of nail involvement, old age and DM have a negative effect on the treatment of onychomycosis to a greater or lesser degree. These results would be helpful to establish therapeutic plan and predict prognosis of onychomycosis.

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Micronized sunscreen particles were not shown to penetrate beyond the stratum corneum in adults and children

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The purpose of this study was to evaluate the absorption characteristics of a topically administered sunscreen product containing micronized zinc oxide and titanium dioxide. Two methods were employed: 1) Confocal Laser Scanning Microscopy (CLSM) which allows for the monitoring of highly scattering endogenous and exogenous moieties within the epidermis; and 2) Raman Confocal Microspectroscopy (RCM) which identifies components at different depths within the epidermis based on molecular vibrations. CLSM in reflectance mode and RCM were used in 12 adults and 10 infants at baseline and after topical application of a proprietary sunscreen containing micronized zinc oxide and titanium dioxide. Eighteen microliters of the sunscreen formulation was applied evenly to the designated 3 cm x 3 cm test site and allowed to remain in contact with the skin for 30 minutes. Residual product was then removed prior to post-treatment measurements. CLSM images showed that particles of zinc oxide and titanium dioxide concentrated on the top of the stratum corneum and at the microrelief lines in both adults and infants after a single application. RCM spectra was used to construct concentration profiles of zinc oxide and titanium dioxide through the stratum corneum and viable epidermis and these did not show penetration of the particles beyond the stratum corneum of adults or infants. While concern has been raised in the literature about the use of micronized particles, little evidence exists regarding the characteristics and behavior of these types of particles within commercially available product formulations. The data presented here are some of the first to demonstrate that following topical application, particles of zinc oxide and titanium dioxide were not detectable in layers of the skin deeper than the stratum corneum.

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NVP-LDE225 inhibits the formation and induces the regression of murine basaloid tumor nests in embryonic and newborn murine skin organ cultures derived from *Ptch*$^{+/-}$ *LacZ* heterozygous mice.

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Patched (Ptch) proteins normally suppress the function of smoothened (Smo), a GPCR-like molecule that activates the Hedgehog (Hh) signaling pathway. Failure of Ptch1 expression is associated with uncontrolled proliferation of epidermal stem cells leading to the development of basal cell carcinoma (BCC) tumors, a distinctive symptom of nevoid basal cell carcinoma syndrome (NBCCS) patients who have an inherited defect of one *Ptch1* allele. In this study, we have used *Ptch1*$^{+/-}$ heterozygous knock-out mice in which one *Ptch1* allele has been replaced with the bacterial *LacZ* gene and thus mimic the genetic situation of NBCCS patients. Embryonic skin punches derived from *Ptch1*$^{+/-}$ *LacZ* heterozygous mice form basaloid nest tumors when cultured and stimulated with small molecule Smo agonist (Hh-Ag-1.3). Using this *in vitro* model, we have evaluated the activity of NVP-LDE225, a novel Smo antagonist, and cyclopamine in blocking the formation of basaloid tumor nests in agonist-induced skin punches. NVP-LDE225 inhibited basaloid tumor nests dose-dependently, with an IC_{50} of %150nM. In comparison, cyclopamine induced less than 50% inhibition of basaloid nest formation at the highest concentration tested (10μM). In a therapeutic setting, basaloid lesions were induced in embryonic skin cultures for 7 days in the presence of Hh-Ag-1.3 and subsequently incubated with NVP-LDE225 or cyclopamine for another 8 days. NVP-LDE225 induced the regression of preformed basaloid lesions with an IC_{50} of %150nM. At the highest concentration of NVP-LDE225 tested (1.5μM), almost complete regression of basaloid nests was observed whereas 10μM cyclopamine caused only about 10% regression. In conclusion, NVP-LDE225 was efficacious to inhibit the formation and mediate the regression of Smo-induced BCC-like tumor nests in murine skin cultures, indicating therapeutic potential for treatment of BCC, in particular in NBCCS patients.

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Novel mechanism for topical treatment of plaque psoriasis – results of a randomized, double blind, concentration ranging, vehicle controlled 12 week study with JAK 1/2 inhibitor INCB018424 cream

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Janus kinases (JAKs) are involved in signal transduction from Th1 and Th17 cytokines implicated in the pathogenesis of psoriasis, including IL-6, IL-12 and IL-23. INCB018424, a novel and selective small molecule inhibitor of JAK1 and JAK2, potently inhibits cytokine-induced JAK signaling and function in both lymphocytes and keratinocytes. In a murine contact hypersensitivity model, topical application of INCB018424 resulted in suppression of STAT3 phosphorylation, edema, lymphocyte infiltration, and keratinocyte proliferation. Additional murine studies demonstrated that topical INCB018424 inhibited acanthosis and the production of IL-22 induced by intra-dermal IL-23. In an open label subtotal inunction study in 25 patients with plaque psoriasis, transcriptional profiling was performed on lesional skin biopsies at baseline and following 28 days of topical INCB018424 treatment. Transcriptional changes consistent with decreased Th1 and Th17 lymphocyte activation, decreased epidermal hyperplasia and dendritic cell activation were observed in this study. A subsequent 12 week phase 2b double-blind, randomized, vehicle-controlled dose ranging study was conducted in 200 patients with mild to moderate plaque psoriasis to explore the safety, tolerability, pharmacokinetics, pharmacodynamics and efficacy of INCB018424. The primary endpoint of Total Lesion Score for all dose groups was decreased > 2 fold over vehicle control at day 84, as was the secondary endpoint for PASI. Systemic exposure was minimal. Topical application of INCB018424 for a 12 week treatment period showed significant clinical activity and was safe and well tolerated in patients with plaque psoriasis.

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A cross-sectional study of skin carotenoid levels in adult patients with psoriasis

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Psoriasis is a chronic inflammatory disease that not only affects the skin but can have systemic implications such as obesity and nutritional deficiencies. Treatment options include vitamin A derivatives. Carotenoids are vitamin A provitamins with anti-oxidant properties. They are present in human tissues including skin and they can be measured through Raman spectroscopy. In this cross-sectional study, we sought to determine whether psoriasis was associated with lower levels of skin carotenoid levels. A total of 44 patients with psoriasis and 72 patients without psoriasis were evaluated between March and June of 2009. Demographic information was also collected as well as information on smoking status, height and weight and multivitamin intake. Patients with psoriasis were evaluated for disease severity and type of therapy. A linear regression model was used to evaluate the relationship between psoriasis and carotenoid levels (primary aim), after adjusting for other factors and to determine if severity of disease was associated with carotenoid levels (secondary aim). The mean carotenoid levels in the psoriasis and no psoriasis groups were respectively 22099 and 29180 and psoriasis was found to be significantly related to lower levels of carotenoids in both the univariable and multivariable analysis ($p<0.05$). In the psoriasis group, the Psoriasis Area and Severity Index was not significantly related to carotenoid levels ($p=0.07$). Patients with psoriasis appear to have lower skin carotenoid counts. The evaluation of the relationship between severity of disease and carotenoid levels may be confounded because most of the patients were on systemic, phototherapy or both.

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Low-dose UVA1 phototherapy sustains antifibrotic response *in vivo* by minimizing photoadaptation

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Low-dose UVA1 phototherapy (10-20 J/cm^2) is reported to be more effective than high-dose UVA1 (110-130 J/cm^2) as an antifibrotic therapy for morphea. Indeed, it has been previously demonstrated that high-dose UVA1 is limited in efficacy because of skin darkening (tanning), which inhibits antifibrotic response, as reflected by attenuation of MMP-1 (collagenase) induction with successive exposures. Here, we theorized that low-dose UVA1 effectively softens fibrotic skin by minimizing photoadaptation (darkening). To test this hypothesis, we irradiated healthy human skin (N=10) with different doses of UVA1 (0, 20, 40, 80 J/cm^2) and measured changes in skin pigmentation, as determined by chromameter (L value). A single exposure to medium-dose UVA1 (80 J/cm^2) caused lightly pigmented skin (L>65) to become medium (L=55-65) in pigmentation (L=74.0 pre-therapy, L=63.1 post-therapy, $p<0.05$). In contrast, a single exposure to low-dose UVA1 (20 J/cm^2) allowed skin to remain lightly pigmented (L=70.9). Given these observations, we irradiated lightly pigmented healthy skin (N=12, L=73.0) at daily intervals (1 to 4 exposures) with low-dose UVA1 (20 J/cm^2). After 4 daily exposures, subjects remained lightly pigmented (L=65.3). With each exposure, skin samples demonstrated incremental MMP-1 mRNA induction, with maximal upregulation seen after 4 daily treatments (47-fold, $p<0.05$). Expression of MMP-3 (stromelysin) exhibited a similar trend (20-fold upregulation after 4 exposures, $p<0.05$). There were no significant changes in expression of type I or type III collagen after 4 exposures. Our observations suggest that antifibrotic responses with low-dose UVA1 are mediated by induction of MMPs (collagenolysis), rather than inhibition of collagen synthesis. Furthermore, in contrast with high-dose exposure, repeat low-dose UVA1 minimizes skin darkening and results in incremental induction of MMPs. Thus, low-dose UVA1 may soften fibrotic skin by inducing sustained MMP elevation.

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High clinical response rate of Sezary syndrome with immune modifying therapies: Prognostic markers of response.

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Sezary Syndrome (SS), the leukemic form of Cutaneous T cell Lymphoma (CTCL), has a poor prognosis. Although there are no uniform standards for therapy of SS, preservation of the immune response through the use of photopheresis (ECP) and biologic therapies is associated with higher response rates. As correlates of response are poorly understood, we sought to identify the important prognostic parameters that affect overall response to treatment in SS. We performed a retrospective cohort study of 143 patients, seen over a 25 year period at the University of Pennsylvania, who had a clinical and laboratory diagnosis of SS. We identified 114 (79.7 %) patients who completed a treatment regimen combining ECP and 1 or more systemic immunostimulatory agents, (interferon alpha, interferon gamma, sargramostim or systemic retinoids) for at least 3 months. A total of 85 (74.6%) patients had significant improvement with multimodality therapy: 26% had complete response (CR); complete resolution of disease and 48% had partial response (PR); >= to 50% improvement but < 100%. Median age at diagnosis for patients with CR was 58 years compared to 63 years for all other patients ($p=0.04$). Differences between the CR group and all other patients were lower, but not statistically significant for the median baseline white blood cell count, serum lactate dehydrogenase and CD4:CD8 ratio. The CR group had a lower median percent CD4+/CD26 negative (21.5 versus 56, $p=0.02$) and CD4+/CD7 negative (12 versus 26, $p=0.076$) cells. Median monocyte percentage at initiation of therapy was higher for patients who showed a CR (9 versus 7.8, $p=0.054$). This is one of the largest cohort studies of SS patients receiving immune modifying therapies. Age at diagnosis, baseline monocyte percent and circulating abnormal T-cell markers were the strongest predictive factors. Baseline monocyte percent and abnormal T-cell markers warrant further examination in a larger cohort.

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Extramammary Paget's Disease of the vulva: A case report of clinical and reflectance confocal microscopy features

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Vulvar Extramammary Paget's Disease (EMPD) is a relatively rare cutaneous neoplasm with unique challenges due to its multifocal nature and sub-clinical involvement. Utilization of reflectance confocal microscopy (RCM) may facilitate early diagnosis and may guide surgical margin selection to reduce repetitive surgical procedures. We present a case report of a 63 year old Caucasian woman with biopsy proven vulvar EMPD. Dermoscopic imaging of clearly affected skin revealed a homogenous pink background with multiple, small, dotted, and corkscrew-type vessels. RCM revealed a disarranged honeycomb pattern interspersed with multiple dark, round to oblong structures of variable diameter with mildly refractile cytoplasm. These structures corresponded to Paget's cells on histopathology. The increased vascularity present on dermoscopy was dramatically captured with real-time RCM video. We utilized RCM imaging to guide placement of scouting biopsies prior to planned surgical excision. While the scouting biopsies revealed no evidence of disease, and prompted a larger than planned excision based upon the visualized clinical extent of involvement, the final surgical excision revealed superficial tumor at the margins, illustrating the multifocal nature of this process. In conclusion, we present the dermoscopic and RCM features of one patient with vulvar EMPD. RCM imaging enables visualization of distinct morphologic features correlating with histopathologic features, and may facilitate early diagnosis of women presenting with non-specific vulvar erythema, facilitate pre-operative surgical planning, and aid in monitoring response to topical therapeutics. Imaging of additional subjects is necessary to fully characterize and validate the dermoscopic and RCM features of EMPD.

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Self-treatment is common and does not correlate with severity of hair loss or quality of life in women with non-scarring alopecia

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Treatments for women affected by non-scarring alopecia such as alopecia areata, telogen effluvium, and androgenic alopecia are usually viewed as sub-optimal, and often lead to various types of self-treatment and/or alternative medicine. Utilization of such approaches to treatment of hair loss may alter the patient's perception of hair loss severity (HLS) and quality of life (QoL). Aims of this study included determination of self-treatment prevalence in patients with non-scarring alopecia, and determination of the relationship between perception of disease severity, type of treatment, and satisfaction with treatment. Participants were diagnosed (n=104) at Northwestern's Hair Loss Disorders Clinic. Subjects completed a questionnaire to evaluate perceived severity of disease (5 point scale of severity, where 5=most severe hair loss), types of treatments used, and satisfaction with treatment (5 point scale of satisfaction, where 5=very satisfied). QoL was assessed using Skindex-16. Although 81% of subjects (n=104) used prescribed treatments such as topical minoxidil (43%) and multivitamins (39%), self-treatment was reported by 29 subjects (28%), including supplemental vitamins (7%), acupuncture (7%), topical minoxidil (6%), and massage (5%). No correlation exists between self-treatment and patient satisfaction (Fisher's exact, p=0.82), and neither prescribed nor self-treatment methods correlated with QoL using Wilcoxon rank sum test (p=0.57). These findings indicate that women with non-scarring alopecias commonly use self-treatment. Moreover, the type of self-treatment used does not correlate with patient-rated hair loss severity. Although both prescribed and non-prescribed therapies do not impact the degree to which patients are bothered by hair loss, physicians are alerted to the diversity and frequency of self-treatment utilized by patients for alopecia.

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Quality of life correlates more closely with patient-rated versus physician-rated hair loss severity in women with non-scarring alopecia

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Although patients with clinically severe alopecia have significantly decreased quality of life (QoL), concordance of quality of life (QoL) and hair loss severity (HLS) as rated by both physician and patient has not been extensively examined. The aims of this study were to assess QoL in subjects (n=104) with alopecia areata (AA, n=22), telogen effluvium (TE, n=33), and androgenic alopecia (AGA, n=41), and to compare QoL to HLS as rated by both patient and physician. Participants in this study were diagnosed at Northwestern's Hair Loss Disorders Clinic. Severity of hair loss was assessed by a dermatologist utilizing SALT scores for AA severity, Ludwig scores for AGA, and hair pull tests for TE. QoL was evaluated with Skindex-16 (SK-16, 100 point scale, where high scores indicate poorest QoL), and a self-administered subject questionnaire to evaluate the perceived HLS (5 point scale, where 5= severe hair loss). Subject-rated mean HLS (3.247 ± 1.11) was more severe than mean physician ratings (2.02 ± 1.31). The entire study population reported a mean SK-16 total score of 57.3 ± 16.2 (n =104); emotional domain had lowest QoL(83.8 ± 15.2), followed by functional (50.2 ± 30.0) and symptomatic (19.9± 19.9) domains. All 3 domain mean scores and total SK-16 mean score strongly correlated with subject-rated HLS ($p<0.05$). Physician-rated HLS did not correlate with SK-16 domain mean scores, however there was a correlation with SK-16 total mean score (p=0.0497). Our data shows that patient-rated HLS more closely correlates with QoL than physician-rated HLS. These findings alert physicians to the variation between patient perceptions of hair loss severity and the physician perception of hair loss severity, as well as the impact that hair loss has on quality of life for women.

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Inducible hyaluronan macropinocytosis by melanoma cells: A useful endocytic route for bioconjugate drug delivery

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The high biocompatibility of hyaluronan (HA) and the expression of the CD44 HA receptor on tumor cells has led to interest in developing the HA co-polymer into a drug delivery scaffold. Our purpose was to: 1) investigate the uptake of HA by melanoma cells, 2) develop a chemotherapeutic HA bioconjugate, and 3) test the efficacy of the HA bioconjugate. B16-F10 melanoma cells endocytosed HA as assessed by transmission electron microscopy and immunogold labeling. Unexpectedly, an antagonistic anti-CD44 antibody failed to inhibit HA uptake showing that CD44 did not play a role in HA endocytosis. A macropinocytosis inhibitor but not inhibitors for clathrin coated pits or lipid rafts, blocked HA uptake. Furthermore, confocal microscopy studies showed that fluorescently labeled HA co-localized with fluorescent dextran (a macropinocytosis tracer) but not with fluorescent tracers for clathrin coated pits or lipid rafts. Our results show that B16-F10 melanoma cells macropinocytosed soluble HA. Interestingly, scanning electron microscopy studies showed that membrane ruffling, a characteristic feature of macropinocytosis, was not constitutive but rather, was induced by treating cells with soluble HA. To test if HA induced macropinocytosis could be used for the delivery of chemotherapeutic reagents into melanoma cells we incubated B16-F10 cells with HA that had been decorated with doxorubicin (DOX). We found that B16-F10 melanoma cells, but not primary keratinocytes macropinocytosed DOX-HA. Moreover, DOX-HA showed time and dose-dependent cytotoxicity. Lastly, we tested the impact of DOX-HA on the growth of established subcutaneous B16-F10 tumors in mice. We found that local treatments of tumors with DOX-HA dramatically reduced their growth rates as compared with free DOX or the vehicle control. In conclusion, our results suggest that HA inducible macropinocytosis may be a useful endocytic route for the delivery of HA bioconjugates to malignant melanoma cells for local tumor treatment.

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Psoriasis prevalence among the 2009 melanoma/skin cancer screening patients

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Psoriasis is a chronic inflammatory skin disorder that affects 2 to 3% of the world's population. There have been population-based studies to assess prevalence of the disease in the United States. Most of these studies are based on self-reported diagnosis. Given that an important number of psoriasis patients do not search for medical care, this method may not be accurate to estimate the prevalence of psoriasis in the general population. The identification of undiagnosed cases of psoriasis is becoming increasingly important, given the availability of newer and more potent therapies. The objective of this study was to determine the prevalence of psoriasis using a self-reported diagnosis and dermatologist skin examination and diagnosis. Information concerning former diagnosis of psoriasis, type insurance and physician's presumptive diagnosis of psoriasis were captured in a modified Melanoma/Skin Cancer Screening form provided by the American Academy of Dermatology. This subset of forms was provided to a limited number of participant sites across the US. Overall and subgroup prevalence of psoriasis was analyzed. The crude results and crude proportions are presented. Among the 2991 participants, 86% answered the psoriasis question. About five percent reported to have a previous diagnosis of psoriasis. As anticipated, this percentage was greater than the prevalence of documented diagnosis of psoriasis (2.8%). This proportion drops to 1.2% if the assumption is made that not answering the question was equivalent to "no psoriasis present"; given that, in more than half of the forms, the examiners did not notate either positively or negatively whether the patient had psoriasis. Weighted averages from the US population yielded similar results. This pilot project, although limited by missing data, reinforces the previous knowledge that psoriasis prevalence is between 2-3% in the US. It also demonstrates the potential of evaluating other skin conditions in a national event such as the Melanoma/ Skin Cancer Screening.

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Topical turmeric extract in a moisturizing cream formula reduces the appearance of cheek texture and fine lines and wrinkles on human facial skin

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Turmeric (Curcuma longa) has a long history of use in Ayurvedic medicine and is known for its potent anti-inflammatory and antioxidant properties. Here we evaluate a stable, almost colorless turmeric extract in a moisturizing cream and demonstrate its ability to provide significant facial appearance benefits. A clinical study was performed to evaluate the efficacy of turmeric extract in cream formulation on the appearance of both skin texture and fine lines and wrinkles. Caucasian women were recruited to a double-blind, 10 week, left-right randomized, split-face study which evaluated turmeric extract in combination with niacinamide in cream formulation compared to a niacinamide only control. The study entailed a two-week washout followed by 8 weeks of twice daily topical product application. Images of each subject were captured at week 0 (baseline), week 4, and week 8 and were evaluated by expert graders for improvements in the appearance of cheek texture as well as fine lines and wrinkles. All changes were calculated relative to baseline. In the clinical study, the formulation containing the combination of turmeric extract and niacinamide was significantly better at reducing the appearance of fine lines and wrinkles than the formulation containing only niacinamide at 4 weeks (p=0.004), directionally better at 8 weeks (p=0.125), and significantly better overall (p=0.009) as judged by expert graders. The formulation containing the combination of the turmeric extract and niacinamide was also significantly better at reducing the appearance of cheek texture (orange peel-like appearance) than the formulation containing only niacinamide at both 4 weeks (p=0.0148) and 8 weeks (p=0.0035). In conclusion, significant improvements in the appearance of cheek texture as well as fine lines and wrinkles were observed from moisturizing creams containing turmeric extract.

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Immature autoreactive B cells may predict treatment response of chronic graft-versus-host disease

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cGvHD after allogeneic bone-marrow transplantation is associated with B cell disturbances/autoreactivity. Clinics of cGVHD can mimic autoimmune diseases, and auto-antibodies derived from B cells have been found. Therapy of cGvHD involves immunosuppressive(IS) agents with differing effects. Identification of specific biomarkers could thus used to address these problems. We investigated B cell subsets in peripheral blood (PB) of 74 pts. Therapies were cyclosporine A (CSA n=24), tacrolimus (n=13), sirolimus (n=18) and ECP (n=19) for moderate (n=29) or severe (n=45) cGVHD(NIH criteria). Organ involvement included: skin(61%), oral mucosa(68%),eyes(38%), liver(30%),lungs(31%).PB leukocytes were analyzed after staining for CD19, CD27, CD21 and surface Ig. Overall, 45 pts (61%) responded to therapy including 83% given CSA, 46% on tacrolimus, 39% on sirolimus, and 63% given ECP, respectively. Prior to IS therapy non-responders had significantly (p=0.03) higher proportions of immature CD19+CD21- B-lymphocytes with a mean of 19.4% compared with a mean of 13.3% in responders. There were significantly higher proportions of immature CD19+CD21- B-cells in non-responders compared to responders to sirolimus (mean of 23% vs 9.3%, p=0.02), tacrolimus (mean of 17.9% vs 8.6%, p=0.02), or ECP (mean of 22% vs 13.7%, p=0.04). After 6 months of immunosuppressives all responding patients had a significant (p=0.01) decrease of immature CD19+CD21- B-lymphocytes (13.3% vs 8.2%. In conclusion, relative amounts of immature CD19+CD21- B-lymphocytes serially assessed prior to start of cGVHD therapy may predict response to systemic therapy. Increased proportions of CD21- B-lymphocytes could be part of the autoimmune pathogenesis resulting in autoreactive B-cells in cGVHD. This novel cellular biomarker should be investigated in larger cohorts of patients.

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Development of a new dendritic cell (DC)-based screening assay for contact allergens

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Recent changes in regulatory restrictions and social views against animal toxicology experiments now force the scientific community to develop reliable in vitro tests for predicting skin sensitizing potentials of large numbers of industrial chemicals. The present study was conducted to satisfy this urgent need. We first determined gene expression profiles in murine skin after topical application of a prototypic sensitizer, dinitrofluorobenzene (DNFB). A total of 84 genes were upregulated significantly (>2-fold, $p < 0.05$, $n = 3$) by DNFB, but not by a skin irritant, benzalkonium chloride (BAC) at 6 h post-treatment. They included a unique set of genes encoding redox regulatory enzymes, such as sulfiredoxin 1 (Srxn1), heme oxygenase 1 (HO-1), and thioredoxin reductase 1 (Txnrd1), suggesting the involvement of oxidative stress. In fact, expression of the same three genes in the XS106 DC line was significantly (2 to 15-fold) upregulated by DNFB as well as by hydrogen peroxide, and a ROS inhibitor, diphenyleneiodonium (DPI), blocked DNFB-induced expression of these genes almost completely. We next developed a HTP-compatible assay system to measure the production of reactive oxygen species (ROS). XS106 DCs were pre-loaded with a ROS-sensitive fluorescent dye, CM-H_2DCFDA, incubated with test compounds, and then examined by FACS. DNFB but not BAC dose-dependently induced rapid (<5 min) and robust (>30-fold) ROS production at sub-toxic concentrations. Moreover, >3-fold ROS production was induced by 22 of the 28 tested contact allergens and by only 3 of the 21 tested skin irritants, indicating relatively low false negative (6/28) and false positive (3/21) rates. Interestingly, four of the allergens that failed to induce ROS are known as pro/pre-haptens. Not only do our results demonstrate the reliability of our new assay platform, they also suggest functional contributions of ROS to various changes known to occur in skin resident DCs during the sensitization phase of allergic contact dermatitis.

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Blockade of the IL-17R with AMG 827 leads to rapid reversal of gene expression and histopathologic abnormalities in human psoriatic skin

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IL-17 (IL-17A) and IL-17F are innate pro-inflammatory cytokines that modulate a broad range of cell types and activate the local expression of cytokines, chemokines, metalloproteinases and antimicrobial proteins/peptides. IL-17 has been implicated in the pathogenesis of several human autoimmune diseases, and both IL-17 and IL-17F mRNA as well as IL-17-positive cells are elevated in lesional skin from psoriasis patients. IL-17 has been demonstrated to induce production of inflammatory cytokines from human keratinocytes. AMG 827 is a fully human blocking monoclonal antibody to the IL-17R, the cognate receptor for both IL-17 and IL-17F. AMG 827 was studied in a single dose (700 mg IV) randomized, double-blind placebo-controlled cohort of subjects with plaque psoriasis [n=10 (8 AMG 827; 2 placebo), PASI > 10 at screening]. Biopsies were obtained from lesional and non-lesional skin pre-dose, and from lesional skin on weeks 2 and 6 post-dose. Microarray analyses from skin biopsies identified a rapid and significant alteration of gene expression in subjects receiving AMG 827 compared to placebo, with the expression of a wide range of genes associated with psoriasis including cytokines and defensins reverting almost completely to non-lesional levels of expression by week 2. Treatment with AMG 827 also led to significant improvements as compared to placebo in multiple histopathologic parameters compared to placebo, including epidermal thickness, Ki-67 and Keratin-16 levels as well as the numbers of infiltrating leukocyte subsets. These responses in the biopsies correlated with remarkable PASI improvements as 7 of 8 subjects achieved at least 75% improvement in PASI score at week 6. These data with single IV doses of AMG 827 validate targeting the IL-17R pathway in psoriasis patients, and identify the molecular consequences of IL-17 activation and subsequent inhibition in human skin.

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Association of autologous sweat allergy and metal allergy with the intrinsic type of atopic dermatitis

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We have previously demonstrated that more than half of atopic dermatitis (AD) patients show skin reactions with intradermal injection of autologous sweat, and that desensitization therapy with the diluted autologous sweat improved the disease. However, several issues remain to be elucidated to further understand the mechanism. We aimed to investigate the type of AD showing positive skin reactions to the diluted autologous sweat, and the relationship between sweat allergy and metal allergy among AD patients. The two types of AD have been proposed. The extrinsic type shows high IgE levels presumably as a consequence of skin barrier damage and feasible allergen permeation, whereas the intrinsic type exhibits normal IgE levels and is not mediated by allergen-specific IgE. There is a possibility that the intrinsic type of AD is associated with metal allergy. We evaluated the prevalence rate of sweat allergy and metal allergy using skin test, patch test or lymphocyte stimulation test in 24 extrinsic and 8 intrinsic AD patients. No significant difference was seen in the sweat allergy test between the two types. On the other hand, a significant increase (80% versus 20%) of the prevalence rate of metal allergy was observed in the intrinsic AD patients with sweat allergy. Furthermore, the nickel concentration in the sweat of the intrinsic AD patients was higher than that of normal control or the extrinsic AD patients. Desensitization with the diluted sweat increased the percentage of CD4(+)CD25(+)FoxP3(+) regulatory T cell in the peripheral blood of the patients who were improved by the treatment. These data suggest that metal allergy is deeply involved in the pathogenesis of intrinsic AD showing allergic reactions to autologous sweat.

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Topical treatment of NVP-LDE225 potently and dose-dependently inhibits anagen induction and Hedgehog target gene expression in mice.

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Aberrant activation of the Hedgehog (Hh) signaling pathway is the pivotal abnormality in skin cancers such as basal cell carcinoma (BCC). In healthy skin, the Hh-pathway is induced during the anagen phase of the hair growth cycle. Binding of Hh-ligand to its receptor Patched (Ptch) activates the 7-transmembrane receptor Smoothened (Smo), leading to transcription of Hh-target genes. Thus, hair growth and Hh-target gene expression can serve as pharmacodynamic read-outs for specific antagonists of Smo, such as the novel compound NVP-LDE225. C57/BL6 mice were depilated in telogen phase and topically treated with NVP-LDE225 or the reference Smo antagonist cyclopamine, each dissolved in propylene-glycol/ethanol (7/3). Depilated skin areas were treated once or twice daily for 14 or 7 consecutive days, respectively, and the intensity of skin pigmentation and hair growth were scored daily. Application of 1% NVP-LDE225 for 14 days completely inhibited anagen induction in all animals up to day 21, while all vehicle-treated animals changed to anagen on day 7. In contrast, 1% cyclopamine only transiently and minimally inhibited hair growth, similar to 0.1% NVP-LDE225, thus indicating an approximately 10-fold higher potency of NVP-LDE225. In a second study, NVP-LDE225 was topically applied at concentrations of 0.3% or 1% of the drug twice daily for 7 days. Hair growth was then evaluated by photography and the levels of expression of Hh-target genes such as *Gli1, Gli2, Sox9, and N-Myc* was determined by quantitative gene expression analysis. While application of a 0.3% solution of NVP-LDE225 was only partially effective, a 1% solution completely inhibited hair growth during anagen as well as the expression of the Hh-pathway target genes. The results demonstrate that the Smo antagonist NVP-LDE225 potently inhibits anagen induction and Hh-pathway gene expression after topical application to mice, indicating therapeutic potential for topical treatment of Hh-dependent skin diseases such as BCC.

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NVP-LDE225 is a potent antagonist of both human and murine Smoothened and blocks pathway activation by Sonic Hedgehog ligand and synthetic agonists.

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Smoothened (Smo) is a GPCR-like molecule which positively regulates the Hedgehog (Hh) signal transduction pathway. Hh pathway activation at or upstream of Smo is linked to proliferation of Hh-pathway dependent tumor types, such as medulloblastoma and basal cell carcinoma. Here we report on comparative studies *in vitro* of the novel Smo antagonist NVP-LDE225 and the reference Smo inhibitor cyclopamine. NVP-LDE225 and cyclopamine were investigated for specific binding to human and mouse Smo protein, determined by their ability to displace a tritium-labeled small molecule Smo agonist ([3H]-Hh-Ag 1.5) or a BODIPY-labeled Smo antagonist (BODIPY-Cyclopamine). In the agonist displacement assay using human Smo, LDE225 and cyclopamine showed IC_{50} values of 11 nM and 280 nM, respectively. The antagonist displacement assay confirmed the superior affinity of NVP-LDE225 for human Smo (IC_{50} 7 nM) when compared to cyclopamine (IC_{50} 45 nM). Similar potencies were obtained in assays using mouse Smo. In cell-based reporter gene assays, NVP-LDE225 inhibited Smo signaling with an IC_{50} of 4 nM as assessed by inhibition of a Gli response element-driven luciferase activity induced by Hh-Ag 1.5. In comparison, cyclopamine showed an 11-fold lower potency (IC_{50} 46 nM). The high potency of NVP-LDE225 to inhibit Smo signaling was also demonstrated in primary human embryonic palatal mesenchymal (HepM) cells in which recombinant Sonic Hedgehog ligand-stimulated *Gli1* gene transcription was inhibited with an IC_{50} of 12.7 nM. Taken together, various *in vitro* assays showed that NVP-LDE225 is a potent antagonist of Hh- and Smo- dependent signaling, with IC_{50} values in the low nanomolar range, and superior to the reference Smo antagonist cyclopamine by factors of 10-20.

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The Smoothened inhibitor NVP-LDE225 has favorable skin penetration/permeation properties *in vitro* and *in vivo*

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NVP-LDE225 is a novel and selective low molecular weight (MW: 485) antagonist of Smoothened, a G-protein coupled receptor-like molecule that activates the Hedgehog (Hh) signaling pathway. NVP-LDE225 demonstrated efficacy to mediate regression of basaloid tumor lesions in skin punch cultures derived from *Ptch*$^{+/-}$ heterozygous knock-out mice and to inhibit anagen induction and Hh-target gene expression after topical application to C57/BL6 mice. In this study, the penetration properties of NVP-LDE225 into and through human *in vitro* and pig skin *in vitro* and *in vivo* were investigated. For *in vitro* studies, saturated 0.3% solutions in propylenglycol were applied epicutaneously to human cadaver skin or pig skin mounted in static Franz-type diffusion cells. At the end of a 48 hours (h) exposure time, drug concentrations were measured in the skin (after removal of stratum corneum) and in the receiver. For *in vivo* studies, a 0.75% experimental formulation was topically applied to domestic pigs to 4 cm^2 and to 10% body surface to determine dermal concentrations at selected time points and to evaluate systemic exposure 24h after application. *In vitro*, the NVP-LDE225 solution was found to penetrate well into human (27+/-8 µg/g) and pig (11+/-3 µg/g) skin and to permeate through human and pig skin with only a low permeation rate of each 6 ng/cm^2/h. *In vivo*, topical application of the 0.75% formulation on the dorsolateral trunk of pigs resulted in dermal concentrations of 1-1.5 µg/g at time points between 1h and 8h. In the 10% body surface application 1 µg/g dermal levels and blood levels %0.15 ng/ml were observed after 24h. Taken together, the data indicate that NVP-LDE225 penetrates well into human and pig skin, but permeates through skin only to a very low extent. Together with its pharmacological profile, NVP-LDE225 is expected to have therapeutic potential for topical treatment of BCC in patients.

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Low dose alemtuzumab (LDA) depletes malignant central memory T cells but spares normal skin resident effector memory T cells in leukemic CTCL

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Malignant T cells in Sézary syndrome and other leukemic CTCL variants (L-CTCL) resemble central memory T cells (Tcm), which normally recirculate through skin, blood and lymph nodes. In contrast, T cells in mycosis fungoides (MF) skin resemble effector memory T cells (Tem), cells that reside in skin for long periods of time and provide local immune memory. L-CTCL patients (n=11) refractory to multiple systemic therapies were treated at 1/3 the standard dose of the anti-CD52 mAb alemtuzumab (1 mg/3xweekly), and responses in blood and skin were monitored over time. Though the duration of treatment varied, all patients ultimately had complete clearance of malignant (and normal) T cells from blood, and full resolution of skin disease. To date, there have been no significant infectious complications. Skin biopsies before and after LDA treatment demonstrated loss of the malignant clone. However, non-malignant skin resident Tem were not depleted by LDA, and remained abundant even when patients had no circulating T cells. These skin resident T cells were $CD52^+$, had a TCR diversity similar to that of normal donors, and had demonstrable populations of Th1, Th2, Th17 and Treg. These findings suggest LDA depletes circulating T cells, but spares non-migratory tissue resident T cells, allowing clearance of the malignant Tcm in L-CTCL without impairment of cutaneous T-cell immunity mediated by non-malignant skin resident Tem. Interestingly, preliminary results suggest that LDA does not appear to be effective in the treatment of MF, consistent with the idea that malignant T cells in MF are non-recirculating, tissue resident Tem. Further studies will determine whether LDA can reproducibly and effectively treat L-CTCL without depleting skin-resident (or other tissue resident) Tem, thus preserving peripheral T cell memory and conferring relative resistance to the infectious complications that are all too frequent with higher doses of this therapeutic antibody.

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The clonal malignant T cells in leukemic CTCL have diverse functionalities and cytokine production profiles

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It has been suggested that leukemic CTCL (L-CTCL) is a uniform malignancy of $FOXP3^+$ regulatory T cells (Treg). However, skin and blood are populated by a varied collection of T cells with differing functional capacities, all of which could undergo malignant transformation. Using antibodies specific for TCR Vβ subfamilies, we identified and selectively studied the clonal malignant T cell population in 8 patients with L-CTCL. Malignant L-CTCL cells had in common expression of the central memory T cell markers L-selectin/CCR7 and the skin addressin CCR4, as previously reported. Functional analyses demonstrated that malignant clones produced significantly more IL-2 and TNFα than nonclonal benign T cells. In the majority of patients, the proliferative index, as assayed by Ki-67 expression, was greater in the malignant clone than in the remaining benign T cells. Clonal T cells from only 1 patient and a small subset of a second expressed Foxp3 at levels suggestive of a Treg phenotype; these patients' cells produced increased level of IL-10. In the remaining patients, there was remarkable functional diversity among malignant clones. Malignant cells from one patient produced high levels of IL-6 and a second patient's clone produced high levels of IL-22. The cytokine production profile in general was unique for each patient. These results suggest that the functional phenotype of malignant cells in L-CTCL is highly diverse and reflects the diversity of central memory T cells from which they arise. Indeed, the varied clinical presentations in CTCL may reflect the functional diversity of malignant clones. A better understanding of the commonalities and differences between patients with L-CTCL may lead to therapies that are effective in all patients and may also allow understanding and treatment of specific symptoms, such as pruritis, that may reflect particular functional characteristics of the malignant clone.

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Th2 cytokines enhance tissue kallikreins and serine protease activity of keratinocytes in atopic dermatitis

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Tissue Kallikrein 7 (KLK7), a chymotrypsin-like serine protease, and other KLKs, are increased in the epidermis of atopic dermatitis (AD) and considered to be involved in AD pathogenesis since Netherton syndrome patients who have a defect of lympho-epithelial Kazal-type related inhibitor (LEKTI), a serine protease inhibitor, develop AD-like lesions, and overexpression of human KLK7 in mouse epidermis results in chronic itchy dermatitis. We confirmed KLK7 protein expression was increased in AD by immunohistochemistry (n=6). We sought molecular mechanisms of KLK7 increase in AD and hypothesized that Th2 cytokines induce KLK7 expression in AD keratinocytes. Clinically, KLK7 protein level correlated with IL-4 level in AD patients' serum (n=21, r=0.874, p<0.01). Cultured normal human epidermal keratinocytes (NHEK) treated with IL-4 (50 ng/ml) or IL-13 (50 ng/ml) increased KLK7 protein (8.16x, p=0.0015, or 8.96x, p=0.0010) and mRNA (2.73x, p<0.0001, or 2.55x, p=0.0007), while IFN-gamma (100 U/ml), a Th1 cytokine, and IL-17A (50 ng/ml), a Th17 cytokine, did not. KLK7 protein was increased by Th2 cytokines in a time-dependent manner. IL-4 also induced mRNA of KLK1 (1.74x, p=0.0219), KLK8 (2.32x, p=0.0043), KLK11 (2.68x, p=0.0045) KLK12 (2.86x, p=0.006) and KLK13 (2.63x, p=0.0026) in NHEK. Furthermore, protease activity assay with a specific substrate for chymotrypsin-like serine protease showed that IL-4 or IL-13 enhanced chymotrypsin-like serine activity in NHEK (3.23x, p=0.0005, or 2.29x, p=0.0172, respectively). Similarly, trypsin-like serine protease activity also increased with IL-4 or IL-13 (1.11x, p=0.0007, 1.12x, p=0.0008). These findings suggest that Th2 cytokines affect skin barrier function through increased KLK expression in AD.

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The impact of pyruvate and cell density on oxidative stress assays

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Burns are dynamic injuries that progress in depth and size over the course of several days. While the exact mechanisms leading to injury progression are unclear, several processes including inflammation and generation of reactive oxygen species likely play significant roles. Since no proven therapy exists to limit burn injury progression, we are developing reliable *in vitro* drug screen assays to act as a springboard for animal studies and clinical trials. Here *in vitro* assays to test the effect of oxidative stress on adult human dermal fibroblasts (ADHF) are refined. Although many studies have been published using oxidative stress assays, little consistency exists in methodology. AHDF were cultured in complete medium (DMEM supplemented with 10% fetal bovine serum, penicillin, and streptomycin) under standard conditions (humidified 5% CO2, 37° C). Cells used were 8-11 passages, and seeded in quadruplicate onto 96-well plates at 500, 1000, 2000, 4000, and 15000 cells/well. After overnight incubation, cells were treated with increasing concentrations of H2O2. Twenty hours following exposure to H2O2, cell viability was determined using the XTT assay (Roche). When AHDF were seeded using DMEM containing sodium pyruvate (110 mg/L), the LD50 of 2000 cells/well was 400 µM H2O2. In contrast, AHDF seeded at 2000 cells/well with pyruvate-free DMEM had a LD50 of 150 µM H2O2. In addition, cells showed increasing resistance to H2O2 as cell density increased from 500 to 15000 cells/well (500 cells/well=LD50, 50 µM H2O2; 1000 cells/well=LD50, 75 µM H2O2; 2000 cells/well=LD50, 150 µM H2O2; 4000 cells/well=LD50, 350 µM H2O2; 15000 cells/well=LD50, 650 µM H2O2). Thus, pyruvate and higher cell density attenuate the effect of H2O2-induced oxidative stress on cultured AHDF. The latter results may be secondary to greater amounts of reduced glutathione in cell culture systems with higher cell densities. These results underscore the importance of proper experimental design of screening assays for drugs that might inhibit burn injury progression.

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Loss of membrane E-cadherin and expression of p53 are molecular markers of progression of sun-damaged skin to actinic keratoses

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Actinic keratoses are premalignant lesions with a 6-10% lifetime risk of developing into invasive squamous cell carcinomas. In a previous study, we found that 43% of AKs clinically regressed without reoccurrence within an 11 month period of time, and that 32% regressed but then recurred, indicating that natural course of AKs is exceedingly variable. The purpose of this study was to identify molecular events associated with persistence and progression of actinic keratoses. E-cadherin, a marker of epithelial-mesenchymal transition (EMT), and p53, which plays a pivotal role in apoptosis and the repair of potentially carcinogenic UV-induced DNA damage, were examined in 35 clinically present AKs, 4 regressed AKs, and 43 sun exposed skin samples from 26 individuals. Clinically present AKs expressed significantly less membrane E-cadherin than sun-exposed skin (1.89±1.81, AKs vs. 3.07±1.75, sun-exposed skin; p < 0.005). When specimens from clinically present AKs and sun-exposed skin were examined for p53, both expressed p53 (2.89±1.45, AKs vs. 2.58±1.68, sun-exposed skin, p=0.40). Less p53 was observed in regressed AKs than in clinically present AKs (0.75±0.96, regressed AKs vs. 2.89±1.45, clinically apparent AK; p<0.01). These data suggest that loss of E-cadherin expression is a key feature associated with the persistence of AKs, whereas loss of p53 is associated with regression. Procedures which enhance membrane E-cadherin expression and/or diminish p53 expression may therefore be effective in the prevention of non-melanoma skin cancers.

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In vitro release of interferon-gamma from peripheral blood lymphocytes in cutaneous adverse drug reactions

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Cutaneous adverse drug reactions are exceedingly common but also diagnostically challenging due to extensive clinical heterogeneity, simultaneous exposure to multiple drugs and the existence of a latent period which differs between patients. These limitations prompted the development of both *in vivo* and *in vitro* tests. The purpose of our study was to evaluate the performance of an *in vitro* assay as an adjunct to the diagnosis of adverse skin drug reactions. In this assay, mononuclear cells derived from patients with cutaneous rashes suspected to have been caused by exposure to medications, were incubated with and without the suspected drugs. Interferon-gamma (IFNG) levels were measured in the supernatant by ELISA. A positive reaction was defined by assessing the increment in IFNG secretion. To validate the relevance of our IFNG release assay, we performed a telephonic survey in which we evaluated the effect of not taking the drugs incriminated by the *in vitro* assay on cutaneous manifestations. Using the data from the questionnaire, we also explored the possible influences of demographic or clinical characteristics on the test results. We assessed 272 patients who used 1035 medications. When assessed against the questionnaire, and using data collected at least 6 months after stopping the causative drug, the test sensitivity was found to be 83.61% and specificity 92.67%. The likelihood ratio for a positive test was 11.40 and for a negative test 0.18. Positive predictive value of the test is 75.37% and negative predictive value is 95.47%. The test was found to perform significantly better in females as compared with males, and in older patients as compared with younger patients. The results of this study indicate that the IFNG release test is a useful adjunct tool in the diagnosis of cutaneous adverse drug reactions.

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Effect of polyurushiol containing paint on an indoor air condition and atopic dermatitis

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Environmental factor is one of the most important factors in the pathogenesis of atopic dermatitis. Among the environmental factors, the westernized residential environment which increases the density of house dust mites and gives rise to sick house syndrome is come into attention for a convincing causative factor. Urushiol compounds extracted from Rhus verniciflua, a lacquer tree, have not only anti-insect and anti-microbial actions but also TVOC (total volatile organic compounds) reducing function. The purpose of this study was to elucidate the effects of the paint containing a novel polyurushiol synthesized from the extract of Rhus verniciflua on an indoor air condition and atopic dermatitis patients. Nine patients with atopic dermatitis who were resistant to the ordinary treatments were enrolled in this clinical trial. Patient's rooms were painted with the paint containing a novel polyurushiol extracted and synthesized from Rhus verniciflua. First, we measured TVOC before and after the painting. After a month of experiences, patients were evaluated with the objective indices such as EASI score, serum IgE and eosinophil levels, and the subjective satisfaction. Among nine patients, the seven patients showed objective or subjective improvements of clinical symptoms and a reduction of TVOC after painting. On the questionnaire after experience, many patients felt an improvement of air condition. This result indicates that painting with a novel polyurushiol synthesized from the extract of Rhus verniciflua could be helpful to improve an indoor air condition and atopic dermatitis.

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Expression of survivin and other anti-apoptotic biomarkers in extramammary Paget's disease

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Extramammary Paget's disease (EMPD) is a neoplasm of apocrine gland-bearing skin. Survivin, a member of the Inhibitor of Apoptosis (IAP) gene family, is overexpressed in virtually every human cancer. It is known that overexpression of survivin correlates with malignancies. We investigated the expression of survivin and other anti-apoptotic biomarkers by Paget's cells and their role in the tissue invasion and metastasis of EMPD. Thirteen patients of EMPD were enrolled into the study. Expression of survivin, Bcl-2, caspase-3 and caspase-9, were analyzed by immunohistochemical stainings. The variables including the expression level of survivin and other anti-apoptotic biomarkers, invasion level of primary site, and metastasis were statistically analyzed. Survivin was positively stained in 12 of 13 cases (92.3%), Bcl-2 in 10 cases (76.9%), caspase-3 and caspase-9 in 6 cases (46.2%). The expression level of survivin was significantly higher in invasive cases than in situ cases ($p=0.047$), but did not correlate with metastasis. There was no difference in the expression levels of other anti-apoptotic biomarkers between in situ and invasive cases. These findings indicate that survivin correlated with early stage of development of EMPD. In conclusion, measurement of survivin will be useful marker of aggressiveness and poor outcome of EMPD.

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The intrinsic type of atopic dermatitis shows normal barrier function, lack of filaggrin mutations, high percentage of Th1 cells, and high frequency of metal allergy

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Atopic dermatitis (AD) is classified into the IgE-high extrinsic and IgE-normal intrinsic types. To clarify the pathophysiology of intrinsic AD, we investigated the barrier function, and Th cell polarization, and metal allergy in AD patients. Enrolled in this study were 33 extrinsic patients (age, 28.3±11.8; M16, F17; IgE, 7041±7880 U/ml; SCORAD, 35.1; and eosinophils, 7.8 %) and 24 intrinsic patients (age, 30.9±11.3; M8, F16; IgE, 125±108 U/ml; SCORAD, 33.3; and eosinophils, 6.2%). The barrier function was assessed by TEWL and surface hydration. Filaggrin mutations were evaluated in the seven common loci among Japanese patients. Circulating Th1, Th2 and Th17 frequencies were examined by intracellular staining for IFN-γ, IL-4, IL-5 and IL-17. Metal allergy was assessed by patch tests for nickel, cobalt, chrome, etc. Barrier function was abrogated in extrinsic but not intrinsic AD. Filaggrin mutations were found in 42.9% of patients with the extrinsic type and in none of the intrinsic patients. While Th2 and Th17 cells were increased in both types at comparable levels, the percentage of IFN-γ-producing Th1 cells was higher in the intrinsic type. In in vitro study, the CD40L/IL-4-promoted class-switching and IgE production of B cells was downregulated by the addition of IFN-γ to culture. Finally, higher percentages of intrinsic AD showed positive patch tests to cobalt (50.0%), nickel (31.3%), and chrome (12.5%) than extrinsic AD (18.2, 27.3, and 9.1%, respectively). The intrinsic type differs from the extrinsic type in that it shows the normal barrier function without filaggrin mutations, increased number of IFN-γ-producing cells, and high percentage of metal allergy. In the intrinsic type, protein antigens are not allowed to penetrate the skin because of the normal barrier function. However, non-protein antigens, such as metals, can be causative and may induce a certain degree of Th1 responses in intrinsic AD.

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Effect of a novel supplemented drink on skin aging: A double-blind, placebo-controlled study

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We have conducted a double-blind, placebo-controlled human clinical study to evaluate the effectiveness of a nutrient-supplemented drink on skin ageing parameters. The drink was supplemented with Soy isoflavones, Lycopene, Beta-carotene, Vitamin C and Vitamin E and was taken 2X a day with a capsule containing ω-3-essential fatty acids for 14 weeks. 101 healthy, non-smoking Caucasian women (skin type II-III), aged 45- 65, were recruited. Skin measurements were taken on the first day of the trial and after a period of 14 weeks, following daily consumption of test or placebo products. Skin replicas were collected at the crow's foot area of the face and were then analysed using Phase Shift Rapid In vivo Measurement of Skin (PRIMOS) to assess changes in skin surface roughness, wrinkles and fine lines. Skin biopsies and chromameter readings were also obtained. At the end of the intervention, a statistically significant reduction in the average peak to valley distance (Rz) in the test group compared to placebo was found, indicating a reduction in overall wrinkle depth in the test group. In addition, total protein was also extracted from full thickness skin biopsies and analysed for pro-collagen I synthesis (new collagen) at the beginning and end of the intervention. There was a significant increase in pro-collagen I in biopsies from the test group at the end of the study when compared to the placebo, with an average increase in pro-collagen levels of 25%. There was also a significant increase in chromameter b* values in the women consuming the test product versus placebo, indicating increased deposition of carotenoids in the skin. This human clinical study has demonstrated that skin anti-ageing effects can be obtained through consumption of a micronutrient-enriched drink. In particular, we were able to demonstrate a reduction in wrinkle depth in the crow's foot area of the eye and that this change appears to be underpinned by an increase in new collagen synthesis over a 14 week period.

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Skin improvement and protection by circadian rhythm restitution. _In vivo_ study

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Circadian rhythm plays an important role in body organ functioning. In skin, circadian-related genes have been shown to be essential for maintaining the necessary functional rhythm of different genes. Recent studies have shown that stress such as UV alters circadian gene expression, which may lead to skin aging. In this study, we evaluated the effect of enhancing skin-related circadian genes using a specifically-designed inducer (IV09.008). A 17-day *in vivo* double blind study was conducted on 12 volunteers. They applied the inducer-containing cream and placebo, twice a day on the thigh, for 16 days, followed by a full solar spectrum irradiation of 2 MED, in order to alter circadian gene functions. Volunteers continued to apply the creams for one more day. Skin renewal time was investigated by skin DHA staining, and other skin conditions were evaluated by *in vivo* confocal microscopy (VivaScope® 1500). In 11 out of 12 volunteers, we observed an increase in skin regeneration on the circadian-induced side compared to the placebo, which confirmed the implication of these genes in skin renewal. This regenerating effect was also confirmed by observation of DHA color vanishing. Indeed, at D14, just before UV, VivaScope® evaluation revealed a regular stratum corneum for the IV09.008-treated side and a better organized granular layer with improved cohesion between cells, indicating healthier skin. Interestingly, after UV exposure, the placebo showed significant signs of stress, such as increased thickness of both the horny layer and epidermis, in contrast to the clock gene-induced side where cells of the granular layer remained unstressed and exhibited good morphology. Most interestingly, we observed a highly significant reduction in the number of sunburn cells on the clock gene-induced side, in contrast to the placebo side. These *in vivo* observations confirmed the importance of cell circadian rhythm improvement in optimizing skin function and resistance.

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Evaluation of the features of normal and dry skin using *in vivo* confocal microscopy

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Skin dryness depends on a variety of different external and internal factors, such as climate, environmental conditions, biological skin aging, and dermatological diseases. Using *in vivo* confocal microscopy (VivaScope®), in this study we compared the changes occurring at a cellular level in dry and hydrated skin. To compare dry and moisturized skin, we studied the morphology of granular cells and the thickness of the horny layer and epidermis. We focused on measurements of the forearm. On this site, the thickness of the horny layer in normal, hydrated skin is around 8µm, while the thickness of the whole epidermis is around 55µm. Corneocytes are well-organized and cohesive, and granular cells possess a cobblestone pattern and polygonal shape. In dry skin, by contrast, architectural disruption of the epidermis is noticeable, and is accompanied by a loss of the cobblestone pattern; cells lose their polygonal shape and appear bigger than in normal skin. In the granular layer, vacuoles and diskeratotic cells are observable at a microscopic level, and the stratum corneum and epidermis are thicker. Keratinocytes in moisturized skin, on the other hand, are arranged in a regular honeycomb-like architecture. Cells are small with a polygonal shape. Moreover, the horny layer and epidermis are thinner than in normal skin, due to the excellent arrangement and cohesion of the cells. These finding by Vivascope correlate well, and explain the clinical known signs of dry and hydrated skin. When skin is properly hydrated, it is visually smooth, plump, and somewhat bouncy to the touch. Dry skin, by comparison, appears dull, lacks brightness, and is flaky and rough with a few visible squames.

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Evaluation of cytotoxicity with a non-animal sourced hyaluronic acid (HA) and an *in vitro* model

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The objective of this study was to evaluate the potential cytotoxicity of a non-animal sourced HA (HA)-based filler, cross-linked in two consecutive steps utilizing a cohesive polydensified matrix™ technology that contains a monophasic gel (Merz Pharmaceuticals, LLC) using an *in vitro* model. An extract of the test article was prepared as follows: Extraction vehicle (minimum essential medium supplemented with L-glutamine, serum and antibiotics), Temperature (37°C ± 1°C), Duration (24-26 hours with agitation) and Ratio test article/vehicle (0.2 g/ml). The test article extract was placed onto triplicate confluent monolayers of L-929 mouse fibroblast cells. Separate monolayers were prepared for triplicate negative and positive controls. After incubating at 37°C ± 1°C in 5 ± 1% CO2 for 24 hours, the cell cultures were stained by a neutral red solution and examined microscopically (100X) to determine cell morphology. Qualitative score for cytotoxicity was based on the following criteria: Response Index 0=not cytotoxic (intact cells, stained, confluent layer), 1=slightly cytotoxic (stained cells with a slight decrease in the cell density or slight morphological alterations), 2=moderately cytotoxic (stained cells with a large decrease in the cell density or severe morphological alterations) and 3=severely cytotoxic (cell lysis or complete absence of neutral red incorporation). The dye was then extracted from the cultures and optical density was measured at 550 nm. Under the conditions of the study, the extract of the HA showed no evidence of cell lysis or cytotoxicity (reduction of the cell density lower than 25%). The negative and positive controls performed as anticipated.

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***In vivo* study of age-related skin change through *in vivo* confocal microscopy**

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Wrinkles, dry skin, and heterogeneity of pigmentation are considered visual signs of skin aging. These macroscopic signs are the consequence of microscopic changes in the epidermis and dermis. In order to trace the stages of these changes, we investigated, through *in vivo* confocal microscopy (Vivascope® 1500), the following skin parameters: horny layer and epidermal thickness; number, height, and morphology of dermal papillae; granular cell morphology and organization, the spreading of melanin in the epidermis, and collagen appearance. Observations were performed on the forearm. Each volunteer was assigned to one of five groups: 20-30; 30-40; 40-50; 50-60; and 60-70. Each group consisted of 5 volunteers. We noticed a significant statistical correlation between age and thickness of the horny layer ($p<0.01$; $r=0.506$); as well as between age and epidermal thickness ($p<0.001$; $r=0.74$). In fact, both became thicker with age, and a change in cell size, shape, and organization was also noticed. In older groups, cells looked larger and more damaged (appearance of vacuoles, dyskeratotic cells, and heterogeneous pigmentation). With aging, dermal papillae became fewer, smaller, less defined, and irregular in shape. We found highly significant reverse correlation between age and number of dermal papillae ($P<0.001$; $r=-0.699$), as well as between age and height of dermal papillae ($P<0.001$; $r=-0.854$). Collagen fibers were observed to be filiform in older groups because collagen decreased and the matrix became thinner. In contrast, younger groups presented dense connective tissue due to the fact that collagen is a major dermal matrix component. In these groups, collagen fiber bundles were very compact and numerous. Moreover, solar elastosis appeared in older groups and was absent in the youngest group. These results demonstrate that VivaScope® is a useful tool and great addition to H&E and macroscopic imaging when characterizing skin changes due to aging.

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Fibroblasts in early striae gravidarum display a biosynthetic phenotype that reflects increased mechanical tension

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Striae gravidarum, or stretch marks, affect up to 90% of pregnant women. While lesions are associated with rapid weight gain, increased body mass index, and high neonatal weight, there is surprisingly little biochemical evidence for increased mechanical tension as a pathogenic factor in striae development. Interestingly, it is well-known that normal dermal fibroblasts under mechanical tension produce increased levels of collagen. As such, we attempted to determine whether newly developed striae demonstrate biochemical evidence of increased mechanical tension, as reflected by induction of collagen synthesis. Punch biopsies were obtained from pregnant women (N=8) in the second or third trimester within 1-2 months of developing reddish abdominal striae. Overall, markers of collagen synthesis were upregulated in abdominal striae (ST), compared with adjacent, normal-appearing "hyperextended" (HE) skin and relatively unstretched hip skin (NL). Compared with NL skin, HE and ST samples demonstrated a 8- and 13-fold increase, respectively, in dermal immunostaining of type I procollagen ($p<0.05$ for ST vs NL). Nearly identical staining was observed for a protein required for collagen biosynthesis, heat shock protein 47 (HSP47), with significantly elevated staining in ST samples ($p<0.05$ for ST vs NL). Compared with NL skin, HE and ST samples demonstrated a 2- and 6-fold increase, respectively, in gene expression of type I procollagen ($p<0.05$ for ST vs NL, $p<0.05$ for ST vs HE). Additionally, mRNA expression of type III procollagen and HSP47 was significantly ($p<0.05$) upregulated in ST samples, compared with NL skin. ST lesions also demonstrated increased mRNA levels of TIMP-1 (an inhibitor of collagen breakdown), but no increase in mRNA expression of MMP-1 and -3, compared with NL skin. These data indicate that fibroblasts in early ST lesions display a phenotype of increased collagen synthesis and reduced collagen degradation, which is consistent with (or driven by) increased mechanical tension.

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Methicillin-resistant Staphylococcus aureus and childhood atopic dermatitis

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Patients with atopic dermatitis (AD) are particularly sensitive to infection with Staphylococcus aureus (SA) which is known to worsen their skin disease. The incidence of methicillin-resistant SA (MRSA) has been increasing and this has important health care implications. The objective of these studies was to assess the incidence and significance of MRSA infection in children with clinically infected AD. To that end, we enrolled children with infected AD and evaluated the amount of SA, staphylococcal protein A, lipoteichoic acid, and a panel of 12 cytokines in wash fluid from a quantitative culture of an infected skin lesion. The SA isolate was tested for antibiotic sensitivities. The inflammation from the tested lesion as well as the total body skin involvement of dermatitis was quantitated by the Ezcema Area and Severity Index (EASI). The subjects were treated with topical corticosteroids, oral antihistamines and the oral antibiotic cephalexin and were then re-evaluated after two weeks and the clinical EASI evaluation and wash fluid studies repeated. Analysis of data from 61 subjects who completed the study revealed that 59 (97%) were positive for SA, and 9 of these 59 isolates (15%) were MRSA. Spearman rank correlation analysis revealed that the amount of SA correlated with clinical lesional and whole body EASI scores, levels of bacterial products, as well as cytokines IL-1beta, IL-8, and TNF-alpha. Infection with MRSA bacteria was not associated with increased lesional EASI, numbers of bacteria, bacterial products or cytokine levels in comparison to lesions infected with MSSA. However, patients infected with MRSA had an increased total body EASI score, indicating worse AD. Finally, treatment with topical corticosteroids and oral cephalexin improved all parameters examined, and this was independent of MRSA versus MSSA. These studies indicate that although the presence of MRSA in AD is associated with an increased burden of skin disease, a regimen using cephalexin and topical corticosteroids is effective in treating AD secondarily infected with MRSA.

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Increasing HMGCoA reductase in skin improves its barrier structure and resistance to stress. *In vivo* study

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In this *in vivo* study we evaluated the effects of enhancing skin 3-hydroxy 3-methylglutaryl coenzyme A (HMGCoA reductase) using a specific compound (IV09.001), previously shown to enhance HMGCoA reductase synthesis and skin lipids, and to increase cholesterol synthesis in stratum corneum. 12 volunteers participated in this double-blind study. They applied IV09.001 cream or placebo for 21 days, twice a day, on the forearm SLS stress was then applied for 24h followed by a one week application of formulae. We used *in vivo* confocal microscopy (VivaScope® 1500) to assess the condition of the skin. Observation at D21, just prior to stress, revealed a decrease in stratum corneum thickness in 90% of volunteers, and a decrease in the thickness of the whole epidermis on the active ingredient-treated side, compared to placebo. These results were highly significant and indicated a better organization and cohesion of cells, both of which are crucial for good barrier function. SLS stress caused moderate damage to the horny layer, generating a decrease in stratum corneum thickness, the appearance of parakeratosis, and the loss of cohesion between corneocytes. At all time points after stress, 24h, 48h, and 1 week, we observed a decrease in horny layer thickness on the placebo side, compared to the IV09.001 zone, where no noticeable change in thickness and cell morphology was observed. These results suggest that increasing HMGCoA reductase improved epidermal lipid content, which led to a reinforcement of corneocyte cohesion. For the epidermis, examination after SLS stress revealed an increase in thickness on both sides, but this increase was less prominent on the IV09.001 side, demonstrating better skin resistance to stress. In conclusion, enhancing HMGCoA reductase level in the skin increased lipid synthesis of the stratum corneum and protected the skin from stress, allowing it to preserve its function and integrity.

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Effect of taxifolin glycoside on atopic dermatitis-like skin lesions in NC/Nga mice

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Increased levels of eosinphils, IgE, IL-4, 5, and 13 and pro-infl ammatory factors (COX-2, iNOS) are observed in patients with atopic dermatitis (AD). Taxifolin 3-O-β-D-glucopyranoside (TAX) from the roots of Rhododendron mucronulatum (RM) was examined to determine whether its immunomodulatory effect was applicable for treating atopic dermatitis. A total of 7 groups of NC/Nga mice with AD were treated by topical application or intraperitoneal injection of TAX for 4 weeks. Follow-up evaluations were done to assess the changes in clinical observations, eosinophil counts, and levels of IgE, cytokines, COX-2 and iNOS. In the clinical observation during the experimental period, TAX treatment signifi cantly reduced the severity of AD-like lesions induced in NC/Nga mice. Eosinophil and IgE levels decreased after treatment of the animals with TAX. TAX may thus be associated with improvement of eosinophil-related allergic diseases. The expression of cytokines (IL-4, 5 and 13) was signifi cantly inhibited in the TAX-treated group, suggesting that TAX might play an immunoregulatory role associated with AD. In RT-PCR, iNOS and COX-2 expression levels were reduced in the TAX-treated group. In western blotting, the expression levels of iNOS and COX-2 were also reduced in the TAX-treated group. These findings suggest that TAX is effective for the treatment of AD by preventing the production of infl amatory cytokines and by reducing skin infl ammation.

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The topical antimicrobial zinc pyrithione is a potent inducer of heat shock response gene expression and TUNEL positivity in reconstructed human epidermis

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Zinc pyrithione (ZnPT) is an FDA-approved microbicidal agent used worldwide in clinical antiseptic products, over-the-counter (OTC) topical antimicrobials, and cosmetic consumer products. Moreover, ZnPT displays antiviral and antipsoriatic activity in human epithelial model systems. Recently, we have demonstrated that cultured primary human skin keratinocytes display an exquisite vulnerability to ZnPT resulting in induction of heat shock gene expression and poly(ADP-ribose) polymerase (PARP)-dependent energy crisis and cell death (Cell Stress Chaperones 2010; Epub ahead of print Oct 7, 2009). Here we present experimental evidence that topical application of ZnPT induces similar effects in reconstructed human epidermis (EpiDermTM). In cultured keratinocytes treated with nanomolar concentrations of ZnPT, expression array analysis revealed massive upregulation of genes encoding heat shock proteins, combined with rapid dysregulation of intracellular zinc ion homeostasis, depletion of ATP levels, and formation of poly(ADP-ribose) polymers. Consistent with an involvement of PARP in ZnPT-induced energy crisis and cell death, ATP depletion could be antagonized by pharmacological inhibition of PARP, and PARP-1 knockout MEFs were resistant to ZnPT-induced ATP depletion and cytotoxicity. In epidermal reconstructs exposed to topical ZnPT expression array analysis demonstrated global upregulation of genes encoding heat shock proteins (HSPA6, HSPA8, HSPA1A, HSPA1L, DNAJA1, HSPH1, HSPCA, HSPD1, HSPE1), antioxidants (SOD2, GSTM3, HMOX1), metallothionein-2A (MT2A), and the cell cycle inhibitor p21 (CDKN1A). IHC analysis confirmed upregulation of Hsp70 in keratinocytes of the basal epidermal layer, and TUNEL analysis indicated loss of DNA integrity in these cells. Taken together our data demonstrate for the first time dramatic effects of topical ZnPT on reconstructed human epidermis that may be of therapeutic and toxicological relevance.

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Atopic dermatitis-like skin lesions reduced by topical application and intraperitoneal injection of hirsutenone in NC/Nga mice

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Atopic dermatitis is a common inflammatory skin disease. The increasing prevalence and severity of atopic dermatitis (AD) during recent decade has promoted the development of safe and more highly effective drugs. Although topical corticosteroids have been used as first line therapy for atopic dermatitis, their potential side effects limits their clinical uses. We have examined to evidence effect of hirsutenone (HIR), among new diarylheptanoid compound, that it is possible reduced of AD-like skin lesions and other factors related to immune response. HIR applicated on the back of NC/Nga mice involved AD-like skin lesions. We measured the clinical symptoms by scoring method well known. Blood samples were collected before and after drugs treatment, and measured the plasma immunoglobulin, cytokines and inflammatory factors. Th2 related cytokines (IL-4, IL-5, IL-13), Eosinophil, IgE inflammatory factors(COX-2, iNOS) were inhibited in serum, lymphocytes, tissue after hirsutenone treatment. This results suggest that hirutenone might be effective in the treatment of AD.

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Determining the extent to which clinically effective treatment, ustekinumab or etanercept, reverses the molecular disease profile of psoriatic skin: comparisons of lesional, non-lesional and normal skin

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ACCEPT, a randomized, active-controlled study, compared the efficacy of etanercept and ustekinumab in 903 patients with moderate-to-severe plaque psoriasis through wk12. Skin biopsies were performed in a subset of patients at baseline, wks1 and 12. Microarray analyses (Affymetrix U133+2 array) comparing non-lesional skin (n=85) to lesional skin (n=85) at baseline showed several thousand probe sets differentially expressed (>2-fold change FDR, $p<0.05$) in lesional skin. An additional 25 healthy skin biopsies were also analyzed. Comparison of nonlesional skin to healthy normal skin showed a series of lesional genes also dysregulated in non-lesional skin (DEFB4, S100A7A, CCL18, SERPINB3). Analyses to understand the impact of p40 cytokine (IL-12/IL-23) or TNF-alpha blockade on resident and inflammatory cells and on the expression of gene circuits that may drive chronic immune activation and inflammation in the skin were completed. In addition analyses to understand the residual molecular profile or "molecular scar" following 12 weeks of treatment were completed. Patients responding to each agent (>=PASI75, n=21 for etanercept, n=19 ustekinumab) had significant changes in ~4000 transcripts compared to untreated lesions, indicating significant resolution of pathological gene circuits back to nonlesional levels. However, genes such as DEFB4, S100A7, CCL18 and SERPINB3 though reduced to levels similar to non-lesional skin were not reduced to that of healthy normal skin by either treatment unlike ADAM10 and HSD3B1. Elucidation of the molecular pathways which remain dysregulated following effective treatment may provide insight into pathological mechanisms that remain active despite appearance of clinical and histologic resolution.

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Quantitative assessment of disease severity in dermatomyositis

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Skin disease in dermatomyositis is a significant source of morbidity and can be challenging to treat. Validated instruments are required to evaluate efficacy of therapies for dermatomyositis in clinical trials as well as routine care. The Cutaneous Dermatomyositis Activity and Severity Index (CDASI) provides such a quantitative measure and has demonstrated excellent inter-rater and test-retest reliability. This study further validates the clinical utility of the CDASI by determining its construct validity, discriminant validity, and association with patient-derived measures of disease severity. Between 2007 and 2009, 106 patients were prospectively evaluated in two tertiary referral dermatology clinics. Spearman's rho (r(sp)) was used to determine correlations between the CDASI and physician and patient global assessments of skin disease (PGA and PtGA, respectively). To assess responsiveness to clinically significant change, CDASI change scores between consecutive clinic visits were compared to the PGA designation of improved, worse, or no change relative to their last clinic visit (PGA-ctlv). CDASI activity (CDASI-a) and damage (CDASI-d) scores were highly correlated with PGA activity and damage scores, respectively ($r(sp) = 0.73$ ($p = 0.000$), $r(sp) = 0.78$, ($p = 0.000$)). Logistic regression comparing change scores for CDASI-a and PGA-ctlv (0 = worse & no change, 1 = improved; 0 = worse, 1 = no change & improved) yielded odds ratios of 0.83 ($p = 0.002$) and 0.75 ($p = 0.006$), respectively. Neither the CDASI-a nor the PGA correlated with PtGA overall disease, skin, itch or pain. Thus, the CDASI correlates with both static and dynamic skin disease status and provides a useful quantitative measure of disease severity. The lack of correlation between physician and patient-derived measures of disease severity highlights the need for further research into patient perceptions of disease activity in dermatomyositis.

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Differences in photo-protected and photo-exposed skin of caucasians; A wrinkle biopsy study

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It is well understood that sun damaged skin ages differently from non-sun exposed skin. It has also been well accepted that this skin is more likely to develop wrinkles, along with other classic signs of photo-aging. Epidermal thickening as a result of sun exposure has been well established, but other physiological changes are less well-understood. Specifically, the etiology of wrinkles is still unclear. There is a scarcity of literature to reach a conclusion as to the physiology of wrinkle formation. Here we aim to describe some of those changes first from non-invasive measurements on the skin, followed by a histological examination of the wrinkle itself. Subjects initially screened for skin type and ages, with a deep wrinkle on the posterior portion of their neck, were chosen for the study. Punch biopsies from three sites were collected: wrinkle, photo-exposed site adjacent to the wrinkle, and photo-protected site on the upper shoulder. Prior to biopsy, imaging of the area, elastin florescence and silica replicas were taken of each site. Histological endpoints assessed for this comparison included pro-collagen, elastin, glycosaminoglycans and morphometric studies using H&E. In addition, immunohistochemical staining was used for comparison of cell proliferation, as well as elastin and collagen synthesis. Initial findings indicate that the expression of procollagen, the precursor to functional collagen, is reduced directly under the wrinkle as well as an observed thinning of a collagen network in the papillary dermis. A similar effect was observed with elastin fibers in the same region under the wrinkle and was reinforced through non-invasive fluorescence measures. Morphometric comparisons between the sites demonstrated no changes in the average number of rete ridges, however there were observable changes in shape and size. Taken together these results suggest that wrinkles demonstrate structural differences from photo-protected skin and could lead to further understanding of wrinkle etiology.

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Molecular tumor characteristics and pharmacodynamic (PD) responses of patients (pts) with advanced basal cell carcinoma (BCC) in a phase I trial of the hedgehog (Hh)pathway inhibitor GDC-0449

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BCC is associated with mutations in Hh pathway genes including patched (PTCH) and smoothened (SMO). A phase I trial of GDC-0449 showed a good safety profile and objective responses in 19/33 pts with locally-advanced (la) or metastatic (m) BCC. Molecular tumor characteristics and drug PD were characterized in this trial. Thirty-three pts with laBCC or mBCC received GDC-0449 orally at 150 (n=17), 270 (n=15) or 540 (n=1) mg/day. Hh pathway target gene expression (GLI1 & PTCH2) in stored tumor tissue was quantitatively profiled by TaqMan® qRT-PCR. SMO and PTCH1 genes were sequenced from pt tumors to identify mutations. PD assessments of GLI1 mRNA expression were conducted on non-involved skin biopsy specimens collected at baseline and at 7 and 21 d after start of therapy. In stored tumor tissue from patients with objective responses or stable disease, expression levels of GLI1 and PTCH2 mRNA were consistent with levels detected in more common cutaneous BCCs. Evidence of Hh pathway dysregulation was further supported by the identification of mutations in PTCH1 or SMO in 9 of 10 evaluable specimens. Supportive evidence of active Hh signaling was not available in one pt who had disease progression. PD down-modulation (>2-fold decrease) of GLI1 mRNA expression was seen in skin samples from 18 of 22 (81.8%) treated patients with BCC, compared with pretreatment biopsies; however the extent of down-modulation did not correlate with plasma GDC-0449 levels. These data provide evidence of active Hh signaling in pts that derived benefit from GDC-0449 treatment, and represent the first reported evidence of Hh pathway activity in the more advanced forms of BCC.

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Evaluation of dermal contact sensitization in a guinea pig model using a non-animal sourced hyaluronic acid (HA)

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The objective of this study was to evaluate the potential of a non-animal sourced HA-based filler, utilizing a cohesive polydensified matrix technology™, to cause delayed dermal contact sensitization [Merz Pharmaceuticals, LLC]. The Dunkin Hartley albino guinea pig was chosen for this study. A preliminary test was conducted with two animals receiving intradermally a range of concentrations (100/50/25/0%) of the HA. Additional dilutions were performed (10/5/1/0.5%). The highest concentration that did not cause any erythema 24 and 48 hrs later was used for the intradermal challenge (5%). 3 additional animals received a range of concentrations topically (100/50/25/0%) of the test article. The highest concentration that did not cause erythema 24 hrs later was used for the topical challenge (100%). The HA was intradermally injected and topically applied to ten test guinea pigs in an attempt to induce delayed sensitization. Five control guinea pigs received a control solution of 0.9% NaCl similarly injected and topically applied. 7 days later the same area was treated with 0.5 ml of 10% (m/w) Sodium Lauryl Sulfate (SLS) solution to provoke a moderate inflammatory reaction. 24 hrs after the SLS administration, all the test animals received a challenge of the HA by intradermal injection and topical application while the control animals were similarly injected and patched with the 0.9% NaCl solution. All sites were scored at 24 and 48 hrs after patch removal and after injection for erythema (0=none, 1=very slight erythema, 2=well-defined erythema, 3=moderate to severe erythema and 4=severe erythema) and edema (0=none, 1=very slight edema, 2=slight edema, 3=moderate edema, 4=severe edema). All scores were 0 (none) at 24 and 48 hs after the patch removal and intradermal injection. The results of this study indicate that the topical application and intradermal injection of this HA does not induce delayed sensitization in this animal model.

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Evaluation of subacute systemic toxicity using a non-animal sourced hyaluronic acid (HA) in a rat model

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The objective of this study was to evaluate the potential subacute systemic toxicity of a non-animal sourced hyaluronic acid (HA)-based filler, utilizing a cohesive polydensified matrix technology™ [Merz Pharmaceuticals, LLC], in Sprague Dawley rats. 40 rats (20 female and 20 male) were randomly assigned to either the test material (n=20) or negative control (n=20). Each of the rats received five intradermal (ID) injections of 0.1 ml of the test article on each side of the back (total of ten injections per animal). The negative control (0.9% NaCl) was similarly injected into the control animals. After completion of the injection procedure, the rats were observed for any adverse reactions. Detailed examinations for clinical signs of disease or abnormality, as well as food intake, were determined at days 4, 7, 11 and 14. Body weight was determined at day -1, 7, and 14. At day 14, the rats were weighed and then euthanized. A macroscopic examination of the target tissues (heart, lungs, kidneys, spleen, ovaries or testes, lungs, thymus, adrenal glands and lymph nodes) and injected sites was conducted. All animals appeared clinically normal throughout the study. No signs of toxicity were noted. Body weight gain and food intake were similar between the two groups regardless of the sex of the rats. The following was observed during the necroscopy: the majority of the sites showed very slight edema and induration, 9/20 treated rats showed at least one site with an eschar and 4/20 treated rats showed sites with marked induration. Under the conditions of this study, there was no evidence of sub-chronic systemic toxicity at 14 days after ID injection of the HA. No biologically significant or gender-based treatment-effects were found between the test and the negative control groups.

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Blood-based biomarker study of human malignant melanoma using gene expression profiling of peripheral whole blood

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Developing analytical methodologies to detect and identify biomarkers in easily accessible body fluids is highly valuable for the early diagnosis and management of cancer patients. Although genetic signatures of melanoma tissues and melanoma cells have been extensively studied, little has been known about blood-based biomarker of melanoma. In order to better understand tumor-associated environment in the blood circulation and to identify potential biomarkers, we conducted gene expression analysis using peripheral whole blood cells of melanoma patients. Seventy-eight candidate genes were selected by microarray analysis of whole blood from stage IV melanoma patients. High-throughput qRT-PCR analysis of 67 genes was performed using whole blood samples from 45 newly diagnosed melanoma patients (stage I to IV) and 50 normal individuals, confirming 39 genes to be differentially expressed in melanoma blood compared with normal blood. Many genes were not previously described in cancer tissue or disease blood, but were differentially expressed in the blood of melanoma patients, even from stage I/II melanoma patients. A stepwise logit analysis using an automated search procedure in the program GOLDMineR® selected the predictor target genes that distinguish melanoma from normals. We have identified a 2-gene signature (PLEK2 and C1QB) that classifies melanoma patients accurately (sensitivity 93%, specificity 90% and R2 0.731). PLEK2 was upregulated in the non-CD45 cells from melanoma patients, whereas C1QB was upregulated in the CD45 cells from melanoma patients and its expression was increased by the supernatants from human melanoma cells. The present findings implicate tumor-associated systemic processes in the blood of melanoma patients and provide the feasibility of using peripheral whole blood gene expression profiles as biomarkers of human malignant melanoma.

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Gene profiling of side population identifies ABCB1 as a chemoresistant factor in human melanoma

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The side population (SP) phenotype is generated by ATP binding cassette (ABC) transporters and identifies hematopoietic stem cells and pluripotent cells from several solid tumors. By effluxing drug, the ABC transporter provides chemoresistance in stem cells and cancer cells. In the current study, we investigated the SP phenotype of human melanoma cells using clinical tumor samples. Tumor tissues were maintained in immunocompromised mice by directly xenografting tumors (direct *in vivo* xenograft model) so that tumors keep proper microenvironment and hierarchical structures. Here we demonstrated the presence of SP cells in human melanoma specimens. SP cells accounted for less than 0.5% of total cells in human melanoma and the frequency was not correlated with melanoma progression and stages. When injected into non-obese diabetic severe combined immunodeficiency (NOD/SCID) mice, both SP cells and non-SP cells were tumorigenic and their growth rates were comparable, suggesting that SP cells were not enriched with cancer-initiating cells. However, cDNA microarray analysis showed a set of differentially expressed genes between SP cells and non-SP cells. These include genes associated with inflammation, regeneration, immortalization and cell adhesion. The analysis of ABC transporters revealed that ABCB6, ABCB7, ABCF1 and ABCG1 were prevalent in both SP and non-SP cells whereas ABCB1 was more upregulated in SP cells. ABCB1 is one of 3 multidrug-resistance genes overexpressed in multiple cancers. The treatment of patient melanoma cells by paclitaxel, a substrate of ABCB1 transporter, *in vitro* induced upregulation of ABCB1 gene expression and 5- to 10-fold increase in the percentile of SP cells, suggesting that SP cells are resistant to paclitaxel treatment. The current study is the first to describe gene expression profiling of SP cells using patient melanoma samples, and will better clarify the molecular mechanisms of multiple drug resistance in human malignant melanoma.

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Minimizing the immunosuppressive effects of histone deacetylase inhibitors (HDACi): Implications for therapy of cutaneous T-cell lymphoma (CTCL)

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Histone deacetylase inhibitors (HDACi) represent a novel class of drugs targeted for the treatment of cancer with several members, including Vorinostat (VOR), approved for the treatment of CTCL. Recent data suggests that HDACi may suppress the immune response via poorly understood mechanisms. As it is desirable to preserve the host immune response of CTCL patients, we sought to comprehensively define the effects of VOR on different arms of the cellular immune response (CMI) in an effort to develop strategies to optimize its effects on the malignant T-cell population while simultaneously minimizing immunosuppression. Peripheral blood mononuclear cells (PBMCs) isolated from CTCL patients and healthy, age matched controls were treated with VOR alone or in combination with immune stimulatory agents (IFN γ, IFN α, IL-21 or a TLR agonist) and malignant cell apoptosis and CMI assessed. VOR greatly increases malignant CD4+ T-cell apoptosis but not apoptosis of CD8+ cells. However, marked inhibition of immune responses by VOR is associated with suppressed production of immune stimulatory cytokines by dendritic cells (IL-12 and IFN α) and NK cells (IFN γ). This suppression is dose dependent and partially reversed by combining VOR with a TLR agonist or IFN γ. Furthermore, VOR profoundly suppresses NK cell activity which in experiments using purified NK cells appears to be directly mediated on this population. NK cell function with VOR can be partially preserved by co-exposure to IFN α, IL-21 or both. In conclusion, while the HDACi VOR induces apoptosis of malignant T-cells, it also potently suppresses multiple arms of the immune system. This immunosuppression can be ameliorated by combining VOR with stimulatory cytokines such as IFN γ, IFN α or a TLR agonist for treatment of refractory CTCL.

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Bone mass, compression fractures, and associated risk factors in patients with epidermolysis bullosa

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The objectives of this study were twofold: 1) to describe the range of bone mass and estimate the prevalence of low bone mass and occult lumbar compression fractures in pediatric patients with generalized forms of epidermolysis bullosa (EB); and 2) to identify risk factors associated with low bone mass and fractures in this population. We prospectively studied 24 subjects (16 male, 8 female) at 2 institutions using clinical evaluations, laboratory studies, spinal radiography, and lumbar dual energy X-ray absorptiometry scans. Subjects were 2 to 20 years old and had the following forms of EB: EB Simplex Dowling-Meara (2), recessive dystrophic EB, severe generalized (20), and recessive dystrophic EB, generalized, other (2). Mean bone mineral density (BMD) Z-score based on chronologic age was -2.6 (range -5.5 – 1.7). BMD Z-score adjusted for bone age (N=16) was -1.65 (range -3.2 – 1.7). Low bone density for age (Z-score ≤ 2) occurred in 14 subjects (64%). 1 occult fracture (4%) was identified. Low BMD correlated with height Z-score, weight Z-score, hemoglobin, serum iron, serum albumin and immobility and inversely correlated with erythrocyte sedimentation rate and c-reactive protein values ($p < 0.05$). A trend of lower BMD associated with increased extent of blistering was noted ($p < 0.1$). 25-OH vitamin D, corrected calcium, and IGF-1 Z-score did not correlate with low BMD. In conclusion, children with generalized forms of EB, particularly recessive dystrophic EB, often have low BMD for age and are at risk for developing lumbar compression fractures. Subjects with low BMD tend to be small for age, less active, and have evidence of chronic systemic inflammation and poor health.

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A potent and selective CRTh2 antagonist is efficacious in a model of atopic dermatitis

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Prostaglandin D2 (PGD2) is a potent prostanoid released from activated mast cells during atopic responses. CRTh2, chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (a.k.a. DP2), a PDG2 receptor, mediates chemotaxis and mast cell-dependent activation of basophils, eosinophils and Th2 lymphocytes. Preclinical data and emerging clinical results suggest CRTh2 antagonists may have utility in allergic diseases. ARRY-006 is a potent, selective, orally bioavailable competitive antagonist of CRTh2 (binding IC50 = 1 nM). ARRY-006 inhibits i) PGD2-mediated chemotaxis of isolated human basophils, ii) PGD2-induced eosinophil shape change in human whole blood and iii) PGD2-induced CRTh2 receptor internalization in human whole blood. In a model of atopic dermatitis (AD) utilizing NC/Nga mice that spontaneously develop symptoms of AD, oral administration of ARRY-006 at 30mg/kg (QD) inhibited ear thickening, erythema, oozing, crust formation, hemorrhaging and pruritus and showed trends in improved skin histopathology. Selective CRTh2 antagonist, ARRY-006, is a potent inhibitor of basophils and eosinophils in vitro and exhibited significant protective activity in a model of dermatitis.

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Fractional photothermolysis treatment triggers a wound healing response and dermal remodeling in photoaged human skin *in vivo*

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Connective tissue damage is a hallmark of photoaged skin and arises primarily from increased degradation and decreased production of collagen I, the major structural protein in skin. Anti-aging treatments aim at increasing collagen I production in photoaged skin. Fractional photothermolysis (Fraxel laser) is a recently developed method used to clinically improve the appearance of photoaged skin. This study was designed to quantify the molecular changes induced by a single Fraxel treatment of photoaged human skin *in vivo*. Evidence of skin thermal injury was demonstrated by presence of microblisters in the epidermis, and collagen denaturation in the upper dermis 24 hours after Fraxel treatment. Histology and laminin γ2 immunostaining demonstrated minimal damage to the basement membrane. Skin thermal injury induced by Fraxel laser was followed by an inflammatory response, evidenced by increased inflammatory cytokine (TNF-α) production and neutrophil infiltrate, 24 hours after treatment. Increased inflammation was accompanied by increased matrix metalloproteinase 1 and 3 production (275- and 90-fold *vs* baseline, respectively, n=10, $p<0.05$). Following waning of the inflammatory response, Fraxel laser treatment induced a robust increase in type I and III collagen mRNA levels (5.0- and 4.0-fold *vs* baseline, respectively, n=10, $p<0.05$), and procollagen I protein (2.9-fold *vs* baseline, n=10, $p<0.05$), 2 weeks after treatment. High levels of collagen I mRNA and protein persisted at least until week 4 post-treatment (3.5- and 6.3-fold *vs* baseline, respectively, n=10, $p<0.05$). Immunohistochemistry indicated that increased procollagen I production occurred throughout the dermis. Taken together, these data demonstrate that a single Fraxel laser treatment induces thermal injury that triggers a wound healing cascade leading to new collagen deposition in photodamaged skin. Clinical improvement following Fraxel laser treatment is likely to result from stimulation of dermal remodeling in photodamaged human skin *in vivo*.

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Combination treatments for psoriasis: A systematic review

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The purpose of this study is to determine the most effective combination therapies for moderate to severe and mild to moderate psoriasis. Data was collected by performing a systematic review of the literature on combination treatments of psoriasis and subsequently performing a series of meta-analyses based on the available data from randomized controlled trials. Article searches of Medline, pubmed, CINAHL, MeSH, and Cochrane Review were performed using the combination of keywords "psoriasis" and "psoriasis, therapy" with "combination," as well as the phrases "drug therapy, combination" and "combined modality therapy". The searches were performed in June 2009 including all available English language articles and produced a total of 1713 results. Articles not related to efficacy of psoriasis combination treatments and all duplicate entries were excluded, resulting in 371 entries. Of these entries, 115 articles were randomized controlled trials investigating the efficacy of one or more combination treatments. 125 entries were prospective non-randomized trials with one or more treatment arm. 18 articles were retrospective chart reviews, 63 were case reports, 28 were review articles, and one article was a meta-analysis of interventions for chronic palmoplantar pustulosis. All randomized controlled trials were reviewed and organized by baseline psoriasis severity using criteria based on a combination of several frequently used scales. 10 randomized controlled trials were excluded from the analysis because severity was not reported. Using advanced statistical techniques to weight the results of each study, the best treatment trends will be assessed. Limitations to conclusions about treatment recommendations will be delineated. These limitations include a lack of homogeneity in the psoriasis severity scales, variation in dosages, treatment length, and treatment frequency, and differences in outcomes reported and length of study follow-up.

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Cytologic "touch preparation" analysis of skin biopsies for rapid evaluation of malignant skin diseases

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Skin-based cytology methods for the diagnosis of epidermal/dermal lesions are under-developed. In the "touch prep" technique, the fresh biopsy specimen is pressed lightly against a glass slide, creating a cytologic imprint that can be air-dried, stained, and evaluated within minutes. Touch prep analysis is commonly used in analysis of lymph nodes, but studies of its potential application to skin biopsies has been limited. We sought to evaluate the use of skin biopsy touch preparations as a method for discriminating malignant vs. non-malignant lesions. Touch preparations were performed on 50 consecutive patient skin biopsies from an academic medical center. Malignant lesions included basal and squamous cell carcinomas (n=12); benign lesions included seborrheic keratoses, blue nevus, neurofibromas, verrucae, melanocytic nevi, and ulcers (n=38). Specimens were processed using Diff-Quik. Three independent pathologists, blinded to the final pathologic diagnosis, reviewed slides and were provided the clinical description and differential diagnosis. The gold standard for the diagnosis of lesions was considered to be the findings on permanent histology. Touch prep analysis identified malignant biopsies with a sensitivity of 92% and specificity of 97%. Detection of basal cell carcinomas had the highest sensitivity and specificity (100%), as BCCs displayed characteristic and reproducible cohesive clusters of small- to medium-sized cells with scant basophilic cytoplasm. In summary, touch preparation cytology of skin biopsies is a rapid, accurate, cost-effective methodology for initial screening diagnosis. Touch preparation cytology may eventually be useful as a tool for medical practitioners operating in locations without routine access to pathology facilities, or as an initial screen for triage of biopsy samples requiring formal histologic analysis.

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Effects of cosmetic ingredients as anti-cellulite agents: Synergistic action of actives with *in vitro* and *in vivo* efficacy.

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In cellulite changes within adipose cells include enhanced lipogenesis together with decreased lipolysis resulting in increased lipid storage within the adipocyte. Changes in the dermal matrix is also known to occur. In this study, the ability of cosmetic agents *Furcellaria lumbricalis*, *Fucus vesiculosus*, retinol, conjugated linoleic acid (CLA) and glaucine were investigated for their effects on the lipolysis in human adipocytes, the production of proCollagen I by mature fibroblasts and for their ability to improve cellulite condition *in vivo*. Combined treatment of *F. vesiculosus* and *F. lumbricalis* stimulated proCollagen I production (5.75ng/ug total protein ± 1.01, p=0.05, n=3) compared to untreated fibroblasts (1.7ng/ug total protein ± 0.03, n=3). Mature adipocytes exposed to *F. lumbricalis* and *F. vesiculosus* induced free glycerol release (15uM ± 3.7, n=4). This was higher after treatment with CLA (32uM ± 8.1, n=4) or retinol (36uM ± 9.4, n=4). The highest level observed was after treatment with glaucine (115uM ± 43.5, n=4). However, all combined ingredients showed a significant synergistic increase in free glycerol release (338uM ± 31.5, n=4, $p<0.05$). In a double blind, fully randomized placebo clinical study conducted on 36 caucasian volunteers with modest cellulite on thighs, significant improvements in cellulite grade was observed by the dermatologist after 8 weeks (-1.2 ± 0.9, $p<0.05$) and 12 weeks (-1.6 ± 0.9, $p<0.05$) compared to placebo treatment. Ultrasound imaging demonstrated a significant decrease in fat thickness (-0.6mm, $p<0.001$) compared to placebo treatment after 12 weeks. The findings in these studies show that this unique combination of active ingredients synergistically increase adipocyte lipolysis. The *in vitro* actions of the ingredients were translated *in vivo*, whereby a clinical improvement of cellulite condition was achieved.

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Cosmetic ingredients stimulate fibroblast contractile capabilities and procollagen-1 production resulting in skin anti-aging and skin lifting properties *in vivo*

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The aim of the study was to determine the effects of retinol, palmitoyl-tripeptide-5 and low molecular weight hyaluronic acid (LMWHA, 10-15kDa) on improving the contractile properties, the ability to stimulate collagen production of fibroblasts from normal aged skin, as well as improving wrinkles and facial sagging. Two models to investigate the improvement of contractile forces by the individual active ingredients were used; a "retracted lattice" and a "tense lattice"-GlaSbox®. An *in vitro* monolayer culture system (with *in vitro* 'aged' fibroblasts) was used to measure Procollagen 1 stimulation by ELISA. In the tense lattice model all three actives showed a significant ability to contract fibroblasts compared to the untreated control (retinoic acid and palmitoyl-tripeptide-5 $p<0.001$, LMWHA $p<0.05$). In the retracted lattice model palmitoyl-tripeptide-5 and retinoic acid stimulated the contraction of the gels compared to the untreated control ($p<0.01$). Pro-collagen 1 production increased following stimulation with all three actives compared to the control (retinol and palmitoyl-tripeptide-5, $p<0.001$, LMWHA, $p<0.05$). In a 12 week, double blind, placebo controlled clinical study Fast Optical *in vivo* Topometry (FOITS) showed significant reductions in the roughness (Ra) and smoothness (Rz) of the wrinkles at 8 weeks (Ra 5%, $p<0.05$) and 12 weeks (Rz 9%, $p<0.05$) respectively for the treatment compared to placebo. Expert visual grading showed that the sagging at the jaw-line improved by 11% at week 4 compared to baseline ($p<0.05$). These findings show that retinol, palmitoyl-tripeptide-5 and LMWHA are capable of stimulating collagen synthesis *in vitro*. They also improve the capability to contract of *in vitro* 'aged' fibroblasts. The *in vivo* results show how the combination of the three actives can reduce wrinkles and improve skin sagging for anti-aging and lifting applications.

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Oral administration of carcinine improves Zucker diabetic fatty (ZDF) rats skin condition

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Carcinine, a small peptidomimetic first isolated from crustacean, is found at low concentration in several mammalian organs including skin. Very similar to the dipeptide L-carnosine, carcinine is endowed with anti-glycation and antioxidant properties, its particular interest residing in high resistance to proteolytic enzymes. Carcinine was given to ZDF rats, a widely used type 2 diabetes animal model that develops progressive insuline resistance and glucose intolerance. A 3 months study was conducted on 12 non-diabetic age-matched Zucker (LEAN) rats as control, and 40 ZDF rats with daily carcinine administration at 0, 1, 10 and 100 mg/Kg. Body weight curves, glycemia progression, and glycated haemoglobin (HbA1C) dosages were in agreement with previously published data with this model. Skin morphological analysis didn't show gross modifications between diabetic and control rats, while histochemical analysis revealed several skin alterations associated with disease progression: increased carboxymethyl lysine (CML), glucose transporter GLUT-1 reduced expression, reduced number of Ki67-positive cells in the epidermis and increased caspase-3 expressing apoptotic fibroblasts. Treatment of diabetic rats with carcinine significantly decreased CML staining in the epidermis, and caspase-3 expressing cells in the dermis (at all concentrations tested). Decorine, a dermal glyprotein involved in collagen fibrillogenesis, and GLUT-1 were over-expressed upon treatment. No significant difference was seen for Ki67-positive cells. Treatment could not reduce significantly glycemia or glycated haemoglobin levels. Nevertheless, our results suggest that carcinine could limit glucose-mediated damages within the skin, or increase defence mechanisms, and thus improve skin condition in diabetic rat. Considering that diabetes induces alterations that mimic some age-related changes in the skin, carcinine may be regarded as an effective anti-aging ingredient.

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Involvement of increased protease-activated receptor-2 expression in the pathogenesis of melasma

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Melasma is a common acquired symmetrical hypermelanosis which often affects the sun-exposed area of oriental woman. The pathogenesis of melasma is not fully understood. Recently, elevated vascular endothelial growth factor (VEGF) expression and increased vascularity were reported as one of the major features of melasma and considered as an important factors in the melasma pathogenesis. Protease-activated receptor-2 (PAR-2) activation has been reported to increase keratinocyte phagocytic activity which is important mechanism of melanosome transfer. To evaluate the role of PAR-2 in the pathogenesis of melasma, we retrospectively examined skin biopsy specimen which had been made a histologic diagnosis of melasma. On the histological examination of immunofluorescent staining for PAR-2, increased expression level of PAR-2, especially at the suprabasal keratinocyte, was observed in several patients with melasma. On the review of clinical photographs, we also found that the patient, whose melasma lesion shows elevated PAR-2 expression, has a propensity to show pronounced telangiectatic erythema confined to melasma lesion. Therefore, we conducted *in vitro* study to investigate the effect of PAR-2 activation on the vasculature of melsma lesion. Increased VEGF mRNA levels were noted in cultured keratinocytes, which were treated with PAR-2 agonist peptide, by using PCR technique. These findings suggest that PAR-2 activation may play an important role in the pathogenesis of melasma partially through the increase of VEGF.

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Analysis of allergic triggers in adult and pediatric atopic dermatitis

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Atopic dermatitis is a common chronic relapsing pruritic inflammatory skin disease. The pathological mechanisms underlying atopic dermatitis are complex and involve both cellular and antibody-mediated phenomena. In many cases IgE antibodies to common foods and microbes develop and exacerbate the condition. Additionally, in a subpopulation of atopic dermatitis patients autoantibodies against IgE develop and may aggravate the disease. Identification of these triggers may be useful in assessing and monitoring the disease process and in patient counseling. Discard and de-identified serum samples from atopic dermatitis patients were evaluated for specific IgE to cow's milk, egg white, soybean, peanut, fish, wheat, Malassezia, Candida, Staphylococcus aureus enterotoxin A, and Staphylococcus aureus enterotoxin B using the Phadia ImmunoCAP system. A specific IgE level of greater than 0.35 kU/L was considered positive. Total IgE was measured using the Immulite 2000. IgG antibodies to IgE were quantified with a solid phase indirect non-competitive ELISA. Almost half of adult (45%) and pediatric (49%) atopic dermatitis patients had IgE specific for at least one microbe. The most common microbes were Malassezia and Candida. The majority of patients (92% adults and 71% of pediatric) positive for at least one microbe had total IgE levels of greater than 100, with many values over 1000. Over half (55%) of adult patients were positive for at least one food with peanut and wheat being the most common. A higher percentage (66%) of pediatric patients contained IgE for common foods; with over half of the samples containing IgE to at least two foods. Adult patients were more likely to have IgG autoantibodies to IgE compared to pediatric patients (18% vs 6%). Identification of the triggers for a patient's atopic dermatitis can be used in patient counseling. For some triggers, such as microbes, this data may be used to direct specific therapeutic options.

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Development of a glycation evaluation method to support *in-vivo* ingredient screening

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Increases in cross-linked collagen are associated with the aging process and diabetes. The demand for improved skin care addressing the aging process requires the discovery and development of new and effective ingredients. Development of new and non-invasive measurement and evaluation methods assists in the prediction of new ingredient efficacy. Changes in the accumulation of advanced glycation-end products (AGEs) in diabetic and non-diabetic populations may be observed using fluorescence spectroscopy. This method may be utilized to determine the effectiveness of glycation-breaking or glycation-preventing active ingredients in skin care products. Diabetic and normal populations, representing elevated cross-linked collagen and normal cross-linked collagen, were evaluated using a fiber-optic probe spectrofluorimeter across a broad spectral range (230 – 480 nm excitation)(300 – 540 nm emission) while avoiding the primary reflection spectral ranges. Normalized maximum fluorescent intensities extracted from subset spectral ranges were obtained from left/right forearm and lower back measurements of study subjects and evaluated for equivalence and proximity relationships. The resulting evaluation provided relationship patterns capable of identifying greater than 70% of the subjects as diabetic or non-diabetic in a blind study. Identifications were compared to study subject HbA1c and blood glucose levels obtained at the start of the study. Non-invasive approaches of this type are considered to be essential in the discovery and evaluation of new and efficacious ingredients related to anti-aging skin formulations.

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A typical daily dose of ultraviolet radiation on human skin treated with an FDA-standardized sunscreen leaves a unique "UVA signature" on the transcriptome

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Currently there are no standard *in vivo* assays to evaluate the effects of ultraviolet A (UVA) on the human skin. This makes it difficult to estimate the extent of UVA contribution to the adverse effects of ultraviolet radiation (UVR), and to assess sunscreens for the UVA protection efficacy. Skins of human volunteers were exposed to solar-simulated UVR at a minimum erythema dose (MED), 0.1 MED or 100 J/m^2, with or without the pre-treatment with an FDA-standardized sunscreen, or to the corresponding doses of UVA. Changes in the skin transcriptome were analyzed using expression microarrays, and genes that were differentially expressed by UVA were identified to create custom UVA gene sets (UP_by_A and DOWN_by_A), which were used in gene set analysis to probe the skin for the signature of UVA exposure. Single-gene analysis demonstrated dose-dependent effects of UVA and UVR on the skin while the sunscreen blocked 99.6% of the UVR effects. Gene set analysis demonstrated the signatures for P53, proteasome and MYC signal activation by 1 MED UVR, and the signatures for RAS signal activation and TGF-β signal inactivation by sunscreen + 1 MED UVR. Both custom UVA gene sets (UP_by_A and DOWN_by_A) detected the UVA signatures in the skin treated with sunscreen + 1 MED, 0.1 MED, or 100 J/m^2 UVR. Gene set analysis revealed a substantial contribution of UVA to the UVR effects on the skin, and demonstrated the persistence of UVA signature when the skin was pre-treated with sunscreen before being exposed to UVR. Custom UVA gene sets provide sensitive measurements of the UVA effect *in vivo*, and shall offer a novel method to evaluate sunscreen formulations for UVA protection efficacy.

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Evaluating quality of life in dermatomyositis

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Quality of life (QoL) for patients with inflammatory skin disease can be significant, but has been evaluated in just one study in dermatomyositis (DM). The purpose of this study is to examine the relationship between the Cutaneous Disease and Activity Severity Index (CDASI) and various QoL study instruments and to determine the impact of DM on QoL. The skin-specific QoL instruments used were the Skindex and the Dermatology Life Quality Index (DLQI). The global medical QoL instrument used was the SF-36. Using the SF-36, DM was compared to cutaneous lupus erythematosus (CLE), CHF, HTN, clinical depression, diabetes type II, recent MI, and the general population. Pruritus was evaluated by a visual analogue scale (VAS) and 0-10 scale in DM and CLE populations, respectively. The CDASI was used to determine the cutaneous severity of DM. There was a significant correlation between the CDASI and each of the Skindex subscores (CDASI v. symptoms r=0.355 p=0.037, v. emotions r=0.481 p=0.003, and v. function r=0.474 p =0.004) and between the CDASI and the DLQI (r=0.331 p=0.002). DM, assessed by the SF-36, was found to have significantly worse QoL scores than the general population with the exception of bodily pain (all subscore p values <0.01). Furthermore, DM had a significantly lower vitality score ((mean:standard deviation (SD)) DM 43.29:11.83), representing energy level, compared to mean HTN, diabetes, and recent MI scores (50.63, p=0.0001; 49.4, p=0.001; and 50.32, p=0.001; respectively). There was a significantly lower mental health score (DM 45.74:11.92), representing overall mood, to all compared diseases except CLE and clinical depression ((disease:mean)HTN:51.5, CHF:49.69, diabetes:50.86, recent MI:50.32; p values < 0.01). We found that DM produces a larger amount of pruritus than CLE ((mean:SD) DM 3.95:3.13 n=82, CLE 2.37:2.60 n=161; p < 0.0001). We conclude that DM has a large impact on QoL, even when compared to other diseases, and that DM skin disease activity correlates with a poorer QoL.

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Gadolinium deposition in disease affected versus unaffected skin of nephrogenic systemic fibrosis

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Nephrogenic systemic fibrosis (NSF) is a rare, progressive, potentially fatal fibrosing disorder affecting the skin and other organs. The mechanism of disease pathogenesis is unknown, but NSF development has been associated with renally excreted gadolinium (Gd) based contrast agent (GBCA) exposure in patients with renal insufficiency. GBCA exposure in patients with CKD 4-5 has been implicated in transmetallation and skin deposition of free Gd. Attempts have been made to identify and quantify Gd in the tissue of NSF patients. To date, Gd quantification has been performed on paraffin-embedded samples, and Gd deposition has not been correlated with disease severity by statistical analysis. The purpose of this study is to quantify the fresh skin and blood Gd levels of NSF patients. We compare the Gd levels in disease affected skin (AS) to unaffected skin (US). We use Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to quantify the Gd in 13 patients with NSF. We compare the skin Gd levels in NSF patients with skin Gd levels in 13 control patients. The AS biopsies had a mean Gd concentration of 71.4 μg/g dry weight ± 89.4 (range 6.3-348.7 μg/g dry weight). The US biopsies had a mean Gd concentration of 10.2 μg/g dry weight ± 19.9 (range 0.6-68.2 μg/g dry weight). Mean ratio of paired Gd concentrations of AS to US was 23.1, ranging from 1.2 to 88.9. Mean serum Gd concentrations in NSF patients was 4.8 ng/ml which is more than 10-times the level in normal patients. A statistically significant correlation existed between serum and AS Gd concentrations (r^2=.74, p<.0001). In conclusion, Gd is found in the skin and blood of NSF patients despite distant GBCA exposure. There is a statistically significant difference in the amounts of Gd in involved vs non-lesional skin of NSF patients. These findings support the role of differential free Gd deposition from GBCA in NSF pathogenesis. Furthermore, the overlap in the range of Gd levels in AS and US implicates factors other than absolute Gd levels in disease pathogenesis.

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Serial poly-L-lactic acid injections for the treatment of HIV-related facial lipoatrophy

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HIV-related lipoatrophy (LA) is a component of the antiretroviral-associated lipodystrophy syndrome, affecting up to 35 % of those patients on highly active antiretroviral therapy (HAART). Numerous studies have investigated the negative impact that facial LA has upon HIV patients, including depression, stigmatization, decreased quality of life, and discontinuation of medication. A variety of techniques have been reported to address this manifestation of HIV treatment, many of which rely upon more invasive procedures. Poly-L-lactic acid is a biodegradable bioabsorbable aliphatic polyester, approved for the treatment of facial LA. This study was designed to examine the efficacy of serial poly-L-lactic acid injections as a minimally-invasive method to correct HIV-associated facial LA. Thirty-two veterans with longstanding HIV were included in the study. Each subject received from 2-8 injections at 6 week intervals. Global LA severity score, facial skin fold thickness, and quality of life measurements were assessed at baseline and at regular intervals throughout the study. Overall LA severity decreased on the standard 4 point scale from 2.2 to 0.5 (p<0.001), with 31/32 patients achieving a post-treatment score below 1. Average cheek skin fold thickness increased over 48 %. All 32 patients reported a dramatic improvement in quality of life. Side effects of the procedure included mild to moderate pain with injection, bruising, and the development of palpable but not visible subcutaneous nodules. Average cost of treatment was $4320 per patient. Several study patients had follow up of over 1 year and maintained a high degree of correction. Poly-L-lactic acid injection is an effective, minimally-invasive and safe intervention in the treatment of HIV-associated facial LA, resulting in dramatic improvements in both objective and subjective physical end points and improvements in quality of life.

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Doxycycline modifies sebum production *in-vitro*

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The *in-vitro* effect of doxycycline on proliferation and lipogenesis of the human sebocyte cell line SZ95 was studied. Regulation of genes involved in differentiation, lipogenesis, inflammation (PPARα and PPARγ) and apoptosis (bcl-2 and bax) was evaluated. SZ95 sebocytes were studied at 6, 12, 24 and 48 h after exposure to 5 concentrations of doxycycline: 0.025– 4 mcg/ml. Lipid synthesis was assessed by Nile red fluorescence, cell growth by crystal violet staining and drug toxicity by MTT assay. Quantitative real-time PCR on mRNA extracted from sebocytes after 12 and 24 h exposure to 0.025 mcg/ml and 4 mcg/ml doxycycline examined PPARα, PPARγ, bcl-2 and bax. Doxycycline reduced cell proliferation after 12 h at all concentrations, in particular at higher doses. Lipogenesis increased at higher doses. At 2 mcg/ml cell growth reduced by 44%, while neutral lipids increased by 126% and polar by 102%. Toxicity assays revealed reduced cell viability after 24 h at higher doses. RT-PCR showed the bcl-2/bax ratio reducing with higher doses of doxycycline, with a ratio of 2 at the lower dose compared with 0.42 at the higher dose at 12 h. Both PPARα and PPARγ were upregulated at both doses, with the largest increase (5-fold) evident at the higher dose at 24 h. Doxycycline reduces SZ95 sebocyte proliferation and viability at higher concentrations while simultaneously increasing lipogenesis. The increased lipid:cell ratio with higher concentrations indicates increasing cell size and differentiation. The increase in both polar (membrane) and neutral (sebaceous) lipids suggests terminal differentiation of the sebocyte. Our RT-PCR results confirm that apoptosis (determined by the ratio between the anti-apoptotic bcl-2 to the pro-apoptotic bax protein) is induced at later timepoints and with higher antibiotic concentrations. Together with upregulation of PPARγ (which plays an important role in sebocyte differentiation, lipogenesis and inflammation) these results provide new insight to the disease-modifying potential of doxycycline.

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Poly-activated tumor-associated macrophages in cutaneous squamous cell carcinoma.

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Squamous cell carcinoma (SCC) is the second most common human cancer, affecting over 300,000 individuals in the United States each year. Aggressive behavior in SCC is associated with immune compromise in the host and manifested by increased morbidity and mortality in organ transplant recipients. We compared tumor-associated macrophages (TAMs) in cutaneous SCC and transplant-associated SCC (TSCC). Most studies suggest that TAMs are M2 type macrophages, polarized by cytokines such as IL-4, rather than M1 macrophages, which are polarized by IFNγ. We were interested in evaluating the polarization of TAMs in SCC. We compared the number and distribution of macrophage markers, CD68 and CD163, in SCC and TSCC by immunohistochemistry. CD163+ and CD68+ cells were significantly increased in SCC and TSCC compared to normal skin (p<0.001 for each comparison). The vast majority of CD163+ and CD68+ cells surrounded the SCC tumor nests. Many CD163+ cells co-expressed CD68 by immunoflourescence. As it is very difficult to obtain TAMs from SCC for functional studies, we treated *in vitro* monocyte-derived macrophages with cytokines to generate M1 and M2 "pathways". We analyzed these pathways in the SCC transcriptome by Gene Set Enrichment Analysis (GSEA), which showed enrichment for IFNγ-induced macrophage genes. We were surprised to find an abundant M1 signature in SCC and evaluated the expression of the IFNγR and the presence of STAT-1, a classic IFNγ-induced protein. We observed co-localization of IFNγR-α in CD163+ and CD68+ cells in SCC and TSCC. Furthermore, STAT1 appeared abundant and nuclear in both groups. Overall, while others have suggested that macrophages are predominantly the M2 type in tumors, our results show IFNγ-polarization of macrophages in SCC. The tumor microenvironment may create a poly-activated macrophage (PAM), and these SCC-associated macrophages could be termed "PAM-TAMs".

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Natural PPAR-alpha agonist and atopic dermatitis: From research to clinical efficacy

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Natural Peroxysome-Proliferative-Activated Receptors ligands are able to restore skin barrier function, demonstrate anti-inflammatory properties and have a potential role in Atopic Dermatitis (AD) management. It has been demonstrated that a patented Sunflower Oleosdisitillate (SO) activates PPAR-alpha, induces epidermal key lipids synthesis and reduces cutaneous inflammation. An emollient containing SO has been formulated for AD skin care and its clinical efficacy has been evaluated according two different studies. Study A: Randomized study on the Steroid-sparing effect and Quality of life (QoL) impact of 2% SO. 86 children (4-48 months) with AD have been attributed to 5 different Topical Steroids (TS) treatment groups during 21 days: desonide 0.05% 2X/D to 1D/2, with or without 2% SO cream. Results showed similar and significant improvement of SCORAD (p<0.01) in all groups: 63% in the TS 2X/D group and 75% in the TS 1D/2 + 2% SO cream group. QoL improvement was at best in the 2% SO cream groups. Study B: Randomized study comparing 2% SO cream 2X/D to TS (hydrocortisone butyro-propionate; 1 mg/g) 2X/D for 21 days among 40 children (mean age 2.3 years), with moderate AD (mean SCORAD 37). In the two groups, SCORAD decrease was significant at D7 and D21 (-70% and –75%), with no statistical difference of SCORAD at D21 (11 vs 9.4). QoL questionnaires were similarly improved in both groups (65 to 75%). For the first time, a cream containing Sunflower Oleodistillate has demonstrated comparable therapeutic properties to a Topical Steroid in a randomized comparative study during 3 weeks. These properties are probably linked to previously demonstrated agonist PPAR-alpha properties.

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Resveratrol protects keratinocytes cells against nitric oxide-induced toxicity

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A growing number of studies suggest that natural extracts and phytochemicals have a positive impact on brain aging. This can be evidenced by exposure of hippocampal cells to the nitric oxide (NO) donor sodium nitroprusside (SNP) which resulted in a decrease in cell survival and the protection by natural polyphenols against these events. More recently, it has been reported that neuroprotective effects of one of these compounds, resveratrol, may be mediated by specific binding sites in the brain. In this work we have transposed to skin these results. Using immortalized keratinocytes cell lines HaCaT, we have here studied the effect of resveratrol in a model of toxicity induced by the nitric oxide (NO) donor, sodium nitroprusside (SNP). A 24-hour exposure SNP (0.3-10 mM) caused a concentration-dependent decrease of cell survival, as revealed by the MTT and calcein assays. A treatment with resveratrol (1-30 μM) inhibited the toxic effect of SNP, the effect being significant from 10 μM. Moroever, resveratrol reduced the increased activity of caspase-3 and the number of apoptotic cells, as estimated by the SYTO 16, suggesting that it protects keratinocytes cells through its anti-apoptotic action. Finally, we have identified [3H]-resveratrol binding sites in HaCaT as well as in human skin sections. Taken together, these data suggest that as for brain resveratrol is a potent agent that offers anti-aging effetcs for human skin. Moreover a similar mechanism could be involved to explain the beneficial effects resveratrol in skin and brain. This can be connected with the hormonal prevention of aging which has also been described to have beneficial effects against age-induced damage in the CNS the liver and the skin through molecular mechanisms reducing oxidative stress and apoptosis.

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Visualization of untreated premature atherosclerosis in a US psoriasis cohort

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Many findings of an association of psoriasis with cardiovascular disease (CVD) are in non-US populations and are retrospective. We used cardiac CT and carotid ultrasound to determine the presence of atherosclerosis, while also measuring serum risk factors and determining psoriasis history and treatment. A cross-sectional study of 89 psoriatics and 44 controls without inflammatory skin disease was performed. The presence of atherosclerosis, defined by coronary artery calcification, plaque present on carotid ultrasound, or carotid intima-media thickness ≥ 75th percentile, was increased in psoriatics compared to controls [27% vs. 12% (aged 20-39), and 74% vs. 50% (aged 40-59)]. Among psoriatics with atherosclerosis, only 39% were treated with aspirin, statins, or ACE-inhibitors. Mean serum hsCRP, a strong and specific marker for future CVD events, was 56% greater in psoriatics (n=100) than controls (n=53) (relative change 1.56 [95% CI: 1.02, 2.38], p=0.041). Adjusting for waist-to-hip ratio and systolic blood pressure attenuated this association. Serum hsCRP was associated with psoriasis severity measured by PASI (relative change 1.04 [95% CI: 1.01, 1.06], p<0.001). Adjusting for CVD risk factors and psoriasis duration did not alter the association with PASI. Of particular interest, the serum hsCRP of psoriatics currently on TNF-α inhibitors was less than half of those not currently on TNF-α inhibitors (relative change 0.41 [95% CI: 0.22, 0.76], p=0.005). To examine the prevalence of CVD in the Murdough psoriasis cohort, we compared our data to the 2005-2006 National Health and Nutrition Examination Survey (NHANES). CVD (presence of hypertension, coronary artery disease, heart failure, or stroke) was 54% in psoriasis (n=325), versus 36% of NHANES patients (n=8x10^{7}); CVD in our cohort's controls comported well with the NHANES defined control group. In conclusion, these data provide direct evidence of premature and untreated atherosclerosis in psoriasis patients.

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Treatment of hypovitaminosis D in veteran patients with psoriasis

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The aim of this preliminary study was to investigate the effect of oral supplemental vitamin D3 on serum 25(OH) vitamin D level in psoriasis patients. The study was a randomized controlled trial and conducted at John D Dingell Veterans Medical center in Detroit Michigan. METHODS: twenty veteran psoriasis patients with hypovitaminosis D [25(OH)D < 20 ng/ml], their mean age, 56.2 years old; range 40-70 with moderate to severe psoriasis [mean Psoriasis Area and Severity Index (PASI) score 9.8; range, 3.8-18.8] divided into four groups, each group received oral vitamin D3 (cholecalciferol) therapy at a dose of 400 IU daily, 1000 IU daily, 4000 IU daily or 50,000 IU weekly respectively for 12 weeks. Blood samples were drawn before, every month and at end of treatment. Serum measurements of 25(OH)D, PTH, calcium, Comprehensive metabolic panel and alkaline phosphatase were taken. RESULTS: Blood 25(OH) Vitamin D level increased in every patient in all four groups, however rapidly and significantly increased in patients receiving vitamin D3 supplement at doses of 4000 IU daily and 50K IU weekly. Blood vitamin D levels did not significantly increased in the two patient groups that receiving 400 IU daily or 1000 IU weekly. No direct correlation was observed between PASI score improvement and vitamin D treatment. In addition, we paired a dark-complexion group with a fair-complexion group according to baseline 25(OH)D levels and found no differences in 25(OH)D increase after treatment. CONCLUSION: Vitamin D3 supplemental at doses 4000 IU daily and at 50K IU weekly are efficient ways to raise deficient serum 25(OH) vitamin D levels to a preferred target levels in psoriasis patients. Our future studies will include health benefits of vitamin D3 oral supplements on metabolic syndrome comorbidities associated with psoriasis in a large number of patients.

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A long-term study of safety and allergic comorbidity development in a randomized trial of pimecrolimus cream in infants with atopic dermatitis

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Little is known about the safety and course of early initiation of treatment for atopic dermatitis (AD) in infancy (ie, ages 3–18 mos and within 3 mos of AD diagnosis). We assessed effects of pimecrolimus 1% cream (Pim) in 1087 infants with AD in a 36-month, randomized, double-blind (DB) study followed by an open-label (OL) extension. We used a stepwise treatment approach from emollients for xerosis (Step 1) to study drug for inflammation (Pim vs Control [C] BID, Step 2) to rescue topical corticosteroid (TCS, fluticasone 0.05% cream, Step 3a) to high-potency TCS and/or oral medication (Steps 3b/4) after 3 days of non-response at each step. At baseline, mean (± standard deviation) age was 7.3 (±3.9) mos; 62% male; 69% Caucasian; 92% had mild or moderate AD. 469 subjects entered the OL extension, during which all patients received Pim. Common (≥ 5%) adverse events (AEs) occurred in similar percentages of patients at the end of the DB phase (89% Pim vs 86% C) and OL (89% Pim vs 87% C). The system organ classes most often affected were similar between the groups. Serious AEs were relatively similar between treatment groups (8.1% Pim vs 7.4% C). No statistically significant differences were noted in incidence of asthma (12.2% Pim vs 9.2% C; p=0.07), food allergy (17.3% vs 14.5%; p=0.07), allergic rhinitis (23.6% vs 21.2%; p=0.29), or allergic conjunctivitis (15.0% vs 13.2%; p=0.44). This study confirms the safety of early initiation of topical pimecrolimus 1% cream and steroid rescue in infants with AD. Early initiation of mid-potency TCS may have obscured differences in reducing allergic comorbidities.

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Epidermal growth factor receptor inhibitors do not suppress the expression of the antimicrobial peptide human β-defensin 3 in the skin of treated patients

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Epidermal Growth Factor Receptor Inhibitors(EGFRI) are a new class of targeted chemotherapeutic agents, which disrupts a critical pathway promoting cellular proliferation, migration, and survival. These medications have proven efficacy in the treatment of numerous advanced malignancies. Unfortunately, significant skin toxicity develops in up to 80% of treated patients. The etiology of EGFRI-induced eruptions remains unclear, but many patients become superinfected with *Staphylococcus aureus* or other bacterial pathogens. One of the multitude of genes induced by EGFR activation is the antimicrobial peptide, human β-defensin 3(hBD3), a potent bactericidal and immunomodulatory gene product that represents a critical component of both the skin's defenses against infection and also normal cutaneous immunologic function. We postulated that the observed susceptibility to infection and exuberant cutaneous inflammation conferred by EGFRI may be secondary, in part, to reduced epidermal expression of hBD3. We first demonstrated that EGFRI markedly reduce hBD3 induction by the EGFR ligand TGF-α in primary human keratinocytes *in vitro*. To investigate whether a similar abrogation of hBD3 expression were demonstrable *in vivo*, we recruited 10 subjects who were preparing to initiate EGFRI therapy with either erlotinib(n=6) or cetuximab(n=4). We obtained whole skin specimens at baseline (pre-EGFRI) and again 2-4 weeks into therapy. We analyzed hBD3 mRNA levels by quantitative real-time PCR and protein levels using immunohistochemistry in formalin-fixed sections. In the six patients who completed the protocol, cutaneous hBD3 expression on EGFRI therapy was not significantly different from pre-treatment levels in site-matched tissues. This finding fails to support the proposed role of antimicrobial peptides in the evolution of EGFRI skin toxicity. The surprising persistence of hBD3 expression despite EGFR blockade suggests that other as yet undefined EGFR independent pathways are central to the regulation of hBD3 in human skin.

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Identification of risk factors in the development of central centrifugal cicatricial alopecia in African American women

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Purpose: Central Centrifugal Cicatricial Alopecia (CCCA) is the most common type of scarring alopecia, primarily affecting black women. The purpose of this study is to investigate if family history of hair loss, hair grooming practices, comorbid medical conditions including infection, hormonal imbalance and autoimmune diseases are potential risk factors in the development of CCCA. Method: A questionnaire about medical and environmental risk factors standardized by the North American Hair Research Society (NAHRS) for evaluation of CCCA was administered to 326 African American women. This was followed by a scalp exam using NAHRS CCCA hair loss scale to grade hair loss. Answers to the questionnaire were reviewed to determine if there is a relationship between CCCA and these various risk factors. Results: Of the 326 responders, 92% relaxed their hair by chemical means. Twenty-eight percent (28%) of the total responders received a grade of 2+ using the NAHRS CCCA grading scale, a score consistent with clinically evident CCCA. Ninety-four percent (94%) of those with clinically evident CCCA (grade 2+) had history of chemical relaxer use. Advanced CCCA (grade 3-5) was seen in 59% of these respondents. There was also an increased rate of adult acne, pregnancy difficulty, bacterial and fungal skin infections in those with advanced CCCA (grade 3+). Diabetes mellitus type II was also significantly higher in those with severe CCCA. Conclusions: This study suggests that inflammation in the form of bacterial infection and acne may also be contributing to the development of CCCA, a finding consistent with the histopathology of this disease which shows a lymphocytic perifollicular infiltrate in early disease. The overrepresentation of diabetes mellitus type II in those with advanced CCCA suggests a possible autoimmune etiology which could lead to a reduced ability to fight infections thus creating a milieu of increased inflammation. The increase in adult acne and pregnancy difficulties in those with advanced CCCA, suggests that a hormonal risk factor could play a role.

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Perioral wrinkles are associated with female gender, aging, and smoking.

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Clinical impression suggests that perioral wrinkling is more pronounced in the female than male population. We investigated whether differences exist in perioral wrinkling between genders and factors associated with these rhytides. 143 subjects (71M, 72F; age range: 21 to 91) were enrolled and standardized photographs were obtained. Standards were selected from each gender group to create a 9-point photonumeric severity scale [0=none, 8=most severe] for perioral wrinkling. Three evaluators independently used the scale to grade the photographs and average of the three ratings served as the perioral wrinkling score. Each subject also completed a survey exploring factors associated with perioral rhytides. Severity of perioral wrinkling was significantly greater for women than men when all study participants were compared (mean: 3.3=F vs. 1.9=M; p=.0002). This difference was not significant between the genders under the age of 45. But over the age of 45, perioral wrinkling was statistically significantly worse for women than men (mean: 4.8=F vs. 2.9=M; p<.001). In addition, perioral wrinkling was significantly worse in smokers [S] than nonsmokers [NS] (mean: 3.4=S vs. 2.1=NS; p=.0005). When stratified by gender, perioral wrinkling for females was significantly worse in smokers than in nonsmokers (mean: 4.4=S vs. 2.5=NS; p=.002). This difference did not exist in males. In females, there were no significant correlations between perioral wrinkling and oral contraceptive use, number of children birthed, and hormone replacement therapy. In both genders, sun exposure history and NSAID use were not correlated with perioral rhytides. Consistent with our findings, a modified multiple linear regression model that predicts the degree of perioral wrinkling selected gender, age, and smoking as the most important variables. Our data support the observation that perioral wrinkling is more prominent in females than males, and that aside from age, smoking is also an independent factor that contributes to this process.

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Modulation of pruritus by visual stimuli. Atopic eczema patients display intensified itch perception and scratching in comparison to healthy controls when exposed to visual imagery of itch.

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"Contagious" itch is a common occurrence in daily life when we see other people itch and scratch. However, the mechanism of this interesting phenomenon is poorly understood. In this study we aimed to investigate whether visual images of itch can intensify the perception of itch induced experimentally, both in healthy volunteers and in chronic itch sufferers with atopic dermatitis, and whether there are any significant differences between these groups. Subjects received either histamine or a neutral saline control delivered by iontophoresis on the forearm. Subsequently, they were asked to watch short video clips of people itching & scratching, while continuously rating their own itch intensity, with the help of a computer-assisted visual analog interface (COVAS). As a control, subjects were also asked to rate their itch while watching neutral video content. While undergoing the study participants were videotaped and spontaneous scratching episodes were analyzed for counts, duration of bouts of itch and their location. Atopic dermatitis patients displayed a significant response to visual imagery of scratching. The ratings of the perception of their own itch induced by histamine and the number of scratching episodes while watching itch videos was higher in comparison with the rating of itch reported by healthy volunteers in the same settings. Moreover, itch intensity, the extension of the skin areas scratched and the number of scratching episodes increased significantly when saline was delivered to their skin while watching itch videos, whereas in healthy subjects no effect was noted. In conclusion, atopic eczema patients display an increased susceptibility to be influenced by visual cues showing people itching & scratching. These results suggest there is a central activation involved in "contagious" itching that is probably associated with the "mirror neuron" system.

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Patient-specific dendritic cell immunization against tumor in Sézary syndrome

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We evaluated CTCL–specific tumor immunity resulting from DC1-based immunization using tumor cells as a source of antigen in a phase I/II clinical trial. Patients with established diagnosis of Sézary syndrome, leukemic blood involvement (B2) and progressive disease were enrolled in to Phase I/II clinical trial. Eleven patients were screened, 7 enrolled. DC vaccine was generated by co-incubation of immature autologous monocyte-derived DCs with the autologous malignant lymphocytes, matured using Th1-polarizing cocktail, and evaluated for expression of costimulatory molecules, IL-12 production and Th1/Th2 skewing *in vitro*. Patients were immunized using high resolution ultrasound-guided intra nodal injections. A single course of therapy consisted of a total of 4 injections administered once a week for 4 weeks. Primary endpoint was the immune responses to the vaccine. DCs in the vaccine demonstrated high expression of maturation markers and costimulatory molecules, produced high levels of IL-12, and induced Th1-skewing in *in vitro* assays. Therapy was well tolerated; there were no unanticipated adverse events. Two patients (2/7) developed DTH to the vaccine. One patient had PR (76% decrease in mSWAT). Three patients had SD and a decrease in CD4:CD8 ratio in the blood. Three patients had disease progression. The group of clinical responders was characterized by younger age (63.0±3.7 vs. 79.8±5.1, p< 0.05), had fewer Sézary cells in the peripheral blood before the vaccination (1140±311 cells/μl vs. 17957±10659, p< 0.05), and had received prior immunotherapy. We show that DCs from Sezary syndrome patients can be obtained in sufficient numbers from monocyte precursors, loaded with multiple tumor antigens by co-incubation with autologous malignant lymphocytes and then matured by DC1-polarizing cytokine cocktail to induce Th1-skewed responses *in vitro* and *in vivo*. The immunotherapy was not associated with significant adverse events and was well tolerated; clinical responses and improvement in quality of life were observed.

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The relationship of PAR2 and pruritus in end stage renal disease patients and the clinical effectiveness of soybean extracts containing moisturizer on epidermal permeability barrier in end stage renal disease patients

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Pruritus is a common problem in continuous ambulatory peritoneal dialysis (CAPD) and haemodialysis patients. The underlying mechanisms are not yet fully understood. Many therapies have been proposed for patients with uremic pruritus. They improved pruritus in some patients, but either had no effect or aggravated symptoms in others. Previous study showed that SC integrity was impaired in dialysis patients and the tendency of increasing the skin surface pH. The close relationships between skin sureface pH, serine protease and sc integrity induce the new treatment for abnormal SC conditions in ESRD patients. We examined that the increased activity of serine proteased and the increased expression of PAR2 in the skin of ESRD patients were closely related to the severe pruritus. We also want to know that serine protease inhibitor therapy improves the pruritus and abnormal barrier function in ESRD patients We evaluated the basal epidermal barrier status of ESRD patients. After 4 weeks soybean extracts containing moisturizer application. we compared the epidermal barreier status, the protease activity and PAR2 expression levels. Severity of pruritus was evaluated by using a traditional visual analogue scale (VAS). The treatment of soybean extracts is effective for improving barrier status, such as TEWL and skin pH. The increased serine protease activity are reduced. We suggested that the soybean extracts containing moisturizer is a new candidate for the treatment of uremic pruritus.

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A comparative fMRI study of brain activation patterns induced by cowhage and histamine itch in atopic eczema patients vs. healthy subjects.

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PET and fMRI brain imaging studies looking into neural networks underlying itch sensation in health and disease have previously used histamine as the sole itch inducer. Cowhage itch is mediated via protease-activated receptors (PAR) subtype PAR-2 and PAR-4 - recently linked to the pathological itch of atopic eczema - and it is transmitted via a separate spinothalamic pathway. Therefore, employing itch stimuli that mimic chronic itch may enable us to understand the central neural pathways of chronic itch. We recently reported that cowhage induced itch presents common features, but also notable differences in brain activation in comparison to histamine induced itch in healthy volunteers. In this report we also expand on our previous findings describing a different processing of histamine induced itch in atopic patients vs. healthy participants. We investigated by functional MRI Arterial Spin Labeling (ASL) the neuronal processing of itch induced by cowhage and histamine in atopic dermatitis patients and healthy volunteers. We have also correlated itch intensity of each stimulus to the maps of brain activation. Results of ASL fMRI analysis suggested that cowhage and histamine in single application reveal interesting differences in brain activation between atopic subjects and healthy participants that could shed light onto the processing of itch in pathological states.

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Long-term outcomes in a cohort of 1263 Mycosis Fungoides (MF) and Sézary Syndrome (SS) patients

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MF and the leukemic variant SS, while rare, are the most common cutaneous T-cell lymphomas (CTCLs) and the subject of active new drug development over the past decade. This retrospective cohort study was conducted to determine long term outcomes of 1263 MF/SS patients (pts) evaluated at our cancer center from 1982-2009. Kaplan Meier curves were used to estimate median overall survival (OS), disease specific survival (DSS), and progression-free survival (PFS). Cox's proportional hazards regression model was then used to test the statistical significance of prognostic factors for survival. Among 1263 biopsy proven MF/SS patients: 890 were in early stages [1A = 522; IB/IIA =368] and 365 were in advanced stages (IIB-III =200; IV =165) at baseline. Patients with stage IA had better OS and PFS than IB (p= .03 and p=.001). Median OS and PFS were 26.26 yrs and have not yet been reached for DSS or IA pts: In IA OS, DSS, PFS were not reported (n/r). In IB: OS 26.26, DSS n/r, PFS 24; IIA: OS 16.91, DSS & PFS were n/r. IIB: OS 9.69, DSS and PFS 14.92; III: OS 4.21, DSS & PFS 18.14; IV: OS 5.31, DSS 9.74, PFS 9.74. For 1255 pts analyzed for OS, 268 died, 987 were alive at last follow-up, 8 were missing mortality data, and 96 (7.6%) had ongoing complete remission. By PFS analysis, 145 (11.5%) progressed (19 stage I-IIA and 126 stage > IIB) or died (58 pts) due to MF/SS by study end. Advanced age (HR 1.03 [CI 1.02-1.04], p<0001), advanced stage at diagnosis (stage II – HR1.97 [CI 1.38-2.79], p<.0002) or (stage IV - HR 2.06, [CI 1.41-3.0], p<.0001) and black race (HR 1.88 [CI 1.29-2.72] p=.0009) were associated with worse PFS by multivariate analysis. In comparison, the published Stanford cohort (n=525) from 2003 had median OS of 11.4 yrs with 67% early pts compared to 26.26 yrs with 70.9% early pts at our site. These data suggest that OS may be improving for MF/SS patients with the availability of new therapies and specialized treatment centers.

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ABT-737 synergizes with bortezomib to kill melanoma cells by neutralizing multiple anti-apoptotic defenses

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The expression of Bcl-2 family proteins play an important role in modulating sensitivity to anti-cancer drugs in melanomas. The BH3 mimetic, ABT-737, is a potent small molecule inhibitor of the anti-apoptotic proteins Bcl-2, Bcl-XL and Bcl-w. In this report, we examined whether ABT-737 is effective in killing human melanoma cells when combined with bortezomib (a proteasome inhibitor) *in vitro* and *in vivo*. We further evaluated the mechanisms of apoptotic action. Apoptosis assays showed that siRNA-mediated inhibition of the anti-apoptotic protein Mcl-1 (not Bcl-2 and Bcl-XL) sensitized melanoma cells to ABT-737, indicating that Mcl-1 is the main mediator of melanoma cell resistance to ABT-737 treatment. In addition, viability and apoptosis assays showed that ABT-737 displayed strong synergistic lethality when combined with bortezomib *in vitro*. Our preliminary *in vivo* studies suggested that the combination of ABT-737 with bortezomib inhibited melanoma tumor growth more than either drug alone in nude mice with a standard xenograft of human melanoma cells. Furthermore, Western blot analysis demonstrated that bortezomib increased expression of Noxa (a pro-apoptotic Bcl-2 member that antagonizes Mcl-1's function). Apoptosis analysis showed that inhibition of Noxa expression (by siRNAs) protected melanoma cells from cytotoxicity induced by this combination treatment. These results indicate that bortezomib neutralizes Mcl-1 function through the up-regulation of Noxa, and sensitizes melanoma cells to ABT-737. In summary, this study indicates the promising therapeutic potential of ABT-737 in treating melanomas, and it validates rational molecular approaches to targeting multiple anti-apoptotic Bcl-2 members in developing cancer treatments.

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Documentation of skin responses in rhytidectomy with spectral imaging

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Rhytidectomy is a cosmetic surgery procedure designed to reposition the facial skin in an attempt to rejuvenate the aging face. Hematoma (ecchymosis) is one of the most common complications after rhytidectomy. The immediate tissue response to traumatic injury involves an acute inflammatory reaction initiated by trauma. Although skin reactions may be evaluated by the clinician an objective method to quantify the changes in skin chromophores, such as hemoglobin, could improves. A sensitive method to assess skin is Diffuse Reflectance Spectroscopy. We developed a MultiSpectral Clininical Imaging System (MSCIS) which takes 6 spectral images at 556, 582, 650, 680, 820 and 970 nm plus visible and cross-polarized images. The apparent concentration of oxy-hemoglobin, deoxy-hemoglobin, melanin, scattering, water and bilirubin from the cross-polarized images is calculated from each set of images. The MSCIS was calibrated using *in vivo* skin response. Oxy-hemoglobin maps were calibrated by induction of erythema in skin with a solar simulator. Deoxy-hemoglobin maps with induction of blood stasis (pressure cuff). Melanin maps with pigmentation produced after solar simulator exposure. Water maps and bilirubin were calibrated *in vitro*. For water maps, PVA hydrogels were prepared at different water concentrations and for bilirubin, chloroform solutions of bilirubin were prepared. A study was performed in order to assess the capability of the MSCIS to quantify skin trauma after rhytidectomy. Five subjects were elected to undergo to rhytidectomy. Pre- and postoperative images were taken using a custom multispectral imaging system. Skin chromophore maps and bilirubin were calculated for each of the patient's visit. Patients were imaged prior to the surgery and at days 1, 3, 6, 8, 10, 15, 22 and 29. The spectral imaging (MSCIS) provides accurate and reproducible documentation of clinically relevant skin parameters such as ecchymosis, erythema, edema and purpura.

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Dermatoproteome arrays – a novel immunoassay method for stratification of systemic and cutaneous lupus erythematosus phenotypes

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Autoantigen arrays can generate comprehensive autoantibody profiles of patients with autoimmune diseases. We have previously shown their abilities to classify systemic lupus erythematosus (SLE) patients based on their disease severity and distinguish SLE patients, incomplete lupus erythematosus patients, first degree relatives, and normal controls. To explore the possibility of stratifying SLE and cutaneous lupus (CLE) patients, we constructed "dermatoproteome" arrays containing 102 purified autoantigens involved in various autoimmune systemic and cutaneous diseases to measure multiple autoantibodies simultaneously in the sera of 17 CLE patients without SLE (13 discoid lupus erythematosus (DLE), 4 subacute lupus erythematosus (SCLE)), 12 CLE patients with SLE (10 DLE, 2 SCLE), 11 SLE patients without CLE, and 14 normal healthy controls. SLE patients without CLE had the highest IgG levels of 11 autoantibodies compared to every other group ($p<0.05$), with nine against nuclear antigens such as dsRNA, dsDNA, histones, ssDNA, and Ro antigens. CLE without SLE patients and human controls had the lowest levels of these autoantibodies; CLE with SLE patients had intermediate values compared to SLE without CLE patients. The stepwise up-regulation of multiple autoantibodies in CLE without SLE, CLE with SLE, and SLE without CLE patients lends credence to previous observations suggesting that DLE and SCLE patients tend to have milder systemic disease. A possible explanation for these findings is that the skin serves as a repository for autoantibody deposition in these patients, thus decreasing the number of circulating autoantibodies that could inflict peripheral organ damage. These "dermatoproteome" arrays can potentially serve as useful tools in distinguishing lupus erythematosus subtypes, aiding in diagnosis of SLE and CLE, and understanding disease pathogenesis.

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Engineering of murine Basal Cell Carcinoma (BCC) allograft as hedgehog (HH) inhibitor screening platform

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There is increasing evidence demonstrating that Hedgehog (HH) pathway is involved in various cancer types, which makes small molecule inhibitors against HH activity have become one of the hottest targets in cancer drug development. As a useful mouse model, Ptch 1+/- mice that develop BCCs after UV or ionizing radiation, provide an excellent system for investigating the HH regulatory system, studying the effect of HH in cancer biology, and evaluating tumor intervention therapies. However, the intrinsic limitations to the use of autochthonous mouse tumors to investigate therapeutic interventions, including inter-tumor variability and long tumor latency, necessitates the enrolling of numerous mice to assess the efficacy of any intervention. As one approach to circumventing these problems, in the present study we aimed to develop BCC tumor allografts, which have not previously been established successfully, and to compare their histology, growth rate, vasculature, and their sensitivity to drug treatment with that of their parental tumors. We inoculated single cell suspensions derived from primary murine BCCs into multiple NOD/SCID mice and characterized the allografts generated in terms of histology (H&E) and immunophenotype (Ki67, Gli1, CD31 and K14) comparing with that of the parental tumors. As expected, the allografts grew much faster and consistently than the spontaneous tumors. Moreover, we found that allografts could reproduce the histology of the parental tumor, exhibiting either an infiltrative subtype or a nested structure. Consistent with their faster growth rate, allograft tumors showed greater Ki67 staining than did primary tumors. Additionally, HH activity was slightly reduced in allografts and extensive vascular distribution was observed in both tumor models. Finally, we tested the tumor allograft sensitivity to HH inhibitor treatments. We conclude that our murine BCC allografts could be useful in pre-screening of HH inhibitors.

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Reducing the pain of lidocaine administration by controlling angle of injection

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Local anesthetic injection into the skin is common practice for biopsies and small surgeries. The injection procedure itself inflicts some discomfort to the patient. Previous studies have addressed efforts to reduce the pain by controlling temperature, pH, depth of injection, and injection rate. Anecdotal reports suggest that physicians inject at an angle of 90 degrees to reduce interaction with multiple nerve endings. The purpose of this study is to examine if the angle of needle insertion during local anesthetic procedures influences the degree of pain felt by patients. Thirty subjects were enrolled at University Hospitals Case Medical Center Skin Study Center. Each subject received two injections, one at 90 degrees and one at 45 degrees in each arm at a constant rate, volume, temperature, and pH. Subjects reported pain score on a 10-point "pain faces" scale. A GEE model was constructed to model the mean pain score. Robust variance-covariance estimates were used. The mean pain score at 45 and 90 degrees and the difference in the mean pain score between 90 and 45 degrees, given that the 45 degree angle of insertion was done first, was estimated. This was done when the 90 degree angle of insertion was done first. The results demonstrate that when the angle of insertion was done at 90 degrees first, the mean pain score was higher at 45 degrees than the mean pain score at 90 degrees ($p=0.005$). On the other hand, when the angle of insertion was done at 45 degrees first, the mean pain score at 45 degrees was similar to the pain score at 90 degrees ($p=0.68$). Overall, an insertion angle of 90 degrees trends toward less pain than that at 45 degrees ($p=0.10$). There may be a clinical benefit to using a 90-degree angle of injection to minimize discomfort to patients, and the angle of insertion used for the injection should remain consistent for patients undergoing multiple injections.

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Stem cell transplantation rare genetic disease consortium for allogeneic transplantation in RDEB

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The Stem Cell Transplantation Rare Genetic Disease Consortium (SCTGDC) is comprised of a multidisciplinary team of investigators across five medical centers and focuses on a highly innovative approach to the treatment of rare genetic skin diseases by exploiting the plasticity of circulating stem cells. Stem cell transplantation has been curative for a number of rare pediatric nonmalignant disorders, but has been limited by the toxicity of the myeloablative conditioning and lack of allogeneic donors. The collection of cord blood and successful use of unrelated cord blood donor grafts was developed to circumvent the lack of alternative allogeneic donors. More recently, the relative safety and success of reduced intensity conditioning prior to AlloSCT in pediatric recipients has been demonstrated. The SCTGDC has embarked upon a multicenter clinical trial using reduced intensity conditioning and AlloSCT in patients with Recessive Dystrophic Epidermolysis Bullosa (RDEB). To date, we have enrolled two RDEB patients, each of whom have had characterization of COL7A1 mutations at the molecular level, and will be followed with detailed analysis of COL7A1 mRNA, protein and appearance of anchoring fibrils in the skin. This investigation will test the feasibility of a new potential curative therapy for RDEB.

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Computer-assisted alignment and tracking of comedones in patients with predominantly comedonal acne indicate that most resolve within 4 weeks and do not become inflammatory lesions

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Few studies have described the life cycle of acne lesions, the primary difficulty being the accurate spatial tracking of lesions over time. The purpose of this study was to describe the natural history of comedones utilizing standardized digital photographs along with alignment software. Twelve subjects with comedonal acne (mean age=23; 75% women) were enrolled in a split-face acne treatment study, where one side was treated with microdermabrasion and the other side was untreated. A series of standardized digital facial photographs were obtained from the untreated side at weeks 0, 2, 4, 6, and 10. Each subject's series of photographs were aligned to the week 0 picture using Picture Window Pro 4.0 (Digital Light & Color, Belmont, Mass). Two evaluators then analyzed a region of interest (ROI, area= 600 pixels2 or 3 cm2 real skin) from the cheek or temple for 6 predefined lesion types (open/closed comedones, erythematous macules, papules, pustules and nodules). A total of 158 comedones (134 closed, 24 open) were tracked from first appearance until resolution. Only a small proportion of comedones (8% total: 6% closed, 17% open) were found to progress to inflammatory lesions. Most comedones resolved within 4 weeks (85% total: 86% closed, 83% open), while some persisted longer than 6 weeks (15% total: 14% closed, 17% open). Our results confirm that in patients with predominantly comedonal acne and minimal inflammation at baseline (average Leeds score 1), the majority of clinically visible comedones do not progress to inflammatory acne lesions, and they mostly resolve within 4 weeks. The timeline of the evolution of comedones may help clinicians in evaluating response to interventions targeted at new comedo development.

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Atrophic acne scars may arise from both inflammatory and non-inflammatory acne lesions.

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Acne scars are thought to be secondary to inflammatory lesions. The objective of this study was to determine what types of acne lesions preceded the development of atrophic acne scars. Twenty two subjects with mild to moderate acne (avg. Leeds severity score = 3.8) were enrolled in a split-face treatment study, where one side was treated with non-ablative laser and the other side was untreated. A series of standardized digital facial photographs were obtained from the untreated side at every 2 week interval from baseline to week 12. Each subject's photographs series were aligned to the week 0 picture using Picture Window Pro 4.0 program, then a region of interest (ROI) measuring 3.5cmx3.5cm from either the temple or cheek was selected. Three predefined atrophic scar types: ice pick (0.5 < 1.5mm), boxcar (1.5-4mm), and rolling (>4mm) were identified and counted independently by three evaluators. Lesions in ROI with consensus reached by at least 2 evaluators were selected for study. At week 12, 104 scars were identified. Of these, 72 [69%] were ice pick, 30 [29%] boxcar, and 2 [2%] rolling scars. When all the atrophic scars were tracked to week 0, 53 were clinically normal skin, 30 were established scars, and 21 were acne lesions (7 papules, 6 erythematous macules, 4 pustules, and 4 closed comedones). No open comedones at baseline corresponded to atrophic scars. Papules, closed comedones, and erythematous macules at baseline were most associated with ice pick, followed by boxcar, then rolling scars. Interestingly, all pustules present at week 0 corresponded with only boxcar scars at week 12. Our results verify that inflammatory acne lesions often lead to atrophic scarring. This study also suggests that some scarring may arise from initially non-inflammatory lesions, and that 12 weeks are long enough to develop and establish atrophic scars. Taken together, aggressive treatment of inflammatory and non-inflammatory acne is warranted to minimize acne scarring.

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Regulatory T cells are associated with the human cutaneous SCC microenvironment and suppress activation of naïve T cells stimulated by CD3/28

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The incidence of SCC is ~265% greater in solid organ transplant recipients (SORs) than healthy patients and SCC is more aggressive in SORs than healthy patients. We studied Tregs with an interest in comparing their number and function in SCC of immunecompetent patients to SCC in immunosuppressed SORs (TSCC). We also studied the Treg associated gene expression in SCC, TSCC and normal skin. Cell numbers were determined by IHC and triple-label immunofluorescence was performed to evaluate the presence of tumor-associated CD4+/CD25+/FoxP3+ cells. The function of Tregs was evaluated by their ability to suppress stimulation of naïve T cells by CD3/28 beads *in vitro*. Gene set expression analysis (GSEA) was used to evaluate the Treg-associated gene expression patterns in SCC, TSCC and normal skin. We found 1) significantly greater numbers of CD3+ T cells associated with SCC compared to TSCC and both exhibit greater numbers of T cells than normal skin. 2) There are significantly greater numbers of CD8+ T cells associated with SCC compared to TSCC and that both are associated with greater numbers of CD8+ cells than normal skin. 3) There are significantly greater numbers of Tregs associated with both SCC and TSCC compared to normal skin. 4) There is a greater proportion of Tregs to CD8+ cells in TSCC compared to SCC. 5) FACS analysis revealed that SCC is associated with greater numbers of CD4+/CD127low/FoxP3+ Tregs. 6) GSEA revealed an increased level of expression of Treg associated genes in SCC, TSCC, PTNL compared to normal skin. The observation of the increased proportion of FoxP3+ Tregs to CD8+ T cells in TSCC may help explain the increased aggressiveness of these tumors. Taken together these results suggest that Tregs may play an integral role in SCC tumor-associated immune evasion and TSCC-associated tumor aggressiveness.

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T4 endonuclease V liposome (T4N5) treatment ameliorates inflammation-related biomarkers and enhances apoptosis in both sun-exposed skin and AKs in chronically immune suppressed renal transplant patients (RTPs)

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Non-melanoma skin cancer (NMSC) is the most prevalent cancer with more than one million new cases diagnosed each year in the United States. Organ transplant patients are at a higher risk of developing NMSCs as well as premalignant epidermal dysplasia including carcinoma *in situ* (Bowen's disease) and Actinic Keratoses (AK). This study describes a multicenter, placebo controlled study for the use of bacteriophage T4N5, a DNA repair enzyme encapsulated in an engineered liposome delivery vehicle, for the diminution of AKs and other NMSCs in RTPs with a history of NMSC. Additionally, we assessed the effects of T4N5 on the modulation of a panel of potential surrogate biomarkers of NMSCs. These biomarkers were assessed in biopsy samples of non-sun-exposed skin, sun-exposed skin and AKs in both placebo and T4N5 treatment arms. We observed that T4N5 significantly prevented the upregulation of IL-10, TNF- and TGF-α. In addition, a slight increase in the repair of pyrimidine dimers was observed in these tissues. T4N5 treatment also increased significantly the number of apoptotic cells in these lesions ($p = 0.058$). We did not observe significant changes in the proliferating cell nuclear antigen (PCNA) or p53 expressions. In conclusion, T4N5 liposome treatment ameliorates inflammation-related biomarkers and enhances apoptosis in both sun-exposed skin and AKs in chronically immune suppressed RTPs.

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Malignancies in ustekinumab-treated psoriasis patients: Observations with up to 3yrs of follow-up & comparisons to the general US population

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The incidences of basal and squamous cell or nonmelanoma skin cancers(NMSCs) and all other malignancies were evaluated in patients(pts) with moderate-to-severe psoriasis treated in Phase 2&3 trials. For all other malignancies except NMSC,SIRs compared observed malignancy rates in UST pts to rates expected in the US population adjusting for age,sex and race based on data available in the NIH SEER database(2000-2004). 3117pts were treated with UST for 4774 pt-yrs of f/u (P-Y) for up to 3yrs(median f/u of 1.7yrs with 1247pts treated for 2yrs).The incidence of NMSC(per100P-Y)for the UST45mg and UST90mg grps was 0.64(95%CI:0.35,1.08)and 0.77(95%CI:0.47,1.19),resp;34 cases were observed and included 28 basal cell and 9 squamous cell skin cancers(basal to squamous cell ratio,3:1).The incidence(per100P-Y) of NMSC occurrence by yr evaluated for the UST combined grp was 0.94(95%CI:0.61,1.41), 0.44(95%CI:0.18,0.90) and 0.47(95% CI:0.10,1.36) for Yrs 1,2 and 3,resp; the respective rates of other malignancies were 0.39(95%CI:0.19,0.72),1.00(95%CI:0.57,1.63),and 0.16(95%CI:0.00,0.86).The incidence(per 100P-Y) of other malignancies for the UST45mg and UST90mg groups was 0.69(95%CI:0.39,1.13) and 0.46(95%CI:0.24,0.81),resp;27 cases were observed and included (≥2 cases prostate, breast, melanoma, colorectal, renal, head and neck. The rate of these malignancies reported in UST-treated pts was comparable to the rate expected in the general population (SIR =1.05[95% CI:0.69,1.53]). Malignancy rates remained low and stable with no observed dose effect.The observed malignancy rate was consistent with the expected rate in the general US population in the SEER database.Additional analyses with 5yrs of f/u are planned to continue examining the impact of IL-12/23 blockade on malignancy rates.

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Association of prediagnostic serum Vitamin D levels with the development of basal cell carcinoma

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We investigated the association between serum 25-hydroxyvitamin D (25(OH)D) levels and basal cell carcinoma (BCC) risk in a nested case-control study at Kaiser Permanente Northern California (KPNC). 220 case patients with BCC diagnosed after serum collection were matched to 220 control subjects. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using conditional logistic regression. Fully adjusted models included body mass index (BMI), smoking, education, sun-exposure variables, x-ray exposure, and personal history of cancer. For each measure of serum 25(OH)D (continuous, clinically relevant tertiles, quintiles), we found an increased risk of BCC in unadjusted models (OR=1.03, 95% CI 1.00-1.05, $p<0.05$; OR= 3.98, 95% CI: 1.31-12.31, deficient vs. sufficient, test for trend p value <0.01; OR=2.32, 95% CI: 1.20-4.50, 1st vs. 5th quintile, test for trend p value 0.03). In fully adjusted models, the values attenuated slightly (OR=1.02, 95% CI 1.00-1.05, $p<0.05$; OR= 3.61, 95% CI: 1.00-13.10, deficient vs. sufficient, t-trend p=0.03; OR=2.09 1st vs. 5th quintile, 95% CI: 0.95-4.58, t-trend p=0.11). Our findings suggest that higher pre-diagnostic serum 25(OH)D levels may be associated with increased risk of subsequent BCC. Further studies to evaluate the effect of sun exposure on BCC and serum 25(OH)D levels may be warranted.

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Health outcome measures in atopic dermatitis: A systematic review of trends in disease severity and quality-of-life instruments 1999-2009

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A number of disease severity and quality-of-life (QoL) instruments have emerged in atopic dermatitis (AD) in the last decade. However, information regarding their use in clinical trials and summary of instrument validity is lacking. The objectives of the study are to (1) identify trends in outcomes instruments used in AD clinical trials from 1999 to 2009 and (2) summarize the instrument dimensions and validation studies for the most commonly used measures. We performed a systematic review examining randomized control trials (RCTs) in the treatment of AD from 1999 to 2009 using the U.S. National Library of Medicine. Among the 195 RCTs reviewed, we identified 18 disease severity scales and 11 QoL instruments. Overall, we observed a 72% increase in the use of disease severity scales from 1999 to 2009. The four most common disease severity scales were SCORAD, EASI, IGA and SASSAD; they were used in 93% of the RCTs. SCORAD, EASI and SASSAD have been validated for sensitivity to change and reliability, whereas IGA has not been validated to date. From 1999 to 2004, SCORAD, EASI, IGA, and SASSAD were used in 35%, 21%, 16%, and 11% of AD trials, respectively. From 2005 to 2009, the use of SCORAD, EASI, and IGA increased to 44%, 31%, and 26% of RCTs, respectively; however, the use of SASSAD decreased to 5%. Among the 195 RCTs, 35 studies employed QoL instruments, and the overall use of QoL instruments in AD increased 83% from 1999 to 2009. The four most common QoL outcomes measures were the CDLQI, DLQI, IDQOL and DFI, and all four instruments have been validated. From 1999 to 2004, among the trials that used QoL measures, CDLQI, DLQI, IDQOL, DFI were used in 25%, 25%, 13%, and 13% of the studies, respectively. From 2005 to 2009, the use of CDLQI, IDQOL, and DFI increased to 32%, 18%, 14%, respectively, whereas the use of DLQI decreased to 14%. Of the large number of outcomes instruments that emerged during 1999 to 2009, few have been thoroughly validated. However, there is an overall increase in the use of disease severity and QoL instruments in AD clinical trials.

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The safety of tumor necrosis factor antagonists in psoriasis: A systematic review and meta-analysis of randomized controlled trials

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Due to their immunosuppressive properties, the risk of infection and malignancy associated with the use of TNF-α antagonists has been of concern. To examine these risks in the psoriasis population, we performed a systematic review and a meta-analysis of randomized controlled trials of TNF antagonists in patients with plaque psoriasis and psoriatic arthritis (PsA). We included therapies currently approved or under investigation for psoriasis: infliximab, adalimumab, etanercept, golimumab, and certolizumab. Data was abstracted for number of malignancies, serious infections, and overall infections (serious and non-serious). Results were calculated using fixed effects models and reported as pooled odds ratios (OR). Homogeneity testing was performed using the chi-square and I-squared tests. A systematic literature search yielded a total of 21 studies that met the study's inclusion criteria, with 4,599 patients in plaque psoriasis and 2,222 patients in PsA studies. The pooled OR for overall infection over an average of 16.8 weeks was 1.18 (95% CI: 1.04, 1.33), and 0.71 (95% CI: 0.41, 1.24) for serious infection. The pooled OR for risk of malignancy was 1.92 (95% CI: 0.93, 3.95); of note, 72% of reported malignancies were non-melanoma skin cancer (NMSC). The pooled OR for NMSC was 1.71 (95% CI: 0.77, 3.77), and 1.32 (95% CI: 0.44, 3.96) for malignancies excluding NMSC. Our study demonstrates that there is a small increased risk of overall infection with the short-term use of TNF-alpha antagonists for psoriasis. There was no evidence of an increased risk of serious infection and a statistically significant increased risk in cancer was not observed. However, larger, long-term studies will be necessary to assess the risk of cancer and serious infection associated with chronic use of TNF inhibitors in the psoriasis population.

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Incidence, survival, and geographical distribution of classic and AIDS-related Kaposi's Sarcoma from 1975-2005

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The incidence of Kaposi's sarcoma (KS) in the US population has significantly increased since the onset of the AIDS epidemic in the early 1980s. During the mid-1990s, the introduction of HAART and HIV-related public health initiatives have led to a marked change in the HIV/AIDS epidemic. The purpose of this study is to examine trends in the incidence, survival, and geographical distribution of classic and AIDS-related KS in the U.S. from 1975-2005. The study population consisted of 12,006 patients diagnosed with KS between 1975-2005 in nine geographic areas of the SEER registry. In accordance with classification methods from prior studies, we classified affected individuals over 70 years at time of diagnosis from 1980-2005 and all KS cases from 1975-1979 (pre-AIDS era) as classic KS cases. Those patients under 70 years of age at time of diagnosis from 1980-2005 were considered to have AIDS-related KS. The incidence of AIDS-related KS is significantly higher than that of classic KS from 1975-2005 (p-value = 0.01). During the mid-1990s, the incidence for AIDS-related KS declined across all nine registries from 4.6 in 1990 to 0.3 by 1998 (p-value = 0.05), while the incidence of classic KS remained at a steady rate. Over the course of the HIV epidemic, the 5-year survival rate for AIDS-related KS improved from 12.1% between 1980-1995 to 54% between 1996-2005 (p-value = 0.05), while survival rates for classic KS remained stable throughout. In both forms, incidences were highest in the metropolitan areas. Specifically, changes in AIDS-related KS incidence were most evident in San Francisco, where rates rose from 0.2 in 1980 to 17.2 in 1990 and then significantly declined to 1.6 by 1998. In contrast, classic KS incidence in San Francisco remained consistent, with rates of 2.0 in 1980, 2.6 in 1990, and 2.3 in 1998. The incidence of AIDS-related KS increased sharply in the early 1990s and declined during the mid-1990s. Notably, the incidence and survival rates for classic KS have remained stable and not significantly impacted by the AIDS epidemic.

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Independent and significant association between severity of hypertension and psoriasis

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The purpose of the study is to elucidate the relationship between the presence of psoriasis and severity of hypertension. In this case-control study, we analyzed longitudinal medical records of 3096 adult patients from the University of California Davis Health System from January 1, 2004 to July 5, 2009. During this period, a total of 774 patients were diagnosed with both psoriasis and hypertension, and these patients were defined as "cases" in this study. In a 1:3 frequency-match, 2322 hypertensive patients without psoriasis were defined as "controls", and these control subjects were matched on age, sex, and BMI to the cases. The severity of hypertension was defined based on the number and classes of antihypertensive agents, in accordance with the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7). Our analysis showed that the severity of hypertension is significantly associated with the presence of psoriasis ($p<0.0001$), and this relationship remains significant after adjusting for diabetes, hyperlipidemia, and race ($p<0.0001$) in a multivariable conditional logistic regression model. Compared to patients on lifestyle modifications alone for control of hypertension, patients on monotherapy antihypertensive regimen were 4.4 times more likely to have psoriasis (95% CI 2.97-6.67), those on dual antihypertensive therapy were 8.1 times more likely to have psoriasis (95% CI 5.27-12.59), those on triple antihypertensive regimen were 16.0 times more likely to have psoriasis (95% CI 9.48–26.94), and those on quadruple therapy or centrally-acting agent were 11.8 times more likely to have psoriasis (95% CI 5.45-25.44), after adjusting for diabetes, hyperlipidemia, and race. The results from this study suggest that there is a significant correlation between the severity of hypertension and presence of psoriasis that is independent of other known cardiovascular risk factors.

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The Africa Teledermatology Project: Clinicopathologic correlation of skin specimens

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The Africa Teledermatology Project employs a multinational teledermatology network to improve the provision of care for skin disease. Access to dermatology and dermatopathology services is scarce in sub-Saharan Africa, and biopsy specimens are performed for cases where the diagnosis rendered makes important clinical distinctions for diagnosis and treatment. This retrospective case review seeks to characterize the conditions diagnosed through histopathologic examination of skin biopsy specimens submitted to the Africa Teledermatology Project over the three-year period since its establishment. It further evaluates the concordance of clinical and pathologic diagnosis, and determines the possible clinical impact of such information on the ultimate course of patient care and treatment recommendations. Fifty-five biopsy specimens met inclusion criteria and represent cases of malignancy (35%), infection (7%), suspected infection (15%), lichenoid tissue reaction (5%), dermatitis (15%), and other various conditions (18%). Three biopsy specimens were non-diagnostic (5%). Clinicopathologic concordance occurred in 32 of 55 cases (58%). The clinical and pathologic diagnoses differed in 21 of 55 cases (38%). Kaposi sarcoma (KS) represents the clinical diagnosis most often suspected in the evaluated biopsy specimens (42%), and was correctly recognized in 13 of 23 cases (57%). Microscopic examination of skin biopsy specimens is important for accurate diagnosis of disease and determination of appropriate treatment strategies.

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Effect of tetracycline antibiotics for acne on the development of inflammatory bowel disease

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Our purpose was to determine if there is an association between tetracycline therapy for acne and inflammatory bowel disease. We designed a retrospective cohort study using the THIN database of the UK. We identified 94,487 individuals with acne who were followed by a GP for 546,151 person-years. At least one-month prescription for minocycline was received by 24,085 individuals, for tetracycline/oxytetracycline by 38,603 individuals, and doxycycline by 15,032 individuals. IBD, either Crohn's disease or ulcerative colitis, was noted in 41 (0.17%) individuals exposed to minocycline, 79 (0.20%) individuals exposed to tetracycline/oxytetracycline, 33 (0.22%) individuals exposed to doxycycline, and 59 (0.12%) individuals not exposed to any of these antibiotics. The hazard ratio (HR) for developing IBD for any exposure to a tetracycline antibiotic was 1.26 (0.93, 1.71). HR's for individual antibiotics were 1.00 (0.66,1.51) for minocycline, 1.34 (0.95, 1.84) for tetracycline/oxytetracycline, and 1.53 (1.02, 2.39) for doxycycline. For UC the associations (HR) were for minocycline 1.02 (0.71, 1.47), tetracycline/oxytetracycline 0.97 (0.69, 1.36) and doxycycline 1.56 (1.08, 2.27). For Crohn's disease the associations (HR) were minocycline 0.80 (0.80, 1.70), for tetracycline/oxytetracycline 1.03 (0.65, 1.64), and for doxycycline 2.03 (1.36 3.01). The results were not significantly changed when adjusted for confounding or when further tested using sensitivity analyses. We concluded that Doxycycline use is associated with the development of inflammatory bowel disease. The true clinical importance of these results need to be investigated by further studies. Furthermore, potential confounding by prior doxycycline exposure should be considered when assessing whether treatment with other acne medications increases the risk of IBD.

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Prevalence of psoriasis severity and predictive value of psoriasis codes in The Health Improvement Network (THIN)– a population based study

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Few studies have analyzed the distribution of psoriasis severity in population-based settings. The purpose of this study was to determine the distribution of psoriasis severity and the validity of electronic codes for identifying psoriasis in THIN, a medical records database representative of 5% of the UK population. A psoriasis cohort was identified in THIN using a previously validated algorithm utilizing 19 diagnostic codes. Patients were included if they were 45-65, had been seen in the last year, and had received a psoriasis code within the last three years. 5,008 patients were eligible of which 4,900 were randomly selected. A questionnaire was sent to their GP to confirm the psoriasis diagnosis, and to describe its severity. Nearly 4,000 surveys have been returned to date, yielding a projected response rate of 97%. The diagnosis of psoriasis was confirmed in 90% (95%CI: 89-91) of patients who had an electronic code for psoriasis. The positive predictive value of 1, 2, or 3+ psoriasis codes was 76% (95%CI: 74-79), 89% (95%CI: 87-92), and 97% (95%CI: 96-98) respectively. Of patients with GP-confirmed psoriasis, 46% (95%CI: 44-47) had the diagnosis corroborated by a dermatologist. The duration of psoriasis was < 2 years in 20% (95%CI: 18-21), 2-9 years in 35% (95%CI: 33-36), and ≥ 10 years in 45% (95%CI: 44-47) of patients. In terms of severity, 52% (95%CI: 50-54) of patients had less than 0-2% of their body surface area (BSA) involved, 36% (95%CI: 34-37) had 3-10% of their BSA involved and 13% (95%CI: 11-14) had greater than 10% involvement according to the GP. Based on query to the GP, 18% (95%CI: 17-19) of psoriasis patients are likely to require systemic treatment. These data suggest that THIN is a valid and useful tool for studying psoriasis and that a significant percentage of psoriasis patients in general practice have objectively severe disease.

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The relationship between neurological disease and bullous pemphigoid- a population-based case-control study

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Previous studies have suggested that neurological disorders may occur more commonly in patients with bullous pemphigoid compared with the general population. Our aim was to assess the risk of developing bullous pemphigoid in patients with prior neurological diseases. Computerised medical records from the Health Improvement Network, a large population-based UK general practice database were used for a case-control study. Cases had a diagnosis of bullous pemphigoid and were matched 4:1 with age, gender and practice matched controls. Conditional logistic regression was used to calculate odds ratios for specified prior neurological disorders compared to the general population adjusted for other comorbidity. Associations with other conditions were examined to assess our findings for specificity. Comparing neurological diseases in cases (n=868) to controls (n=3453), stroke was seen prior to diagnosis of BP in 8% (n=73) of cases vs. 5% (n=171) of controls, dementia in 7% (n=62) and 2% (n=81) respectively, Parkinson's disease in 3% (n=26) vs. 1% (n=36), epilepsy in 2% (n= 19) and 1% (n=44) and multiple sclerosis in 1% (n=8) vs. 0.1% (n=3) respectively. The adjusted odds of developing bullous pemphigoid were increased in people with prior multiple sclerosis (OR 13.6 (3.6 to 51.4)), Parkinson's disease (OR 3.7 (2.2 to 6.1)), epilepsy (OR 2.1 (1.2 to 3.6), stroke (OR 2.1 (1.6 to 2.8)) and dementia (OR 3.7 (2.6 to 5.1)). These estimates were not altered greatly when only diagnoses more than 3 years prior to BP were studied. In contrast to neurological disease we did not find any associations with renal, peptic ulcer or connective tissue disease or cancer. Strong associations were observed between specific neurological diseases and the later development of BP supporting possible causal associations. Mechanisms for disease occurrence based on these findings include exposure of neuronal neoantigens with neurological disease or age-related autoimmunity.

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The role of nutrition in acne pathogenesis: Youtube as a reflection of current popular thought.

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The role of diet in acne pathogenesis remains a topic of controversy both in the medical world and layman's literature. Despite recent and remote studies, there is still no indisputable proof of the role of diet in acne. In a study meant to elicit public opinion, we examine what the lay-public observes and thinks on the pathogenic role of diet in acne, in the hope that this will shed light and offer direction for future study. The popular video website www.YouTube.com was searched, with 87 videos deemed relevant and included in our study. 76 videos, over 85%, suggest at least a moderate correlation between diet and acne, while only 9% suggest no correlation exists. 29% of videos suggest that dairy products aggravate acne; 34% recommend increasing intake of fruits and vegetables; 31% recommend dietary supplements; 21% suggest decreasing consumption of sugars/ sweets and 13% suggest decreasing consumption of oily/ greasy foods. The fact that most dermatologists do not manage acne by modifying diet frustrated many video makers, who stated that the source of the problem is not adequately addressed by clinicians. Until there is clarification and consensus on the relationship between nutrition and acne, an open and curious mind can only help in treating our patients. With better understanding of common perceptions, research direction may be guided to address the lay public's theories and suspicions, and optimize patient care and compliance.

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Gender in examination and counseling of patients in primary care

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Primary care clinicians are well positioned to have a substantial impact on melanoma mortality by detecting these lesions earlier. We sought to evaluate the role of gender in melanoma detection in primary care. We report on 55 primary care physicians (PCPs) and 1434 of their patients from 4 geographically diverse centers who were recruited for a randomized trial. Skin cancer control activities by PCPs were assessed by physician survey, chart review, and patient telephone interview about their recent visit to their PCP. Female PCPs reported more often performing total body skin exam (P=.07) and negotiating a patient goal regarding skin self-examination (P=.005) than male PCPs, and male PCPs were more likely to report that counseling on skin cancer prevention or detection was not a priority (P=.02). Chart data confirmed that female PCPs were more likely than men to perform any skin exam (P<.0001) and to come to agreement with the patient on skin self exam (P=.01). Patients reported that female PCPs were more likely to get them partially or completely undressed (OR=2.2, 95%CI 1.0-4.8, P=.06) and to perform a skin exam (OR=2.4, 95%CI 1.3-4.4, P=.005). Female PCPs were more likely to tell patients to examine skin (OR= 1.9, P= .01) and to tell patients how to do a thorough self-skin exam (TSSE) (OR=2.2, P=.01). Female PCPs were also more likely to ask patients if they usually examine skin (OR=2.0, P=.07) and ask patients regarding their last TSSE (OR=1.7, P=.02). Male patients had greater odds of being undressed by their PCP (OR= 1.8, P=.002), having a skin exam (OR=1.3, P=.07), and being told to examine their skin by their PCP (OR=1.4, P=.02). After accounting for the above, male PCPs were more likely to undress (OR=2.3, P<.0001) and perform a skin exam (OR=1.3, P=.07) on their male (vs female) patients and female PCPs were more likely to tell male patients to examine their skin (OR=1.7, P=.04). We describe, with multiple independent measures, key physician and patient gender differences in early detection practices that may be useful to address for control of melanoma.

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Genetic polymorphisms and gene-environment interactions influence extrinsic skin aging

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Extrinsic skin aging is caused by exposure of human skin to UV and IR radiation, tobacco smoke and airborne particles. However, little is known about the importance of genetic polymorphisms for extrinsic skin aging. In this study, the influence of genetic polymorphisms in genes of collagen metabolism and of melanin synthesis was assessed. We also asked whether genetic-environment interactions exist in genes coding for DNA-repair enzymes, antioxidant enzymes and proteins of the biotransformation. The study cohort included 400 German women aged 70 to 80 years. Skin aging was assessed by SCINEXA, a validated score. UV exposure and smoking habits were assessed by questionnaire. Exposure to particles was determined by distance of residency to the closest major road and by taking soot, particles from traffic and background PM_{10} concentrations into account. The impact of genetic polymorphisms and gene-environment interactions on skin aging was analyzed by linear regression and adjusted for potential confounding variables. We obtained strong evidence that genetic polymorphisms contribute to extrinsic skin aging. Women with the Del/Del genotype of the SNP rs1799750 in the MMP-1 gene had less wrinkles in comparison to women with the Del/G or G/G genotypes. We also identified several SNPs in genes involved in melanin synthesis, which were significantly associated with age spots. In addition, the following gene-environment interactions were observed: women with the T/T genotype of the SNP rs13181 of the DNA reapir gene XPD were better protected against UV-induced age spot formation than women with the G/T or G/G genotype; for smoking and particle-exposure we found gene-environment interactions with genes of the DNA-repair enzymes XPC and ERCC6; and for airborne particle-induced skin aging we found further interactions with genes of the biotransformation such as the CYP1B1 gene. These results show for the first time that genetic polymorphisms and genetic susceptibilities are relevant for extrinsic aging of human skin.

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Nutritional antioxidants and extrinsic skin aging in Japanese and German women

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The clinical manifestations of extrinsic skin aging markedly differ in Asians versus Caucasians. Wrinkle formation starts earlier in Caucasian women whereas Japanese women develop earlier more pigment spots. These differences have first been described two decades ago but the underlying reasons have remained enigmatic. The two ethnic groups differ in their dietary habits. Hence, we investigated whether the level of antioxidants in blood differs between Japanese and German women and whether this could explain the different phenotypes of extrinsic skin aging. In 2007/08 we examined 39 German women in Düsseldorf and 48 Japanese women in Nagoya. The skin aging signs were examined by means of a validated clinical score (SCINEXA). In addition, dietary habits were assessed by questionnaire and in fasting blood samples a panel of carotenoids was measured by HPLC. Associations were statistically analysed by regression procedures. We initially confirmed that wrinkle formation was more pronounced in German than in Japanese women of the same age. Interestingly, Japanese women showed up to three-fold higher levels of carotenoids in blood than German women. This difference was most pronounced for α- and β-carotene. Also, in both populations, a diet high in fruits and vegetables was positively associated with increased carotenoid levels. More than 40% of the ethnic difference in the wrinkle grades under the eyes and on upper lip could be explained by differences in carotenoid levels. The protective effect of carotenoids was also seen for pigment spots, but for the Japanese women this effect occured only in the older women not in the younger ones. Thus, differences in plasma carotenoid levels may explain the fact that Japanese women have fewer wrinkles than German women of the same age. They also imply that nutrition-based antioxidant strategies may be a healthy, feasible and effective way to delay extrinsic skin aging.

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A systematic review investigating whether healthy adults require exposure to ultraviolet radiation in order to maintain adequate vitamin D levels

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Ultraviolet radiation (UVR) is often cited as the most important source of vitamin D in man. However, UVR can cause skin cancer and there are obvious concerns regarding liberal use of natural and/or artificial UVR to prevent hypovitaminosis D. A systematic review was therefore undertaken to establish whether there is evidence in the scientific literature to support the claim that UVR is the main source of vitamin D in man and secondly, whether healthy adults can achieve and maintain adequate levels of vitamin D without regular UVR exposure. Twenty-one literature databases were searched for original studies using synonyms of vitamin D, UVR, diet and supplementation, resulting in the detection of 29,095 articles on this topic. Each of these were screened by two independent reviewers, leading to the identification of 247 interventional and observational articles which reported on the effect of UVR on serum vitamin D levels in healthy adults of different skin types and ethnicities worldwide. Analysis of the published data indicates that certain populations are able to sustain adequate serum levels of vitamin D despite negligible exposure to UVR. Additionally, in some groups that are largely vitamin D deficient, there exist individuals who are able to maintain their vitamin D levels irrespective of the scant UVR exposure that they and their cohort receive. In view of the risk of skin cancer associated with UVR exposure, any recommendation on UVR needs to consider whether acceptable vitamin D levels can be reached by other means. Our findings suggest that it is possible for adequate levels to be maintained without regular UVR exposure, something which should be considered during the development of public health strategies on vitamin D and skin cancer.

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Identification of patients at increased risk of amelanotic melanoma

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In this case-only study, we compared histologically defined amelanotic melanoma (AM) to pigmented melanoma (PM) in relationship to clinical characteristics among 2,917 patients with first or subsequent incident cutaneous melanoma in the international Genes, Environment, and Melanoma study. The subjects were 56.6% male and had a mean age at diagnosis of 58.4 years; the melanomas had a median Breslow thickness of 0.59 mm and 227 (7.7%) were classified as AM. In multivariate analyses controlling for study site, AM was associated with histologic subtype favoring nodular melanoma (p<0.001), increasing Breslow depth (p–trend <0.001), and mitoses (OR 2.06, CI 1.34-3.17; p=0.001), and inversely associated with histologic regression (OR 0.62, CI 0.42-0.93; p=0.02). In a separate multivariate model including demographic and phenotypic characteristics, AM was associated with age > 72 years (OR 1.81, CI 1.29-2.54; p=0.001), fewer than 5 back nevi (OR 1.32, CI 0.96-1.81; p=0.09), many freckles (OR 1.59, CI 1.06-2.36; p=0.02), light colored eyes (OR 1.60, CI 1.03-2.47; p=0.04), and a decreased ability to tan (OR 1.46, CI 1.06-2.00; p=0.02) controlling for study site. Amelanotic melanomas were more likely to have histologic markers indicative of poor prognosis, making early detection critical for improving outcomes. Health care providers with knowledge of the potential predictors of AM may more effectively screen susceptible patients and thus identify AM earlier in its course.

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Risk factors for lymphedema: the Iowa Women's Health Study

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Lymphedema, a late-effect of several cancers and their treatments, poses health risks and impacts quality of life for survivors. Risk factors for lymphedema in breast cancer (BrCa) survivors have not been examined using a large prospective population-based cohort. Methods: The Iowa Women's Health Study (IWHS) collected self-reported data for diagnosed lymphedema in 2004, and data for cancer diagnosis, treatment, behavioral and health characteristics in 1986-2003. We studied 812 women, ages 55-69 at baseline, who developed unilateral BrCa. We used cross-sectional analyses to describe the prevalence of lymphedema and age- and multivariate-adjusted logistic regression to examine risk factors for lymphedema (OR [95% confidence interval]) between two groups: n=104 with lymphedema and n=708 without lymphedema. Results: The mean time between BrCa diagnosis and data collection of lymphedema diagnosis was 8.1±0.2 (mean±SE) years. Positive associations for cancer characteristics with lymphedema were identified for: tumor stage (regional vs in situ: 3.92[1.6-9.5]), number of excised lymph nodes (highest vs lowest quintile: 3.52[1.3-9.3], P_{trend}=0.003), presence of positive nodes (yes vs no: 2.18[1.2-3.9]), and adjuvant chemotherapy (yes vs no: 3.05[1.8-5.3]). Associations were not identified for: extent of surgery (exclusive of excised nodes) or tumor hormone receptor status; a borderline association was identified for a history of tamoxifen use. Positive associations for behavioral and health characteristics with lymphedema were identified for: baseline body mass index (BMI) (highest vs lowest tertile: 2.87[1.6-5.2]) and self-reported general health at baseline (fair/poor vs excellent: 2.70[1.1, 6.5]); associations were not identified for hypertension, heart disease, diabetes, alcohol use, physical activity or demographics. Conclusions: In the IWHS, obesity, worse general health, and markers of more advanced cancer were risk factors for lymphedema in BrCa survivors.

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39-year follow-up of xeroderma pigmentosum: Skin cancer, neurologic degeneration and mortality

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Skin cancer is thought to be the major cause of mortality in xeroderma pigmentosum (XP), a rare recessive disease with sun sensitivity and defective DNA repair. Some XP patients also have neurologic degeneration. To determine the major causes of XP mortality, we conducted a retrospective follow-up study of 106 XP patients seen at NIH from 1971 to 2009, the largest study of its kind. 55% (n=58) of the patients were female. The majority of the patients (72%, n=76) were non-Hispanic Whites, followed by Blacks (15%, n=16), Hispanic Whites (6%, n=6), Asians (4%, n=4), and Native Americans (2%, n=2). The median age at last observation or death was 22 yr (range 1 to 73 yr). XP-C was the most common DNA repair complementation group (43%, n=46) in all ethnic groups. 65% (n=69) had a diagnosis of skin cancer, with non-melanoma skin cancer (NMSC) being much more common than melanoma (93%, n=64) versus (55%, n=38). NMSC were diagnosed in all ethnic groups; however, no melanomas were diagnosed in Black patients. The median age at diagnosis for melanoma [23 yr (range 2 to 47 yr)] was significantly older (p<0.001) than NMSC [9 yr (range 1 to 32 yr)] in the XP patients, a relative age reversal from the general population. Neurologic degeneration was present in 23% (n=26) of the patients; 62% (n=16) of the patients with neurologic degeneration were in group XP-D. 29 patients (27%) died. The median age at death was 32 yr (range 6 to 73 yr), which is a 30 year reduction in survival compared to the general population (p<0.001). The most common causes of death were skin cancer (34%, n=10), neurologic degeneration (31%, n=9), and internal cancer (17%, n=5). Patients with XP-D had the highest frequency of death (30%, n=9). These data indicate that, in addition to cancer, neurologic degeneration is a major cause of XP mortality implicating DNA repair in preventing cancer and neurologic degeneration.

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Utility of relaxing music and guided imagery in reducing intraoperative pain and anxiety during Mohs surgery

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There is evidence that patients who are provided relaxing music and taped instructions during surgical procedures under general anesthesia experience reduced intraoperative bleeding and blood pressure, and reduced postoperative pain and healing time. Since Mohs surgery can be associated with bleeding and pain, and since intraoperative stress may be significant for conscious Mohs patients, this study examines whether pain, anxiety, or blood pressure were reduced when Mohs patients were exposed to intraoperative relaxing music or guided imagery, as versus white noise. Partly-blinded randomized control trial. Each of 3 subgroups of Mohs patients (155 patients total) were exposed, via earphones delivering a pre-recorded message, to either intraoperative: (1) white noise (n=51); (2) relaxing music (n=54); or a (3) guided imagery exercise in which a psychologist shared relaxing thoughts pertaining to the surgery (n=50). Outcomes measures were serial measurements of blood pressure and pulse, a pain rating on a 1-10 scale, and completion of two psychometric measures, Skindex and the State-Trait Anxiety Inventory (STAI). Operating physicians also completed the STAI after each procedure. There was no consistent difference in blood pressure, pulse, pain level, anxiety level, or overall skin well-being among patients in the 3 groups. Physicians did report higher anxiety when operating on patients in the white noise group. Additionally, younger women (<45 years) appeared to obtain more relief from music and imagery, but these differences were not statistically significant, possibly because of the small number in this sample. Relaxing music or guided imagery during Mohs surgery may help occupy the patient, and hence reduce surgeon anxiety. It is possible that patients are themselves more relaxed, but these differences are not easily detected. A targeted study may be helpful in confirming that younger women have benefit more from music or guided imagery.

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ABO blood group and incidence of skin cancer

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Previous studies have examined associations between the ABO blood group and risk of some malignancies. However, no prospective study to date has examined the specific association between ABO blood group and the risk of skin cancer. Using two large cohorts in the US, we prospectively examined ABO blood type and incidence of skin cancer, including melanoma, squamous cell carcinoma (SCC), and basal cell carcinoma (BCC). Study participants reported their blood type in 1996 (61,829 women and 21,954 men), and were followed up on their diagnosis of incident skin cancer (1996-2006). During the follow-up, 303 participants developed melanoma, 942 developed SCC and 9,714 developed BCC. We used Cox proportional hazards models to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs) of each type of skin cancer. Compared to those with blood group O, participants with blood group A had a HR of 0.83 (95%CI=0.71-0.95) for SCC in the two cohorts combined. Overall, we did not observe any strong associaiton between blood type and risk of melanoma or BCC. Additional studies are needed to confirm these associations and to define the mechanisms by which ABO blood type or closely linked genetic variants may influence SCC risk.

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Obesity and the risk of skin cancer

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Previous epidemiological studies have found associations between obesity and the risk of skin cancer. Our objective in the present study was to examine the risk of skin cancer, including melanoma, squamous cell carcinoma (SCC), and basal cell carcinoma (BCC), in the Nurses' Health Study (NHS). Weight and height were assessed by using the validated questionnaire from 1980, and body mass index (BMI) was calculated. Participants were followed up on their diagnosis of incident skin cancer from 1980 to 2006. The study included 480 cases of melanoma, 973 cases of SCC, and 14,445 cases of BCC with 1,778,510 person-years of follow up. Cox proportional hazards models, adjusting for age, skin cancer risk factors, sun exposure at different ages, state of residence, and physical activity, were used to estimate the relative risk and 95% confidence intervals (CI). For BMIs in the range of 25-29.5, 30-34.5, and 35 and over, the multivariate relative risk of melanoma was 1.15 (95% CI 0.93-1.43), 1.05 (95% CI 0.73-1.51), and 0.98 (95% CI 0.56-1.70), respectively, compared to those with a BMI under 25. For BMIs in the range of 25-29.5, 30-34.5, and 35 and over, the multivariate relative risk of SCC was 0.77 (95% CI 0.66-0.91), 0.67 (95% CI 0.50-0.91), and 0.67 (95% CI 0.42-1.07), respectively, compared to those with a BMI under 25. Finally, for BMIs in the range of 25-29.5, 30-34.5, and 35 and over, the multivariate relative risk of BCC was 0.86 (95% CI 0.82-0.89), 0.77 (95% CI 0.72-0.83), and 0.69 (95% CI 0.62-0.78), respectively, compared to those with a BMI under 25. These results suggest that there this no association between obesity and melanoma risk. Being obese is, however, associated with a decreased risk of both SCC and BCC, which may be mediated by less cumulative outdoor activities and hence sun exposure.

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How do sun protective habits (SPH) relate to vitamin D? A cross-sectional study using NHANES

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SPH and their associations with serum 25-hydroxy-vitamin D (25(OH)vitD) concentrations in the US population have not been described in detail. We analyzed National Health and Nutrition Survey (NHANES) data from 2003 to 2006. Survey participants age 20 to 59 were asked questions about SPH (hat, shirt, shade, and sunscreen use), skin reaction to sun exposure, and dietary and supplement vitD intake. Participants also received a physical exam and lab tests to measure serum 25(OH)vitD levels via Diasorin assay. We dichotomized vitD levels for deficiency (<10 ng/ml) and insufficiency (<32 ng/ml). We utilized multivariable logistic regression to examine the relationship between a summary SPH score and vitD deficiency and insufficiency controlling for other factors such as demographics (age, gender, education, income), body mass index (BMI), milk intake, and sun-sensitivity. Results: The summary SPH score did not predict deficiency (OR 0.97, 95%CI: 0.82-1.16). The likelihood against being vitD deficient was with decreasing BMI, male gender, non-Hispanic (NH) white, Mexican American, Other Hispanics and Multiracial (vs. NH black) race, increasing milk use, greater number of sunburns in the past year and a skin type that mildly burns after brief exposure to the sun (vs. no reaction). The summary SPH score did significantly predict insufficiency (OR 0.75, 95%CI: 0.64-0.89), as did male gender and decreasing BMI. Factors that increased the likelihood against being vitD insufficient were the same as for the deficiency model (race, milk, sunburns, skin type) as well as education. Conclusion: SPH were not significantly associated with vitD deficiency but did show association with insufficiency even after adjusting for expected factors in the NHANES population. Future research needs to analyze these issues prospectively in order to determine whether patients compliant with SPH are at risk for low vitD.

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Second primary melanomas have similar characteristics to the initial primary melanoma on the same patient.

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Although the risk factors for development of a second primary melanoma have been established, there are no studies to the best of our knowledge investigating whether second primary melanomas are more likely to be similar to the patient's first melanoma than would be expected by chance. To address this question, we performed a series of analyses of 180 patients who developed a second primary melanoma at least six months after having presented with an initial primary melanoma to the Pigmented Lesion Group of the University of Pennsylvania from 1972 – 2005. 63% of the subjects were male, with a mean age of 48.65 years (sd=14.81). The median time interval between the patient's first and second melanoma was 3.82 years. Although the overall anatomic distribution for first and second primary melanomas was almost identical (p=0.838), patients whose initial melanoma developed on a particular body site were significantly more likely to have their second melanoma develop on the same body site when adjusting for sex, age, and Clark's level (for trunk: OR=1.97, 95% CI=1.04, 3.73; for head and neck: OR=2.78, 95% CI=1.13, 6.81; for the extremities: OR=3.25, 95% CI=1.57, 6.74). A similar pattern appeared when examining microscopic subtype. Excluding the 22 patients without a recorded subtype, those whose initial melanoma was of the superficial spreading subtype were significantly more likely to have a second melanoma of the same subtype when adjusting for sex, age and Clark's level (OR=2.82, 95% CI = 1.16, 6.89). Similarly, those whose initial melanoma was of the lentigo maligna subtype were more likely to have a second melanoma of the same microscopic subtype (OR=2.94), which trended towards but did not reach statistical significance (p=0.078) in this relatively small sample. Patients' second melanomas appear to resemble their first melanoma, which may have implications in terms of methodologies and emphases of strategies for screening and prevention.

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Effect of sun protective behaviors on vitamin D levels in the US population: NHANES 2003-2006

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Sun protection is recommended for skin cancer prevention. Yet little is known about the role of sun protection on vitamin D levels, or how this relationship varies by type of sun protective behavior or race. Our objective was to examine and quantify these associations in the general US population. We analyzed cross-sectional questionnaire responses and serum measurements from 5920 US adults aged 18-60 years who participated in the National Health and Nutrition Examination Survey 2003-2006. Sun protective behaviors examined included the frequency of staying in the shade, wearing long sleeves, wearing a hat, and using sunscreen. The main outcome was serum 25-hydroxyvitamin D level (ng/mL) among participants who reported low, moderate, or high frequency of use of sun protective behaviors. Staying in the shade and wearing long sleeves were significant predictors of lower 25-hydroxyvitamin D. The multivariate adjusted 25-hydroxyvitamin D difference was -3.2 ng/mL (95% CI -3.8, -2.6, ptrend<0.001) for high versus low use of shade and -2.0 ng/mL (95% CI -3.2, -0.8, ptrend=0.002) for high versus low use of long sleeved shirts. These associations were strongest for whites, and not significant among Hispanics or blacks. Neither wearing a hat nor using sunscreen was associated with vitamin D levels. Wearing long sleeves and staying in the shade are strong predictors of low vitamin D in the general US population, especially in whites. Individuals who practice these behaviors may require higher oral vitamin D supplementation. However, using sunscreen and wearing a hat do not significantly impact vitamin D levels.

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Lifetime UV exposure and risk for lentigo maligna melanoma

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The effect of sunlight in the etiology of lentigo maligna melanoma (LMM) may be distinct to other types of melanoma. We evaluated the impact of lifetime exposure measured by UV index of residence on the development of LMM compared to other subtypes of melanoma. Data were pooled from the Nurses Health Study (NHS) that includes 121,700 female nurses followed biennially since 1976 and the Health Professionals' Follow-up Study (HPFS) that includes 51,529 men followed biennially since 1986. The main exposure of interest was UV index of residence at birth, age 15, and age 30. We estimated cumulative UV index for participants that lived in the same location from birth through adulthood. The primary outcome measure was pathology-confirmed incident melanoma. Melanomas were sub-divided based on pathology as LMM or non-LMM. Multivariate analyses simultaneously adjusted for age, gender, number of moles, hair color, skin reaction to sun, number of sunburns, family history of melanoma, prior cancer history including systemic and skin cancers. A total of 1810 incident cases of malignant melanoma (278 LMM and 1532 non-LMM) were included. For lifetime residence in a high UV index location, the age-adjusted relative risk of LMM was 1.97 (95% CI: 1.22-3.16, p 0.005) compared to non-LMM (RR 1.10, 95% CI: 0.89-1.37, p 0.37). The multivariate risk of LMM associated with lifetime residence in a high UV index location was RR 1.50 (95% CI: 0.93-2.43, p 0.10) versus non-LMM (RR 1.01, 95% CI: 0.81-1.25, p 0.96). In multivariate analyses, men had a significantly higher risk of LMM compared to women (RR 2.46, 95% CI: 1.81-3.34, p <.0001). Our findings suggest a differential and independent effect of UV index of residence on LMM compared to other subtypes of melanoma. This may have implications for our understanding of the pathophysiology of melanoma as well as public health and prevention recommendations.

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Vitamin D and nonmelanoma skin cancer in a cohort of Caucasian health maintenance organization osteoporosis patients

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Epidemiologic investigation of vitamin D and nonmelanoma skin cancer (NMSC) has shown an inconsistent relationship. Our aim was to characterize the relationship of baseline serum 25-hydroxyvitamin D (25-OHD) with squamous cell (SCC) and basal cell carcinoma (BCC) incidence in a cohort of Caucasian health maintenance organization (HMO) osteoporosis patients. A computerized claims database of a large HMO was used to identify NMSC patients diagnosed between January 1, 1997 to December 31, 2007 from a cohort of 3,333 Caucasian patients seen for osteoporosis advice. Vitamin D insufficiency was defined as <30 ng/mL of serum 25-OHD. Multivariate logistic regression analysis was performed, adjusting for age, gender and co-morbidities (e.g. solid organ transplant and HIV). During follow-up, 19 SCC and 35 BCC were diagnosed in 51 subjects. Those with 25-OHD levels <30 ng/mL were significantly less likely to have NMSC [unadj OR=0.399 (95% CI 0.299, 0.694)], and this remained after adjusting for demographics and co-morbidities [adj OR=0.375 (95% CI 0.214, 0.658)]. Vitamin D insufficient patients were significantly less likely to have SCC [unadj OR=0.192 (95% CI 0.073, 0.508); adj OR=0.177 (95% CI 0.066, 0.474)]. There was an inverse, but not statistically significant, association between vitamin D insufficiency and BCC [unadj OR=0.629 (95% CI 0.318, 1.241); adj OR=0.595 (95% CI 0.300, 1.180)]. Patients with vitamin D insufficiency were significantly less likely to be diagnosed with NMSC or SCC. An explanation could be that sunlight exposure is a confounder as it is directly related to both serum vitamin D levels and NMSC, especially SCC. Further investigation examining the relationship of vitamin D with NMSC is needed.

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High-dose topical tretinoin for reducing multiplicity of actinic keratoses

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Topical retinoid compounds are used to treat signs of chronic photodamage. We previously reported that topical tretinoin was ineffective for the prevention of keratinocyte carcinoma (basal and squamous cell carcinoma of the skin) in a population with Actinic Neoplasia Syndrome; in this report we evaluate whether high dose tretinoin is effective over the long term in reducing the multiplicity of actinic keratoses in this high-risk population. The VATTC Trial was a randomized vehicle-controlled trial of 0.1% tretinoin cream applied to the face and ears up to twice daily if tolerated for the prevention of keratinocyte carcinoma. Control participants applied an identical cream (except for the absence of the active ingredient, tretinoin) up to twice daily as tolerated. Participants were 1131 patients (96% men, median age 71 years, 60% with some college education) at six V A Medical Centers who had 2 or more keratinocyte carcinomas in the 5 years prior to enrollment; all were encouraged to use sun protection throughout the trial and were offered free sunscreen. Participants were followed for up to 5.5 years (mean 3.5 years in each group) on treatment with semiannual examinations by study dermatologists who counted all actinic keratoses on the face and ears at each visit. No actinic keratosis was treated unless a biopsy was taken prior to treatment to confirm the diagnosis. About half developed a new keratinocyte carcinoma during the trial. Repeated measures analysis of variance revealed no evidence of any effect of tretinoin on AK count. At no time point during the trial was there a difference in AK counts between randomized groups, and there was no difference in number of actinic keratoses biopsied between groups. We conclude that long-term use on the face and ears of high dose topical tretinoin is ineffective in reducing the multiplicity of actinic keratoses in a population at high risk for keratinocyte carcinoma.

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Incidence of melanoma in coastal versus inland counties of California

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The incidence of melanoma in the United States has been steadily rising for the past decade, with stabilization of overall mortality in recent years. We evaluated the incidence and mortality of melanoma on coastal versus inland counties of California using the registries of the Surveillance Epidemiology and End Result (SEER) from 2000-2006. Using data from the SEER 17 registries, county-based data on the following were collected on non-Hispanic Caucasians: incidence and mortality of melanoma, and tumor thickness. Markers of county-based socioeconomic status included median household income, percent non-Hispanic Caucasian, and percent with at least a high school education. Latitudes of counties were obtained using geographical data from the 2000 U.S Census. Coastal counties were defined as those having any shoreline on the Pacific Ocean; inland counties were defined as those not having shoreline. The $\chi 2$ test, Pearson's correlation, and univariate and multivariate regressions were used with counties as the unit of analysis. From 2000-2006, there were a total of 41,246 (incidence rate of 48.8) and 19,602 (incidence rate of 40.5) melanomas reported in xx coastal and xx inland counties, respectively ($p<.001$). The annual percent change in coastal versus inland counties was 2.8 versus 0.8. The difference in incidence rates was significant ($p<.001$) even after controlling for socioeconomic ?. There was also a difference in incidence rates of melanomas <1 mm between coastal (43.1) and inland (32.9) counties ($p<.001$); no difference existed in melanomas with thicknesses of 1.01 mm- 2 mm, 2.01-4mm, and >4mm. The mortality rate for coastal and inland counties was 2.6 and 2.4, respectively. Latitude did not correlate with melanoma incidence. Incidence of thin melanoma is greater in coastal versus inland counties of California, possibly reflecting increased accessibility to beaches and UV exposure, but thicker melanomas did not follow this trend.

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The compact SF-12 can detect both physical and mental impacts of psoriasis severity

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The SF-12 Health Survey, a shorter alternative to the SF-36, is validated to assess general health status through Physical Component Score (PCS) and Mental Component Score (MCS), each measured on a 0-100 scale with higher scores representing better quality of life (QoL). Comparative norms from Quality Metrics show a mean PCS of 54.3 and MCS of 52.2 in healthy U.S. population and lower scores for chronic conditions like diabetes (PCS 41.5; MCS 47.3) and ulcer/stomach disease (PCS 43.2; MCS 45.1). Our goal was to determine if the SF-12 is a useful tool to assess the impact of psoriasis severity on QoL. We used survey and physical exam data from 325 adult psoriatics enrolled in the Murdough Family Center for Psoriasis. Psoriasis Area Severity Index (PASI) was used to assess psoriasis severity. Other covariates included age, sex, BMI, psoriatic arthritis (PsA), presence of psychiatric disorders (self-reported or psychiatric medication use), and number of co-morbidities measured by the Charlson co-morbidity index (CCI). Linear regression models were used to estimate effect sizes ±95% CIs. In our psoriasis patients, mean PCS was 45.0 and MCS was 47.1. For every unit increase in PASI, there was a 0.22±0.14 unit decrease in PCS ($p<0.01$) and a 0.13±0.14 unit decrease in MCS ($p=0.07$). The presence of a psychiatric disorder decreased the physical and mental QoL (PCS -3.14±2.98, $p=0.04$; MCS -9.60±2.73, $p<0.01$) and additional co-morbidities (CCI) decreased the physical and mental QoL (PCS -3.56±0.68, $p<0.01$; MCS -0.76±0.76, $p=0.05$). The presence of PsA contributed to a decreased physical (PCS -7.90±3.19, $p<0.01$) but not mental QoL (MCS 0.67±3.23, $p=0.68$). Psoriasis severity remained associated with PCS and MCS after adjusting for covariates although the strength of the relationship was attenuated in some models. Our study confirms prior reports that psoriasis severity is associated with decreased QoL, and suggests that the SF-12 may be a useful psychometric tool for assessing QoL among psoriasis patients.

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Prevalence of vitiligo in China: A population-based study in six provinces

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Vitiligo is a acquired skin disorder characterized by depigmentation of the skin. The prevalence of vitiligo varied among different races. In China,although there were some single-city or single-province studies, no multi-city and population-based study had been reported. We had performed a community-based survey in 6 cities of China and obtained the prevalence of vitiligo in China. Each city was chosen from one province. The cluster sampling method was used. Subjects were required to fulfill the self-report questionnaires and also received physical examination by dermatologists. The age- and gender-standardization of the prevalence was based on the China Population Composition in 2003. 17 345 individuals completed the questionnaires. 122 were found to have vitiligo. The overall prevalence of vitiligo was 0.70%. After standardization, the prevalence of vitiligo was 0.56%. In male, the standardized prevalence of vitiligo was 0.69%. In female, the standardized prevalence of vitiligo was 0.45% that was significantly lower than in male($P<0.01$). In both male and female, the prevalence increased with age. 97.54% patients were vitiligo vulgaris.Segmental vitiligo account for only 2.46% of the patients. In patients with vitiligo vulgaris, generalized vitiligo account for 18.03% and acrofacial vitiligo account for8.20% of the patients. 9.84% patients had positive family history. 31.97% patients reported that the vitiligo had influenced their quality of life.

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Usage of indoor tanning beds and risk of melanoma, squamous cell carcinoma and basal cell carcinoma

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The usage of indoor tanning beds is considered as a risk factor for skin cancer development. We examined the association between the tanning device usage and the risk of melanoma, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) in a cohort of women aged 24-44 at baseline in 1989. The information on tanning device usage was collected in 2005 follow-up questionnaire. During the follow-up of 102,663 women from 1989-2007, there were 343 melanoma cases, 276 SCC cases and 5,404 BCC cases. Cox proportional hazards models were used to estimate the relative risk and 95% confidence intervals (CI). Usage of tanning beds 6 or more times per year during ages 25-35 was associated with an increased risk of each type of skin cancer. After adjustment for pigmentary risk factors and history of natural sun exposure, the relative risk was 1.36 (95%CI, 0.91-2.03) for melanoma, 1.90 (95%CI, 1.22-2.96) for SCC, and 1.27 (95%CI 1.13-1.42) for BCC. These data indicate the involvement of the usage of indoor tanning beds in the development of skin cancer.

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Cause-specific mortality in severe psoriasis: A cohort study using the general practice research database

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Severe psoriasis is associated with excess mortality and an increased risk of cardiovascular disease. The purpose of this study was to determine cause-specific mortality in patients with severe psoriasis. Using the General Practice Research Database in the UK, we performed a cohort study from 1987-2002. We identified patients >18 years with a psoriasis code and a history of systemic therapy consistent with severe psoriasis (N=3603) and compared them to an unexposed (control) population of patients with no history of psoriasis (N=14330). Control patients were selected in a 4:1 ratio from the same practice and date in the practice as patients with psoriasis. Cox models adjusted for age and sex were used to calculate the hazard ratio of time to death for each of the leading causes of death defined by the Centers for Disease Control. Two investigators blinded to study group (RSA and ALN) independently determined the cause of death based on diagnostic codes. Discordant cases were reviewed by JMG, and there was agreement between > 2 investigators in 98.5% of cases. There was a statistically significant increased risk of death in patients with severe psoriasis for cardiovascular disease (HR 1.57; 95%CI 1.26-1.96), malignant neoplasms (HR 1.41; 95%CI 1.07-1.86), chronic lower respiratory disease (HR 2.08; 95%CI 1.24-3.48), diabetes mellitus (HR 2.86; 95%CI 1.08-7.59), dementia (HR 3.64; 95%CI 1.36-9.72), infection (HR 1.65; 95%CI 1.26-2.18), kidney disease (HR 4.37; 95%CI 2.24-8.53), and unknown/missing (HR 1.44; 95%CI 1.09-1.88). There was a trend for an increased risk of death from intentional self-harm/suicide (HR 3.35; 95%CI 0.21-53), accidents/unintentional injuries (HR 1.03; 95%CI 0.21-4.96), and liver disease (HR 2.03; 95%CI 0.37-11.11). Severe psoriasis is associated with an increased risk of death from a variety of causes. Future studies are needed to determine the degree to which these excess causes of death are due to the disease itself, its treatments, or other factors.

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Forming standards for informed consent

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Informed consent (IC) is a process for which guidelines are lacking in dermatology. We aimed to assess the method by which practicing dermatologists obtain IC for common procedures, predicting a more rigorous IC process for more invasive/cosmetic procedures. We also measured the opinions of dermatologists regarding the minimum standard of care (MSOC) for IC for these procedures. A random sample of 500 practicing dermatologists in the US received a mailed survey, on which 19 common procedures were listed. For each procedure, responders selected the method of IC – none, verbal only, written only, or written and verbal (W&V) – representing their usual practice. They also selected the IC method that constitutes the MSOC for each procedure. We grouped the procedures into (1) Destruction of non-malignant lesions (including cryosurgery, intralesional injections, and medical chemical peel), (2) Biopsy, (3) Electrodessication and curettage, (4) Excision (including Mohs surgery), and (5) Cosmetic. Of 97 responders, 58.9% were female and 91.8% were Caucasian. The mean (SD) age was 50.6 (10.7) years. The most common practice duration was >25 years (33%), subspecialty was medical dermatology (72%) and practice setting was private single-specialty group (42%). Verbal IC represented the most common practice and opinion about MSOC for groups 1 (65.7%, 66.1%, p=NS), 2 (44.4%, 55.2%, p<0.05), and 3 (48.9%, 53.2%, p=NS). W&V IC was most common, both in practice and in opinion about MSOC, for groups 4 (61.2%, 46.7%, p<0.01) and 5 (70.6%, 50.9%, p<0.001). In general, W&V IC was more common for more invasive and cosmetic procedures. Opinions about MSOC were not significantly different from reported practice in groups 1 and 3. For groups 2, 4, and 5, practice responses exceeded (i.e. were more likely to include W&V IC) MSOC responses (p<0.001). Limitations included low sample size and response rate (19.4%). Our findings may serve as a basis of IC guidelines for dermatologic procedures. Opinions about MSOC tended to be more rigorous for more invasive/cosmetic procedures and reported practices exceeded those opinions.

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Prevalence of cutaneous manifestations in POEMS syndrome patients

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The purpose of this study was to determine the frequency and characteristics of cutaneous manifestations of POEMS (polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes) syndrome. From January 1, 2000 through October 1, 2009, we identified 107 patients in the Mayo Clinic dysproteinemia database. The medical record was reviewed for documentation of cutaneous manifestations and serum levels of VEGF (vascular growth endothelial factor) and IL-6 (interleukin 6). Prevalence of skin findings and correlations with other manifestations of POEMS including peripheral neuropathy, monoclonal gammopathies, VEGF and IL-6 levels were determined. Percentages were calculated out of the total numbers of patients who had clear documentation of the presence or absence of the clinical sign and therefore the denominators may differ. There were 68 males and 39 females with a mean age at diagnosis of 51 years. 98% of patients had at least one cutaneous manifestation. Hemangioma was the second most common cutaneous manifestation with 77% of patients presenting with at least one. Vascular skin changes presenting as acrocyanosis, Raynaud's syndrome, rubor, or purpura/petechiae were noted in 68%. Sclerodermoid changes, hyperpigmentation and hypertrichosis occurred in 65%, 56% and 51% of patients respectively. Mean VEGF and IL-6 levels were 549 and 153 pg/mL. VEGF levels in patients with vascular skin changes were abnormally high as compared to those without (100% vs 63%, p<0.001, Fisher's exact test). 85% of patients with vascular skin changes had a monoclonal gammopathy compared with 100% of patients without these skin changes (p=0.05). Skin findings in POEMS syndrome are quite frequent with a high prevalence of vascular proliferations and vascular skin changes. Disease activity is generally monitored by following VGEF and IL-6 levels. Our data demonstrate a significant correlation between VEGF levels and the presence of vascular cutaneous manifestations of the disease. POEMS syndrome should be considered when any one or more of these skin findings are identified.

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Predictors of biologic therapy, phototherapy, and traditional systemic therapy use among individuals with moderate to severe plaque psoriasis

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More research is needed to better understand indicators associated with initiation of therapy among individuals with moderate to severe plaque psoriasis (PsO). The study objective was to determine the factors associated with the initiation of biologic therapy (BT), phototherapy (PT), or traditional systemic therapy (TST) among individuals with PsO. This was a retrospective analysis using claims data from January 1, 2004 to December 31, 2008 from the HealthCore Integrated Research Database®. Index date (ID) is the date of first medical or pharmacy claim for a BT, PT, or TST between January 1 and December 31, 2006. Included pts were aged ≥20 to ≤62 yrs; had ≥2 medical claims for PsO (ICD-9=696.1) prior to ID; and continuous medical and pharmacy eligibility for ≥24 months before and after ID. Medical and pharmacy claims were examined for 2 yrs prior and following the ID. A multivariate logistic regression model was specified to examine possible factors (e.g., demographics, comorbidities, prior medication use) associated with initiation of a specific index medication (IM). There were 1,541 individuals with PsO (mean age=47 yrs; 46% female). BT, PT, and TST were the IM for 20% (311), 41% (632), and 39% (598) of the pts, respectively. Being male [OR=1.71, 95%CI (1.30-2.25)] and living in the Midwest [OR=1.79, 95%CI (1.26-2.56)] were associated with an increased likelihood for the initiation of BT vs. PT or TST. Receiving other topical therapies (e.g., coal tar) during the 2 yrs prior to the IM was associated with a decreased likelihood [OR=0.51, 95%CI (0.30-0.86)] for initiating BT vs. PT or TST. In this retrospective analysis of community-based practice, demographics were found to be associated with the initiation of BT for the treatment of PsO. Treatment guidelines identifying both clinical and non-clinical characteristics (e.g. pt demographics, geographic variations in clinical practice, prior medication use) to initiate specific therapies may be useful to guide clinicians in the treatment of PsO.

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Skin cancer risk perceptions among African-Americans

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Racial disparities in skin cancer diagnosis have recently been highlighted in medical literature. In African-Americans (AA), melanoma is diagnosed at later stages leading to increased morbidity and mortality. It is unclear what factors lead to such disparities. The purpose of this study was to evaluate skin cancer perceptions of AA compared to other races to discover differences that may contribute to racial disparities in skin cancer diagnosis. Skin cancer-related perceptions of Americans surveyed via the Health Information National Trends Survey 2005 (HINTS) were examined across races (AA, white, and Hispanic). One-third of survey respondents with no prior history of skin cancer were randomly selected to answer questions assessing perceived risk and knowledge of preventive strategies and symptoms of skin cancer. Logistic regression was performed to investigate associations between measures of the mental model of skin cancer and race, controlling for age, sex, education, and income. Compared to whites, AA view their likelihood of developing skin cancer in the future as low (p<0.0001) and they worry less about getting skin cancer (p=0.0044). AA are less likely than whites to believe that skin cancer is caused by behavior (p=0.004), but they are more likely to think it is preceded by pain (p=0.0011). When presented with the statement "There are so many different recommendations about preventing skin cancer that it's hard to know which ones to follow." AA are more likely to agree than whites (p=0.0083). Finally, AA are more likely to think that skin cancer's 5-year survival rate is low (p=0.012). These findings indicate that uncertainty and misconceptions exist in AA perception of skin cancer. Such misconceptions may help to explain the advanced presentation of melanoma in AA. Also, though AA risk of developing skin cancer is small, the perception of low/no risk coupled with the erroneous expectation of pain may lead AA to ignore early melanomas, thereby contributing to delayed diagnosis.

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Classification of skin manifestations of adult T-cell leukemia/lymphoma useful for staging and predictive for prognosis

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Adult T cell leukemia/lymphoma (ATLL) is a malignancy of mature CD4+ T cells caused by the human T-cell leukemia virus type I. Cutaneous involvement is seen in up to 50% of patients, and even the cutaneous type of ATLL has been proposed to indicate skin-limited lesions. Although ATLL is listed in the new WHO classification and WHO-EORTC classification for cutaneous lymphomas, the subdivision, staging and prognosis of the skin-involved ATLL remain unclear. We aimed to define the subtypes of skin-involving ATLL by adopting the classification and staging of mycosis fungoides (MF) and Sézary syndrome (SS), because utilization of MF/SS classification is generally acceptable for physicians. The prognosis of the cutaneous subtypes was also statistically analyzed. We performed a retrospective analysis of 119 patients with ATLL involving skin seen from 1979 to 2010 at our hospital. The skin manifestations were divided into nodulotumoral, patch, plaque, multipapular, erythrodermic, and purpuric types. According to MS/SS classification, they were classified into: T1 (patch/plaque, <10% of body surface), T2 (patch/plaque, 10%≥), T3 (nodulotumoral), and T4 (erythrodermic). The percentages of the smoldering type in each group were: T1, 66.7; T2, 35.0; T3, 29.5; and T4, 0 (all erythrodermic). The blood levels of LDH and sIL-2R were significantly increased from T1 to T4. A survival rate analysis revealed that significantly high prognosis was found in the order of T1 to T4. The survival rates of multipapular and purpuric types were comparable to that of T2, suggesting that these eruptions that are not seen in MF/SS can be categorized into T2. These results suggest that T classification of MF/SS is useful for subdivision of skin-involved ATLL, and ATLL-specific eruptions can be categorized to one of the T groups. The modified T classification may provide evidence that skin manifestation is an independent factor to predict the prognosis of skin-involved ATLL.

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The epidemiology of adolescent acne in North East China

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Adolescent acne impacts self-esteem and quality of life in adolescents and its etiology is not fully understood. To describe the epidemiological features of adolescent acne in North East China and determine the impact of genetic and environmental factors on the pathogenesis of acne. Data were collected from 5696 undergraduates (2920 patients and 2776 controls) by questionnaire. The survey data were analyzed using SPSS13.0, and to calculate heritability by Falconer's method. Total prevalence of adolescent acne was 51.30% (52.74% in males, 49.65% in females). The difference between genders was statistically significant (p <0.05). Adolescents with a family history of acne had earlier age of onset (p < 0.001). The prevalence of acne in first- and second-degree relatives of acne patients was 22.5% and 7.19% respectively, significantly higher than in controls (p < 0.001). Heritability of adolescent acne was 78.47±2.05% in first-degree relatives and 75.05±3.18% in second-degree relatives. Risk factors to the acne suffers were, in descending order of occurrence: acne family history, mental stress, menstrual disorder, frequent insomnia, high fat diet, being male, dysmenorrhea, anxiety, sleeping less than eight hours per day, depression, fried food, study pressure, spicy food, oily skin and mixed type skin. Protective factors were, in descending order: dry skin, neutral skin, frequent fruit consumption and computer access time < two hours daily. Adolescent acne includes a familial genetic predisposition. Additional environmental factors of psychological stress, skin oiliness and high caloric diets may also contribute to the onset of acne in Chinese adolescents.

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Methods to account for actinic keratoses (AK) burden

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AKs represent a significant burden. They were the 2nd most common dermatologic complaint in 2001. Development of AK therapies requires reliable measurements. We sought to validate evaluation methods on facial AKs which were previously tested on upper extremities. Methods included counting individual lesions, first irrespective of size (total count) and then greater ("large") and less ("small") than 2.5 mm diameter; assessing body surface area (BSA) by mentally "scooping" the AK into a continuous mat of lesions. Raters were 12 board-certified dermatologists who are investigators for a larger VA Cooperative Study investigating the efficacy of field treatment of AKs. 9 subjects with AKs were recruited, one for demonstration purposes. The 8 remaining were divided into a pre- and post-consensus group. The raters assessed the subjects independently using the methods detailed above for AKs on the face and ears. Then the raters assessed and discussed each subject in a consensus format. Finally, the raters independently assessed the post-consensus AK subjects. We used a one-way intraclass correlation coefficient (ICC) to evaluate agreement. The total count method had the highest pre- (ICC 0.181, $p<0.05$) and post-consensus (ICC 0.658, $p<0.001$) agreement. The BSA did not demonstrate any agreement even after consensus. The "small" and "large" counting were not significant pre-consensus, but after discussion, both demonstrated agreement; small: ICC 0.208, $p<0.05$ and large: ICC 0.621, $p<0.001$. We conclude that the BSA method should not be utilized to assess AK burden on the face and ears; the small surface area most likely precludes meaningful application of the BSA method. Total count appears to generate the highest level of agreement; however, it is clear that an educational demonstration is necessary to bring raters into agreement. Dividing AK into large and small lesions does not appear to enhance agreement.

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Risk for multiple non-melanoma skin cancers among US women and men

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Non-melanoma skin cancers (squamous cell carcinoma and basal cell carcinoma) are the most common cancers in the United States. Individuals diagnosed with one non-melanoma skin cancer are at risk for subsequent multiple skin cancers. We evaluated phenotypic risk for multiple non-melanoma skin cancers among men (Health Professionals' Follow-up Study) and women (Nurses' Health Study) in the United States. The outcome was self-reported lifetime multiple non-melanoma skin cancers treated surgically, categorized as none, 1, 2-4, 5-10 and ≥11. Exposure was based on known phenotypic risk factors for skin cancer that have been well-characterized in previous studies. We had information on phenotype prior to onset of skin cancer. Participants with a history of other cancers were excluded. Number of sunburns was associated with a significantly higher multivariate risk of ≥11 skin cancers (≥10 sunburns RR 3.38, CI: 2.30-4.97). Inability-to-tan was also associated with a significantly higher risk of ≥11 skin cancers (painful burn with 2 hours of sun exposure RR 2.37, CI: 1.88-2.99). Men had a significantly higher multivariate risk of 5-10 (RR 4.44, CI: 3.90-5.05) and ≥11 (RR 5.16, CI: 4.15-6.42) skin cancers. Hair color was associated with a significantly higher multivariate risk of ≥11 skin cancers (blonde hair RR 1.69, CI: 1.30-2.19 and red hair RR 1.80, CI: 1.25-2.59). Similarly, number of moles on the arm was associated with a significantly higher multivariate risk of ≥11 skin cancers (3-5 moles RR 1.39, 1.01-1.92 and ≥6 moles RR 1.85, CI: 1.31-2.63). Family history of melanoma was also significantly associated with risk of ≥11 skin cancers (RR 1.39, CI: 1.03-1.88). Individuals at risk for multiple skin cancers have a distinct phenotype. Further work is needed to better understand mechanisms behind phenotypic risk for multiple skin cancers and identify strategies for prevention.

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Evidence of reliability, reproducibility, and validity of the ItchyQoL in the veteran population

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Chronic pruritus has significant quality of life (QoL) impact. The ItchyQoL, developed by our group, is a 22 item pruritus-specific health status QoL instrument grouped into 3 subscales: symptom (sx), emotion (em), and function (fx). The ItchyQol has been demonstrated to be reliable, responsive and valid in patients recruited from a tertiary academic center, but little is known about the psychometric properties of the instrument in other populations. From March 2008 to February 2009 veteran patients with chronic pruritus, (>6 weeks) were recruited from the dermatology clinic at the Atlanta VAMC. Of the 54 pruritus patients, 85% were male, the mean age (SD) was 63.8 (15.5) years, and 79% were Caucasian. 75% of subjects experienced pruritus most or all of the time with 93% experiencing pruritus for > 6 months. The mean ItchyQol score was 2.63 (0.94) with questions scaled from 1 (never) to 5 (all the time) and higher scores translating to worse QoL. The overall ItchyQoL instrument score demonstrated internal consistency reliability with Cronbach α coefficient of 0.95. Similar findings were observed for the Cronbach α of each of the three subscales: 0.79 (sx), 0.94 (em), and 0.87 (fx). Reproducibility was demonstrated with intraclass correlation coefficient (ICC) ranging from 0.644-0.729 for sx, 0.676-0.883 for fx, and 0.738-0.860 for the em subscale. Confirmatory factor analysis was used to confirm that the 22 individual items mapped to the three constructs. Both our correlated and uncorrelated models affirm the proposed matching of items to constructs with a root mean square error of approximation of 0.149 and Bentler Comparative Fit Index of 0.703. This study demonstrates that the ItchyQol is a reliable, reproducible, and valid pruritus-specific health status QoL instrument in the veteran population. Further studies are warranted to affirm the psychometric properties of the ItchyQol in other unique populations.

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Depression and risk of incident psoriasis in US women: A prospective study

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Depression is a common mental health condition that has been associated with psoriasis. In the absence of prospective data, it is unclear whether depression precedes psoriasis as a risk factor or is a result of psoriasis. We evaluated depression as a risk factor for incident psoriasis among 82,869 women from the Nurses' Health Study 2. Depression was the main exposure, assessed via the Mental Health Index (MHI-5), a subscale of the Short-Form 36 health status survey that was asked in 1993. MHI-5 scores range from 0 to 100, with lower scores associated with increasing depressive symptoms. MHI-5 was categorized into four strata: 0-52, 53-75, 76-85, and 86-100 (referent). Participants were also defined as having depression if they reported ever taking fluoxetine, sertraline, or a tricyclic anti-depressant. Self-reported incident psoriasis was the main outcome measure. In 2005, we asked participants if they had ever received a physician diagnosis of psoriasis, and if so, the year of diagnosis. We excluded participants with a history of psoriasis prior to 1993 and adjusted for body mass index, smoking status, and alcohol intake. We documented 928 incident cases of psoriasis over 12 years. Women with MHI-5 scores ≤ 52 had a significantly increased risk for developing psoriasis compared to women with MHI-5 scores ≥ 86 (multivariate relative risk [RR] 1.49; 95% CI 1.11-2.00). Women who reported taking an anti-depressant had a significantly increased risk for developing psoriasis compared to women who never took an anti-depressant (multivariate RR 1.36; 95% CI 1.12-1.66). Women with both an MHI-5 score ≤ 52 and who reported taking an anti-depressant also had a significantly increased risk for developing psoriasis (multivariate RR, 1.59; 95% CI 1.21-2.08). We found that depression is independently associated with an increased risk of psoriasis in a population of US women.

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Cancer worry (CW) in Atypical Mole Syndrome (AMS) patients utilizing Total Body Digital Photography(TBDP): Does previous history of melanoma (MM) matter?

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CW is the emotional reaction to the threat of cancer. For those at elevated risk for MM, moderate CW may be beneficial, leading to more regular self skin exams and total body skin exams by dermatologists. Little is known about how the level of CW compares in AMS patients with and without a history of MM before and after TBDP surveillance is implemented. From June 2005 to October 2009, AMS patients ± MM at the Arizona Cancer Center and Emory University were recruited to complete surveys at baseline and again 3-6 months following TBDP. CW was evaluated using the Revised Impact of Events Scale (RIES, range 0-75) and the Melanoma Worry Scale (MWS range 4-16). Baseline CW of AMS patients with and without a history of MM were compared using t-test. Paired t-tests were used to compare baseline and follow-up CW values in the two groups. Of 136 AMS patients 38% were male, the mean age (SD) was 42 (11) years, 99% were Caucasian, 48% had a history of MM and 26% has a family history of MM. Sixty-six completed follow up surveys. AMS patients with a history of MM had significantly higher levels of baseline CW using both scales (REIS: 0.73 (3.2) vs. 15.8 (16.52), $p=<0.0001$; MWS: 7.46 (2.7) vs. 9.61 (3.5), $p=0.0001$). Evaluating the level of CW after TBDP revealed an overall decrease in CW in patients with MM in all scales, but was only significant using the MWS (mean difference 1.05 (2.6), $p=0.02$). For those without MM history, the RIES and MWS also showed a decrease in CW but were not significant. AMS patients with MM and without MM had a similar change in CW levels after TBDP ($p=0.94$). AMS patients with a history of MM have significantly higher levels of CW at baseline. TBDP caused a similar decrease in CW for AMS patients with and without a history of MM, indicating that AMS patients without MM derive the same benefit of TBDP as do those with MM. Physicians may consider using TBDP to reduce CW in AMS patients regardless of MM history.

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HIV+ patients in Botswana find mobile teledermatology an acceptable method for receiving dermatologic care

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Whether HIV+ patients in sub-Saharan Africa are willing to receive skin care via mobile teledermatology is unknown. We conducted a cross-sectional study of HIV+ adults with mucocutaneous complaints in Botswana. Enrolled patients received face-to-face and mobile teledermatology evaluations and completed a questionnaire on their acceptance of mobile teledermatology. We screened 89 patients of whom 75 completed the survey. Time(76%,95%CI66-86%), cost(57%,95%CI46-69%), and distance(41%,95%CI30-53%) were the major barriers in seeking skin care. 45%(95%CI33-58%) of patients need 1-3 hours to see a dermatologist, while for 52%(95%CI 39-64%) it takes >3 hours. 99%(95%CI96-100%) of patients reported complete comfort with mobile skin consultation. Most patients were willing to wait 1-3 days (40%,95%CI28-51%) or up to 7 days(27%,95%CI17-38%) for a response from the mobile phone consultation. 58%(95%CI47-70%) of patients accepted facial photos while 92-97% accepted chest, genital, leg and body as a whole photos. Acceptability of mobile consultation for facial lesions versus all the other body sites was significantly different (paired t-tests p<.05). Most patients cited reduced cost of travel(85%,95%CI76-93%) and reduced time away from home or work(65%,95%CI53-76%) as reasons to prefer mobile phone consultations over face-to-face consultations. Mobile teledermatology consultations are well accepted by HIV+ patients with mucocutaneous conditions in Botswana. Patients cite time and costs as major barriers to receiving skin care and view mobile teledermatology as an acceptable method for obtaining skin care that leads to reductions in these barriers. Most patients accept mobile teledermatology consultations for all parts of their body. However they are most sensitive about facial photography. These concerns need addressing in future strategies to increase access to skin care through in this population.

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Vitamin D levels and oral supplementation update in patients with skin cancer

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Given an increased risk of skin cancer (CA), dermatologists do not recommend UV exposure to maintain vitamin D (vitD) status, but can recommend oral vitamin D supplementation (POvitD). We investigated the relationship between vitD status and photoprotection (PP) while controlling for POvitD in patients with skin CA. Subjects recruited from dermatology clinics were queried for demographics, skin CA history, PP behavior, and POvitD intake (both supplementation and dietary). Patients who were monitored for vitD status were excluded. VitD status was determined by serum 25-hydroxyvitamin D level (25(OH)D) with ELISA. VitD insufficiency (INS) and deficiency (DEF) were defined as 25(OH)D <32 and <20 ng/ml. Adherence to PP was measured by the Glanz Sun Protection Habits (SPH) Instrument, using the median (2.8) of our subjects, into adherent (ADH<2.8) and nonadherent groups (nonADH≥2.8). Students T-test compared 25(OH)D between groups. Linear regression determined predictors of 25(OH)D. A p-value of <0.05 was considered statistically significant (SS). Of the 144 subjects, 53% were male, 99% were Caucasian, 55% had a history of melanoma, and the mean(SD) age was 55(16) years. 25(OH)D ranged from 11-73 ng/ml with a mean(SD) of 29(10)ng/ml. Overall, 17% of subjects were vitD DEF and 51% were INS. Mean(SD) 25(OH)D in the ADH (29(11)ng/ml) and nonADH groups (28(10)ng/ml) was not SS different. Of subjects, 60% were taking POvitD. Mean(SD) 25(OH)D was higher (31(11)ng/ml) in the POvitD group vs 25(8)ng/ml in the group not taking POvitD (p<0.01). There was no SS difference in the dietary intake of vitD between groups. In a multivariate regression, POvitD was the only significant predictor of 25(OH)D (p<0.01). Age, gender, CA type, skin type, SPH score, and hours outside/week were not SS predictors. Within our sample were unable to demonstrate that PP adherence plays a SS role in vitD status. A majority of subjects had vitD DEF or INS. Since POvitD influenced vitD status, dermatologists should identify patients at risk for vitD INS and recommend oral supplementation.

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Getting to burden of disease: Willingness-to-pay (WTP) and Dermatology Life Quality Index (DLQI) in Psoriasis and Psoriatic Arthritis

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Psoriasis is a common chronic condition with skin and musculoskeletal manifestations. Previous studies have demonstrated considerable impact of psoriasis on health-related quality of life (QoL) using tools such as Skindex and DLQI. However, these tools do not assess the QoL impact of musculoskeletal disease, and are not true indicators of burden of disease. We evaluated overall burden of disease and compared impact of skin and musculoskeletal disease using a novel WTP tool that we have previously tested in individuals with psoriasis and psoriatic arthritis. In this study, a total of 233 participants with psoriasis and 161 with psoriatic arthritis responded to questionnaires. In the psoriasis group, 181 participated in DLQI and 207 in WTP. In the psoriatic arthritis group, 133 participated in DLQI and 154 in WTP. Out of these, 125 individuals with psoriasis and 72 with psoriatic arthritis responded to both DLQI and WTP. About half of the participants were female (51.20%). In both groups, most participants responded to the physical comfort (45.49% with psoriasis vs. 40.37% with psoriatic arthritis) and social comfort (40.34% with psoriasis vs. 32.30% with psoriatic arthritis). The median amount patients were willing to pay for a hypothetical cure of psoriasis specific to a particular domain was highest for intimacy, physical comfort and social comfort (median $500 each), and lowest for ability to concentrate (median $100). In psoriatic arthritis, WTP was highest for intimacy, physical comfort, social comfort, emotional health and ability to sleep (median $1000 each), and lowest for work or volunteer (median $300). In contrast, median DLQI score was 5.0 for psoriasis (mean 7.05) and 3.0 for psoriatic arthritis (mean 6.39). WTP is a useful measure of overall burden of disease and goes beyond simply measuring QoL impact. This study also demonstrates that WTP can be used to measure burden of disease for both skin and musculoskeletal aspects of psoriasis.

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Skin color in the US population: An analysis of the distribution and validity of dermatologist-assessed Fitzpatrick skin type

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Our objectives were to provide an estimate of the distribution of Fitzpatrick skin types in the United States, to explore the relationship between Fitzpatrick skin type and phenotypic skin characteristics, and to evaluate the reproducibility of the Fitzpatrick scale as used in a representative sample of the US population. We analyzed cross-sectional questionnaire responses and dermatologist-assigned Fitzpatrick scores in US adults aged 20-59 years who participated in the National Health and Nutrition Examination Survey 2003-2006. Analysis included data on self-reported race, hair color, number of moles and skin reaction to sun. Two independent dermatologists examined 2,696 digital photographs and assigned a Fitzpatrick score to each subject. We used Cohen's kappa statistic to determine the level of interobserver agreement between the two dermatologists. Kappa analysis consisted of both unweighted and weighted kappas with unweighted kappas representing the level of absolute interobserver agreement and quadratic weighted kappas representing the relative agreement. We found that 59% of Americans have Fitzpatrick skin type 3. Most Hispanics have skin type 3 or 4 and most whites have skin type 2 or 3 while over 80% of blacks have skin type 6. Self-reported race is highly correlated with dermatologist-assessed Fitzpatrick skin type (r=0.84, p<0.001). The absolute interobserver agreement between two dermatologists was generally low (Ku=0.45), however the agreement within 1 value on the Fitzpatrick scale was high (Kw=0.85). Individuals who self-reported a tendency to burn had lower Fitzpatrick scores (lighter skin) than those who tend to tan (r=0.44, p<0.01). Since dermatologists who assigned Fitzpatrick scores did so using only the digital images without access to self-reported answers to a participant's tendency to burn or tan, it appears that phenotypic characteristics alone are enough to predict burning or tanning tendency and hence risk for skin cancer.

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Association of IgE levels and eczema in the US population: Results from NHANES 2005-2006

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Recent research has shown that defects in filaggrin are associated with development of atopic eczema and asthma. Exposure to environmental and other allergens through the skin may thus lead to IgE-mediated allergic sensitization. We sought to determine whether subjects who have eczema are more likely to have elevated IgE levels compared to subjects without eczema. NHANES participants were asked if they had ever received a diagnosis of eczema from a physician (N=9804) and if they had a rash in areas typical for eczema including the antecubital, popliteal fossae and wrists. Total allergen-specific IgE levels for 19 different allergens were measured using ImmunoCap fluoroenzyme immunoassay. Logistic regression models were used to examine the association of eczema with the odds of having elevated IgE levels. Adjustments were made for gender, race, poverty, and season of the year. Roughly 2.8 million Americans reported having eczema. Fifteen percent of children have eczema compared to 7.4% of adults. The majority of eczema was reported in non-Hispanic white patients (78.9%) and was inversely associated with poverty (p<0.05). Serum total IgE levels did not differ between subjects with and without eczema, however we found significant differences in allergen-specific IgE levels. Children with eczema were more likely to have elevated IgE to peanuts (OR=1.2, 95% CI 1.0, 1.4), eggs (OR=1.6, CI 1.1, 2.2), cats (OR=1.3, CI 1.1, 1.5), and dogs (OR=1.5, CI 1.2, 1.7). Adults with eczema were more likely to have elevated IgE to dust mites (OR=1.2, CI 1.1, 1.4), cats (OR=1.3, CI 1.1, 1.4), and dogs (OR=1.2, CI 1.1, 1.4) compared to subjects who did not report having eczema. Children with eczema are more likely to have allergic sensitization to food as well as common household pets, whereas adults with eczema are more likely to be sensitized to environmental allergens.

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Identifying persons at highest risk of melanoma using self-assessed risk factors: Results from a case-control study in Washington State

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We sought to develop a self-assessed melanoma risk score to target high-risk persons for melanoma screening. We used data from a 1997 case-control study of melanoma in white participants aged 35 to 74 living in Washington State. There were 386 cases with invasive cutaneous melanoma (excluding lentigo maligna melanoma) and 727 controls selected by random-digit dialing. Melanoma risk factors were assessed by telephone interview. A logistic regression prediction model was developed on 75% of the data and validated in the remaining 25%. We calculated the c-statistic (also known as the area under the receiver operating characteristic curve, a measure of predictive accuracy that ranges from 0.5 to 1, with higher scores indicating better prediction) in the validation set and in a 10-fold cross-validation. A risk score was calculated for each individual. Controls were ranked by risk score to develop risk strata. Sensitivity and specificity for various risk cutoffs among these strata were calculated on the entire dataset. Risk factors in the final model included sex, age, hair color at age 15, density of freckles at age 20, number of sunburns in childhood and adolescence, number of raised moles on the arms and history of non-melanoma skin cancer. The c-statistic was 0.70 in the validation set and 0.74 in the 10-fold cross-validation. In this dataset, screening the top 10% risk group would capture 43% of melanomas (sensitivity 43%, specificity 90%), and screening the top 15% risk group would capture 52% of melanomas (sensitivity 52%, specificity 85%). This self-assessed melanoma risk score could be used to identify persons at highest risk of melanoma in primary care and the general population. Our study suggests that it is possible to capture a large proportion of melanomas by screening a relatively small high-risk group.

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Screening for Psoriatic Arthritis and Psoriasis phenotypes in the nurses' health study 2

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The Psoriatic Arthritis Screening and Evaluation (PASE) tool was developed to help dermatologists screen for active psoriatic arthritis (PsA) among individuals with psoriasis. In this study, we used the PASE to screen for active PsA among participants who self-reported a diagnosis of psoriasis in the Nurses' Health Study 2 (NHS 2), an ongoing US cohort study of over 116,000 women. We also confirmed psoriasis self-reports and collected information on psoriasis phenotype using the Psoriasis Screening Tool (PST). The PASE has 15-items that screen for active symptoms of PsA with 93% sensitivity and 80% specificity. The PST has eight 'yes' or 'no' questions and a scoring algorithm that assigns a diagnosis of psoriasis with 99% sensitivity and 94% specificity. Color images of skin, nail, and scalp changes are included on the PST. We mailed the PASE and PST to 1886 NHS 2 participants who had self-reported a physician-diagnosis of psoriasis in 2005. A total of 1637 (87%) women responded with completed questionnaires. Of these, 1511 (92%) were confirmed to have psoriasis according to the PST. Of these confirmed cases, 347 (23%) women met criteria for PsA according to the PASE. Of these PsA cases, 48 (13.8%) reported having plaque-type psoriasis, 35 (10%) reported having scalp psoriasis, 12 (3.5%) reported nail changes, 5 (1.4%) reported inverse psoriasis, and 213 (61.3%) reported having a combination of phenotypes. Of these, 21 (9.9%) reported having nail and plaque type psoriasis. No phenotype was reported in 34 (9.8%) of PsA cases. Women with PsA reported having nail changes (p <0.0001) and scalp psoriasis (p = 0.03) more frequently than women without PsA. We found that PsA was prevalent among confirmed cases of psoriasis in this population of women. Clinicians and investigators may choose to use the PASE tool to screen for PsA among individuals with psoriasis.

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Characteristics and survival of Kaposi sarcoma patients in the pre-AIDS, AIDS epidemic, and HAART eras

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This study describes the incidence, characteristics and survival of patients with Kaposi sarcoma (KS) in the pre-AIDS era (1973-1980), the AIDS epidemic (1981-1995), and the highly-active antiretroviral (HAART) (1996-2006) periods, as HAART is reported to favorably impact the natural history of KS. Using data from NCI's SEER (Surveillance, Epidemiology, and End Results) Program, we analyzed cases of KS. A total of 19,695 cases of KS identified, and 90.0% were male, with 1.4% occurring in the pre-AIDS era, 70.2% during the AIDS epidemic and 28.4% during HAART. For males with pre-AIDS KS, 64.1% occurred above age 70. Most (90.4%) were white, while 5.3% were black. With the onset of AIDS, most cases (75.6%) occurred between 30 and 49, and only 11.7% occurred above age 50. Most still were whites (86.7%), but cases in blacks rose to 9.9%. In the HAART-era, most cases occurred in the age group 30-49, but cases over 50 comprised 24.6%. Cases of KS rose to 22.3% in blacks, with 70.4% occurring in whites. Skin was the primary site in most cases over this time, but involvement of oral mucosa and GI tract as primary sites rose from 0% in the pre-AIDS era to 4.9% during the AIDS era, and 6.2% during HAART. Relative 5-year survival for males with pre-AIDS KS was 84.5% (sem=4.9%), declining to 14.2% (sem=0.3%) during the AIDS epidemic, and rising again to 57.9% (sem=0.9%) with HAART, 53.4% (sem=1.2%) in the early HAART era and 61.3% (sem=1.2%) in the late HAART era. The difference in survival in the AIDS era versus HAART era was significant ($p<0.001$), as well as in early versus late HAART ($p<0.001$). There was no difference in survival in blacks between early and late HAART ($p= 0.165$), whereas other groups had improved survival. As the HIV population ages in the era of HAART, KS patients are diagnosed at more advanced ages and survive longer. Disparities exist between blacks and whites with regard to incidence and survival. KS during the AIDS epidemic and the HAART era increasingly involves sites beyond the skin, a factor thought to impact prognosis.

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Rate of positive Sentinel Lymph Node Biopsy (SLNB) in shave vs. punch biopsies of thin (<1mm) melanomas

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The decision for biopsying a pigmented lesion by shave or punch/excision approach is controversial. Under current guidelines, if a melanoma is transected (positive deep margin), patients may be recommended to undergo SLNB, even if the melanoma is thin. We investigated the proportion of shave biopsies of thin melanomas which had a positive SLNB compared to punch biopsies and whether a positive deep margin influenced this proportion. Patients who underwent SLNB for primary cutaneous melanoma with a Breslow thickness <1mm between 2000 and 2006 were identified from the Emory Prospective Melanoma Database. Charts were reviewed for diagnostic and therapeutic data. Of the 251 thin melanomas, 156 (62%) were shave biopsies of which 85 (34%) were transected with positive deep margins. Sixty-one (72%) of these were Clark's Level (CL) IV and 5 (6%) were ulcerated. Thus 22% (n=19) of patients with positive margins from shave biopsy underwent SLNB with no other indication. Of the 85 transected shave biopsies, 3 (3.5%) had a positive SLNB compared with 2 (2.8%) of the remaining 71 non-transected shave biopsies. All 5 positive SLNB cases had a CL IV. Of 95 melanomas diagnosed by punch biopsies, 25 (26%) were transected. Seventeen (68%) were CL IV and 3 (12%) were ulcerated. Overall 20% (n=5) of patients with a transected punch biopsy underwent SLNB with no other indication. Three (3.2%) transected punch biopsies had positive SLNB and all cases had a CL IV. Although this was a small sample size, we were able to demonstrate similar rates of positive SLNB among the transected and non-transected shave and punch approach. Every positive SLNB had other indications aside from a positive deep margin, suggesting that transected thin melanomas comprise a significant proportion of biopsies undergoing unnecessary SLNB. Given the higher rates of transection with shave biopsies, clinicians using the shave technique should be aware of the pitfalls of transecting thin melanomas.

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Skin cancer risk perception in renal transplant recipients

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Renal transplant recipients (RTRs) are at increased risk to develop cancer. Non-melanoma skin cancer is the most common malignancy after transplantation. Risk perception is paramount for behavior modification and skin cancer prevention. Our goal was to identify variables which positively correlate with cancer risk awareness. A face-to-face questionnaire was administered to 666 RTRs during routine transplant follow-up visits between 2007 and 2010. Responses were entered into the Generic Clinical Research Database and queries were made regarding demographics, skin cancer history and screening, sunburn, and risk perception. The average time from transplantation was 9 years. Median age was 54 years. Seventy one percent of the patients were white. Patients reporting skin cancer history was 20%. The prevalence of patients who reported history of skin cancer increased progressively from 9% to 73% after 5 years and 30 years of immunosuppression, respectively. White ethnicity, history of smoking, and history of blistering sun burns were all associated with skin cancer. Patients with a history of cancer were 2.69 times more likely to report knowledge of increased cancer risk than those who did not have cancer, suggesting that patient risk perception reflects the increased morbidity of cancer. Caucasian patients were 4.34 times more likely to have a history of cancer. Furthermore, Caucasian patients with skin cancer history who perceived their skin to be darker than white were 0.31 times less likely to know that sun exposure increases the risk of skin cancer. Patients who demonstrated knowledge of skin cancer risk were 3.82 times more likely to have seen a physician for skin cancer screening. Interestingly, more patients associated increased skin cancer risk with sun exposure than transplantation (78.23% vs. 43.09%). In summary, risk perception is increased with skin cancer history, skin cancer screening and associated with lighter skin color. Education tailored to differences in patient perceptions and risks would likely improve skin cancer awareness in RTRs.

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2009 US tanning and sun protection magazine images

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Despite awareness of increased skin cancer and cutaneous aging risks, many young women continue to tan with ultraviolet radiation. This study examined whether fashion magazine images differentially promote tanning to young women compared to older women. Previous studies of magazine images have come to contradictory conclusions as to whether younger models are portrayed with more darkly tanned skin and more skin exposure. We examined images of Caucasian models in the highest circulation fashion magazines directed at adolescent girls (*Seventeen*) and mature women (*In Style*) during the months of May through July 2009 (n = 345 images). Photographs were independently coded by two of the authors and differences were resolved by consensus. Most models in *Seventeen* appeared to fall in the age range of 12 to 24 years-old, while most found in *In Style* appeared older than 24 years-old. Compared with standardized photos of models with varying degrees of a tan, 32% and 30% of models had no tan, 53% and 56% had a light tan, and 15% and 13% had a medium tan respectively in *Seventeen* and *In Style*. Of models portrayed outdoors, two thirds of *Seventeen* models were in unshaded settings and none wore hats, while three quarters of *In Style* models were in unshaded settings and 11% wore hats. There was a trend toward decreased clothing cover among models in *Seventeen*, with most models having no arm cover, some chest exposure and their legs mostly exposed. Some of the darkest models were in sunscreen advertisements. To its younger female audience, *Seventeen* demonstrated fewer sun-safety practices with regard to hat and clothing coverage, but did not portray models more frequently in the sun or with tanner skin.

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Cumulative ultraviolet radiation flux and risk for incident skin cancer in the United States

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Sunlight is a known environmental risk factor for both melanoma and non-melanoma skin cancers. However, individual exposure to sunlight over time is particularly difficult to quantify. We evaluated the association between cumulative ultraviolet (UV) radiation flux and incident basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma in the Nurses Health Study. UV radiation flux has previously been established as a measure that can be used to assess cumulative sun exposure that is less influenced by recall bias than traditional modalities. Exposure was defined as UV flux measured in a geo-coded population of women living across the United States and updated every two years with address changes. Outcome was defined as incident self-reported BCC (greater than 90% validity of self-reports for BCC has previously been demonstrated in this population) and incident SCC and melanoma confirmed by pathology review. Multivariate analyses were simultaneously adjusted for age, hair color, mole count, susceptibility to burn, and ability to tan. Multivariate risk for melanoma was not significantly different when comparing women living in the lowest quintile of cumulative UV flux (RR 0.66, CI: (0.30 - 1.48)) to women living in the highest quintile. However, multivariate risk was significantly lower for women living in the lowest quintile of UV flux for SCC (RR 0.04, CI: (0.01 - 0.27)) and for BCC (RR 0.42, CI: (0.33 - 0.52)) compared to women living in the highest quintile. Using a spline function, smoothing demonstrated that SCC was most sensitive to cumulative UV flux level, and risk continued to rise with increase in cumulative UV flux, whereas BCC was less sensitive and melanoma risk increased slightly. We found that melanoma, SCC, and BCC have distinct associations with UV exposure, suggesting a variable role of UV radiation in the carcinogenesis of the three major skin cancers.

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Synthetic mechanisms of various ceramides in differentiated keratinocytes

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Ceramides (Cer) are major components of intercellular lipid in human stratum corneum (SC), and play a critical role in its barrier and water holding capacity. Recently, we reported that there are 11 Cer classes in human SC (the total number of Cer species is approximately 350) and that the Cer composition of individuals with atopic dermatitis or psoriasis is different from healthy skin controls. However, the synthetic mechanisms of many of the human SC Cer species remain unknown. For this reason, we analyzed Cer species and the gene expression of molecular factors related to Cer synthesis in a differentiated keratinocyte cell (KC) model. KC's were cultured until confluence in EpiLife-KG2 media, changed to a differentiating medium (DMEM: HamF12=2:1, 10% FBS, 10μg/ml insulin, 0.4μg/ml hydrocortisone, 50μg/ml ascorbic acid) (day 0) and maintained for 10 days. Cer species were quantified by LC-ESI-MS, gene expression levels were analyzed by real-time PCR. Of the 11 Cer classes in human SC, 2 classes (Cer[NDS], Cer[NS]) were synthesized on day 0 while, 10 Cer classes (Cer[NDS], Cer[NS], Cer[NH], Cer[NP], Cer[ADS], Cer[AS], Cer[AH], Cer[AP], Cer[EOS], Cer[EOP]) were synthesized on day 10. Human SC contains Cer[NS]C32-54 (32-54 carbons), however Cer[NS]C32-46 (32-46 carbons) species were synthesized on day 0 while, Cer[NS]C32-52 (32-52 carbons) species were synthesized on day 10. In conjunction with the increase of the number of Cer species, RNA expression levels of DEGS1 and DEGS2, enzymes in the Cer[NS] and Cer[NP] synthesis pathway, respectively were increased. Similarly, expression levels of FATP4, a long-chain fatty acid transporter, and ELOVL4, an elongase of long-chain fatty acid, were also increased. These data indicated that all of the Cer species found in human SC are not synthesized in differentiated human KC throughout 10 days of *in vitro* culture. Rather, these data suggested that different Cer species are synthesized in a KC differentiation-specific manner.

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Dermatoremediation of iron overload: Sequestering iron in epidermis ameliorates hepatic accumulation of iron in mice with hemochromatosis

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Several billion corneocytes are shed from human skin daily. Metabolic studies from 50 years ago showed that corneocyte shedding had no measurable effect on systemic protein or iron status. This was true even in hyperproliferative skin conditions and even though 20-25% of daily iron loss normally occurs through epidermal desquamation. We asked whether, under special circumstances, epidermal desquamation could be made to impact metabolic homeostasis. We created mice that accumulate iron in epidermis as a result of overexpression of the transferrin receptor (TfR1): iron in epidermis of the K14-TfR1 line is 3 times normal; iron in the epidermis of the Inv-TfR1 line is nearly 2 times normal. These mice have no clinical or histological phenotype. Hemochromatosis is caused by recessive mutations in the human HFE gene, and mice lacking this gene (Hfe-/-) have features of the human disease, including progressive accumulation of iron in liver. Transgenic TfR1 mice were crossed with Hfe-/- mice and parameters of iron homeostasis measured. Groups of a dozen Hfe-/- mice expressing the TfR1 transgene were compared with littermates lacking the transgene. In the K14- series, iron in liver of Hfe-/- x TfR1 transgenics was reduced by a statistically significant 22% at 7 weeks of age, and there was a concomitant decrease in serum transferrin saturation but no change in hematocrit. In the Inv- series, which had a more modest increase in epidermal iron than the K14- series, iron in liver of Hfe-/- x TfR1 transgenics was unchanged at 6 weeks and reduced by a statistically insignificant 6% at 12 weeks of age. A twenty-fold increase in dietary iron abrogated the ability of the K14-TfR1 epidermal transgene to reduce hepatic iron accumulation. Under the appropriate circumstances of diet and genetics, epidermal desquamation has a measurable impact on systemic metabolism. By implication, systemic effects of circulating toxins might be remediated by sequestering them in epidermis.

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Topical hesperidin improves epidermal permeability barrier function in murine model

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Previous studies have demonstrated that 1). epidermal permeability barrier function is regulated by both peroxisome proliferator-activated receptor (PPAR) gamma and epidermal lipids; 2). hesperidin increases expression of PPAR gamma and fatty acid synthase in addition to inducing cell apoptosis. In present study, we determine whether topical hesperidin regulates epidermal permeability barrier function. Hairless mice were topically treated with either 2% hesperidin or 70% ethanol twice daily for 6 days. At the end of treatment, basal transepidermal water loss, and barrier recovery rates were measured at 2 and 4 hours post barrier disruption with TM300 probe connected to MPA5. In addition, epidermal proliferation and differentiation were also assessed by H&E and immunohistochemistry staining. Following hesperidin treatment, the gross appearance of mouse skin appeared normal. There was no difference in skin surface pH and stratum corneum hydration between hesperidin and vehicle treatment. However, hesperidin significantly decreased basal transepidermal water loss as compared with vehicle. Moreover, pretreatment with hesperidin significantly accelerated barrier recovery at both 2 and 4 hours after barrier abrogation with tape-stripping. In contrast, barrier recovery was delayed when hesperidin was applied after barrier disruption. Furthermore, topical hesperidin also induced a dramatic increase in epidermal thickness, which was accompanied by increased epidermal PCNA positive cells. Finally, expression of epidermal filaggrin and loricrin were increased following repeated hesperidin applications. These results suggest that topical hesperidin could be an alternative approach to improve epidermal function such as epidermal permeability barrier function and epidermal differentiation.

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Ultraviolet B irradiation induces the expression of hornein, a member of the S100 fused-type protein family in human skin

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Hornerin is a member of the S100 fused-type protein family. The structural features of hornerin are quite similar to those of filaggrin, an essential protein for keratinization. The hornerin protein was detected in the granular cells of both regenerating skin and psoriatic skin, but not in normal skin. The exposure of human skin to a ultraviolet B (UVB) dose corresponding to a mild sunburn reaction induces epidermal hyperproliferation and alternation of several constitutive differentiation markers. This study investigated the effect of UVB irradiation on the expression of hornerin. Skin samples obtained from a healthy volunteer, were transplanted to the backs of nude mice. A dose 500 mJ/cm2 UVB irradiation was administered to the skin graft two months after transplantation. Grafted skin samples were removed at various times after irradiation, and then analyzed by immunostaining. The expression of hornein was induced in the granular layers of the UVB exposed skin 48 hours after UVB exposure. Filaggrin expression was altered together with the formation of abnormal honey layers in the same sample. In addition, keratin K6, which is expressed in hyperproliferative epidermal keratinocytes, was also detected in the suprabasal layers of the UVB exposed skin. Filaggrin returned to the granular localization and the number of K6 positive keratinocytes was markedly decreased 7 days after UVB exposure. However, hornerin was still detected in the granular layers at day 7. These results indicate that exposure the skin to UVB to could be one of the triggers of hornerin expression. In addition, the expression of hornein might be a possible maker of acute UV-damaged skin.

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A topical Chinese herbal mixture improves epidermal permeability barrier function in normal murine skin

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Systemic Chinese herbal medicines (CHM) have been used to treat a variety of skin disorders for centuries, but recent studies demonstrate that topical CHM also has beneficial effects for the treating inflammatory skin disorders. Yet, how CHM exert their benefits is unclear. Since all inflammatory dermatoses are accompanied by abnormal permeability barrier function, we asked whether topical applications of these herbal extracts improve permeability barrier function and the mechanism(s). We investigated the effects of a topical CHM extract containing radix paeonlae rubra, cat nut, phelloden dron, etc., on permeability barrier function in normal murine skin. In addition to comparing changes in barrier recovery rates, we also assessed ultrastructural changes, tracer permeability, and epidermal lipid content following topical CHM extract applications twice daily for 7 days to normal skin. The topical CHM extract accelerates barrier recovery following acute barrier disruption. The basis for enhanced barrier function appears to be increased lamellar body production followed by accelerated secretion. Accordingly, epidermal cholesterol content and mRNA expression of the lipid transport protein, ABCA12, increase by topical CHM extract treatment. Moreover, topical CHM extract treatment reduces the penetration of both nickel nitrate and lanthanum chloride, into the stratum corneum, further indicating improved barrier function. Finally, the CHM extract increases mBD3 mRNA expression in intact skin and β-defensin 4 mRNA expression human keratinocyte cultures. These results demonstrate that this topical CHM extract enhances permeability barrier function and antimicrobial defensin, suggesting that topical CHM could provide an alternative regime for the preventing or treating inflammatory dermatoses accompanied by barrier abnormality.

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Different characteristics of reactive oxygen species produced by HaCaT cells treated with DNCB and BKC

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Contact hypersensitivity (CHS) is the result of the activation of both innate and adaptive immunity in response to haptens. Reactive oxygen species (ROS) are involved in the activation and maturation of dendritic cells during antigen presentation in CHS. Keratinocytes (KCs) are required to initiate and amplify the immune response; however, the role of ROS on KCs in CHS remains unclear. In this study, we investigated 1) the production of ROS by hapten-treated HaCaT cells, human KCs line 2) ROS-induced protein carbonylation 3) cellular sources of hapten-induced ROS and 4) the effect of antioxidant on hapten-induced ROS. ROS production were detected using CM-H(2)DCFDA after 60 minutes treatment 2,4-dinitrochlorobenzene (DNCB), DNFB, TNBS, $CoCl_2$, thimerosal, SDS and benzalkonium chloride (BKC) in a concentration-dependent manner without reducing cell viability. Among ROS-producing chemicals, DNCB and BKC were selected to be the representative of allergen and irritant, respectively. With a western blot using anti-DNP antibody, ROS-dependent protein carbonylation was detected in response to DNCB, however, not in BKC. The potential sites of ROS generation were evaluated with the pretreatment of 1) diphenylene iodonium (DPI), an inhibitor of NADPH oxidase 2) rotenone, an inhibitor of mitochondrial electron transport chain complex and 3) allopurinol, a xanthine oxidase inhibitor. DNCB-induced ROS was partially blocked by both rotenone and DPI, but not allopurinol. BKC-induced ROS was completely blocked by DPI, but by neither rotenone nor allopurinol. DNCB and BKC-induced ROS were not inhibited by the pretreatment of antioxidants (glutathione, selenium, N-acetyl cysteine, vitamin E, catalase) in HaCaT cells prior 40 minutes treatment of DNCB and BKC. Although this study concerning the role of ROS on KCs in response to haptens were inconclusive, the results suggested that the characteristics of ROS produced by KCs in response to haptens might be different each other.

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Essential roles of the calcium-sensing receptor in epidermal differentiation and barrier function

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Extracellular calcium (Ca^{2+}_o) is an important regulator of keratinocyte differentiation. The Ca^{2+}-sensing receptor (CaR), a G-protein coupled receptor, plays an essential role in mediating Ca^{2+}-induced keratinocyte differentiation *in vitro*. To investigate the function of the CaR in epidermal development *in vivo*, we generated keratinocyte-specific CaR knockout (^{Epid}CaR-/-) mice by breeding a floxed CaR (CaRfl/fl) mouse line with transgenic mice expressing Cre recombinase under the control of the human keratin 14 promoter targeting cells in the stratum basale (SB) of the epidermis. The expression level of CaR was reduced >90% in ^{Epid}CaR-/- epidermis as compared to CaRfl/fl controls. Ion capture cytochemistry detected a steep Ca^{2+} gradient increasing from the SB to the stratum granulosum (SG) in the normal epidermis, which was lost in the ^{Epid}CaR-/- mice. CaR knockout profoundly decreased the expression of the intermediate and late differentiation markers. The expression of several key enzymes mediating epidermal sphingolipid synthesis, transport and processing was suppressed in the ^{Epid}CaR-/- epidermis, as were the number of lamellar bodies in the keratinocytes of the upper SG and their secretion into the stratum corneum. These changes were accompanied by a 35% reduction in the thickness of the lipid-bound cornified envelopes. Moreover, lanthanum perfusion revealed that the integrity of the permeability barrier in ^{Epid}CaR-/- epidermis was compromised. Although the basal rate of trans-epidermal water loss in ^{Epid}CaR-/- mice was comparable to the controls, ^{Epid}CaR-/- epidermis displayed a significant delay in barrier recovery after acute barrier disruption in mice maintained on a low Ca^{2+} (0.02%) diet. Deleting CaR *in vivo* perturbs the epidermal Ca^{2+} gradient, impairs keratinocyte differentiation, and down-regulates permeability barrier homeostasis, indicating a critical role for the CaR in normal epidermal development and barrier function.

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Identification and characterization of trichohyalin-like1 (TCHHL1), a novel S100 fused-type protein in human skin

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The genes that code for the S100 fused-type protein (SFTP) family are clustered within the epidermal differentiation complex (EDC) on human chromosome 1q21. The SFTP family is an essential component that maintains epithelial homeostasis and barrier functions. A total of six genes have been identified within the EDC in humans, including filaggrin, trichohyalin, hornerin, repetin, corunulin and filaggrin-2. The present study identified trichohyarin like protein 1 (TCHHL1), a novel member of the SFTP family, coded for by a gene between those of trichohyalin and S100A11. The TCHHL1 gene is composed of three exons 45, 158 and 3400 bp in length and contains an open reading flame of 2715 nucleotides, encoding a protein of 904 amino acids. The deduced amino acid sequence contains an EF-hand domain at the N-terminus and one trans-membrane domain at amino acid 66-88. The calculated molecular mass is 99 kDa, and the pI is 4.48. TCHHL1 mRNA was detected in the normal skin, but not in any other organs by RT-PCR analysis. Antibodies were generated against the C-terminus of TCHHL1 protein to examine the expression of TCHHL1 protein. These antibodies detected a band corresponding to the expected size of TCHHL1 protein by a Western blot analysis. An immunohistochemical study showed that the TCHHL1 protein was expressed in the basal layer, but not in the suprabasal layer in normal epidermis. The signals of TCHHL1 protein were co-localized with those of cytokeratin 14. Furthermore, the TCHHL1 protein was detected around the nuclei in cultured normal human keratinocytes. These results indicate that TCHHL1 protein might be involved in the proliferation and differentiation of keratinocytes.

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Regulation of asymmetric cell divisions in developing epidermis

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Stratification of the developing epidermis is driven by proliferation of basal progenitor cells. Initially these cells divide symmetrically, but concomitant with stratification a balance of symmetric and asymmetric divisions occur. This allows for continued surface area growth during stratification. These processes must be tightly controlled to ensure that stratification begins at the correct time and that a tissue of the correct thickness is achieved. We have found two important levels of regulation that control asymmetric cell division. Both the commitment to asymmetric divisions and the choice between symmetric and asymmetric divisions is controlled by expression of the asymmetric cell division gene, Inscuteable. Forced expression of this gene is sufficient to cause asymmetric cell divisions in single-layered epidermis and increases the ratio of asymmetric:symmetric divisions later in development. However, division orientation choice is under robust control as the ratio of division orientations returns to normal after prolonged expression of Inscuteable. This suggests a novel control point that is able to "turn off" the asymmetric cell division machinery post-translationally. p63, a master regulator of epidermal stratification, does not control Inscuteable expression. It is, however, required for asymmetric divisions because of its roles in promoting cell-substratum adhesion and cell polarity. We conclude that the regulation of Inscuteable expression is a major control point in the commitment to stratification and in epidermal progenitor's choice of division orientation.

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Assessment of corneocyte desquamation and determination of cellular vs. extracellular dimensions in normal human stratum corneum by a novel fixation/embedding method

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Standard fixation and embedding of skin for ultrastructural studies results in unacceptable artefacts in stratum corneum (SC) structure. These artefacts are due in part to the non-polar lipids in the SC extracellular matrix, which result in substantial extraction during hydration, as well as poor visualization of extracellular membrane structures. While ruthenium tetroxide (RuO_4) post-fixation allows visualization of lamellar bilayers, the extraction artefacts persist with current RuO_4 post-fixation. We developed a novel protocol that both avoids tissue processing artefacts, and provides excellent visualization of lamellar bilayers. Five sequential D-squame tape strippings from normal young adult males (n=3) were exposed to RuO_4 vapor for one (1) hr at room temperature, and then the first and fifth stripping were placed directly in an epoxy resin mixture for embedding. Using the first stripping, we could show that lamellar bilayers remain intact even at the cell surface. Thus, normal desquamation does not require prior loss or disorganization of lamellar bilayers. With images from the fifth strippings, we quantitated by sterological (morphometric) methods the volume contribution of the corneocyte vs. the extracellular matrix compartment in SC. In contrast to all other epithelia, where the extracellular compartment comprises <0.5% of tissue volume, the SC interstices comprise 9.75±0.63% (SEM; n=26) of SC volume. In summary, utilizing a new, minimally-invasive, artifact-free fixation and embedding protocol, we have shown that desquamation does not require prior dissolution of lamellar bilayers, and that the SC extracellular matrix comprises a uniquely-expanded tissue compartment. This protocol could have wide applications, including the assessment of mechanisms of abnormal desquamation, evaluation of skin care products, and modeling of transdermal drug delivery.

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Epidermal barrier abnormalities and pathogenesis of Ichthyosis Prematurity Syndrome

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Ichthyosis Prematurity Syndrome is a rare form of autosomal recessive congenital ichthyosis (ARCI) characterised by a itchy non-scaly lichenified skin with atopic manifestations, including high numbers of eosinophils and elevated level of IgE in peripheral blood. It is caused by mutations in SLC27A4 gene encoding Fatty Acid Transport Protein 4 (FATP4); a protein functioning as both a fatty acid transporter and an acyl-CoA synthetase. In this study we investigated the cutaneous abnormalities in IPS to better understand how the deficiency in FATP4 functions results in the IPS phenotype. Skin samples were obtained from 9 IPS patients (range 12-44 years), and during autopsy of one premature newborn (34 weeks) and one aborted foetus (23 weeks) with IPS. Light microscopy revealed acanthosis and prominent hyperkeratosis in all cases. Importantly, variable inflammation with eosinophilia in underlying dermis was observed in 7 out of nine patients, as well as in newborn and foetal skin. Ultrastructural studies revealed that the principal abnormalities in IPS skin were associated with the lamellar body secretory system: though the density of lamellar bodies is normal, the internal content of individual organelles often contains microvesicles. As a result, the lamellar bilayer formation is affected with extensive domains of lamellar/non-lamellar phase separation, leading apparently to impaired barrier function of the skin. Our data indicate that disturbed lipid metabolism associated with FATP4 mutations leads to a proinflammatory state of the epidermis already *in utero* and may predispose for development of allergic disorders.

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Effect of inducible misexpression of Dlx3 in basal keratinocytes on epidermal differentiation and hair development

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Dlx3 homeodomain transcription factor plays a crucial role in mouse embryonic development, especially during placental formation, skeletal organogenesis, and development of ectodermal appendages such as hair and teeth. In humans, a frameshift mutation in the coding sequence of the DLX3 gene is etiologic for an autosomal dominant ectodermal dysplasia, Tricho Dento Osseous (TDO), characterized by aberrations in hair, teeth, and bone development. We previously showed that constitutive, ectopic, over-expression of Dlx3 in basal keratinocytes results in runted neonates displaying a reduced skin barrier function, a decrease in proliferation and a premature differentiation of basal skin cells. Due to the perilethality of these neonates, a tetracycline inducible system was generated to temporally control Dlx3 over-expression in basal keratinocytes under control of the Keratin 5 (K5) promoter. Immunohistochemical analysis and ex vivo differentiation assays reveal that Dlx3 over-expression in the epidermis leads to aberrations in the expression pattern of differentiation markers, including a premature expression of filaggrin. Since the K5 promoter induces Dlx3 over-expression in the outer root sheeth of the hair follicle, we induced Dlx3 over-expression in basal keratinocytes during crucial stages of hair cycling to examine its role in hair formation and regeneration. After hair waxing of these animals, hair follicles display an impaired transition from exogen to a new anagen phase. Using our inducible model, we show that mis-regulation of Dlx3 expression interrupts hair follicle cycling. This inducible model will help 1) increase our understanding of Dlx3's role in promoting keratinocyte differentiation and in the development of ectodermal appendages, such as hair and 2) identify direct targets of its transcriptional activity.

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Compositional differences between infant and adult stratum corneum determined by *in vivo* Raman confocal microspectroscopy

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Infant skin continues to develop during the first years of life and has been shown to differ from adult skin in structure and function. We have previously reported that although infant stratum corneum (SC) appears to contain more water, its content of natural moisturization factors (NMF) is lower compared to adult. In this study we compared the compositions of infant and adult SC based on concentration profiles of individual components using *in vivo* Raman confocal microspectroscopy. Raman spectra were acquired in both the high wavenumber and the fingerprint region from the volar forearms of 13 infants (3-24 months) and 16 adults (25-43 yrs). The SC thickness was calculated from the high wavenumber spectra based on the water profiles. The fingerprint region spectra were analyzed for the concentration profiles of various biochemical components. After normalization to account for variation in the SC thickness, our results show that infant and adult SC contain similar amounts of urea, lipids (cholesterol and ceramides), and protein (keratin). However, whereas adult SC contains higher amounts of amino acids, pyrrolidone carboxylic acid, and urocanic acid, infant SC contains significantly more lactate. These data are in agreement with our previous report of lower concentration of NMF in infant SC, as well as other reports on the decreasing NMF amount with aging in adult populations. Moreover, the role of lactate in controlling several SC properties including water-binding and stiffness, as well as its potential to induce cell proliferation in deeper skin layers, underscores the significance of these data in explaining the functional differences between infant and adult skin.

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Intracellular Ca2+ release is the initial signal for epidermal differentiation after acute epidermal permeability barrier perturbation

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Ca+ fluxes control epidermal permeability barrier repair. We show here that barrier perturbation releases Ca2+ from Stratum Granulosum (SG) intracellular Ca2+ stores, leading to accelerated secretion of lamellar bodies, coincident with the transition of SG cells into cornified stratum corneum (SC) cells. These findings suggest that intracellular Ca2+ store release, from the endoplasmic reticulum (ER), specifically signals terminal differentiation. To test this mechanism, we first showed that acute barrier perturbation stimulates ER stress, as measured by Xbp1 expression. Next, we found that pharmacological emptying of the ER Ca2+ pool, using the Ca2+ ATPase inhibitor, thapsigargin (TG), induced lamellar body secretion and transition cell (TC) formation, without perturbing the epidermal permeability barrier. TG treatment also increased epidermal caspase 14 expression and activation, enhanced loricrin expression, and increased cornified envelope thickness. However, cornified envelopes only appeared 2-3 layers above the initial formation of transitional cells. Since lamellar body secretion and TC formation precedes cornified envelope formation, these findings require revision of long-held hypotheses about the sequence of events that lead to terminal differentiation. Moreover, since the status of ER Ca2+ sequestration seems to control lamellar body secretion and transitional cell formation, agents that specifically control either ER stores or store operated Ca2+ currents could provide new therapeutic targets in skin conditions associated with impaired barrier function, such as atopic dermatitis, aged skin or psoriasis.

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Neutral lipid storage leads to acylceramide deficiency, likely contributing to the pathogenesis of Dorfman-Chanarin syndrome

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Dorfman-Chanarin syndrome (DCS) is a neutral lipid storage disorder with ichthyosis that displays an epidermal permeability barrier defect due to loss-of-function mutations in CGI-58, an activator of certain triglyceride (TG) lipases. Yet, the mechanisms accounting for abnormalities in barrier function in DCS are unknown. Fatty acids (FA) derived from TG appear to be utilized for ω-O-esterification, leading to ω-O-acylceramides (acylCer) formation, essential lipids that mediate barrier function. Morphological analysis of the skin of a DCS patient (male, 62 yrs) carrying a novel missence mutation in CGI-58 showed abnormalities of both lamellar body contents and extracellular lamellar membrane structures, and accumulation of lipid droplets in the stratum corneum (SC). Lipid analysis revealed increased TG and decreased FA levels in DCS SC scale *vs.* normal subject. However, only trace amounts of acylCer were present, although cholesterol and total ceramide (Cer) content were not altered. Moreover, only trace levels of covalently-bound ω-OH Cer and ω-OH FA that form the corneocyte lipid envelope (CLE), were evident in DCS. Morphological studies revealed lack of CLE structures in the SC of this and other DSC patients. Finally, blockade of CGI-58 expression (*si*RNA) diminished the acylCer precursor (acylgluosyCer) production in cultured human keratinocytes. Pertinently, the alteration of TG and bound ω-OH Cer levels correlated with the severity of ichthyosis, consistent with acylCer deficiency contributing to DCS pathogenesis. Thus, CGI-58 not only facilitates TG lipolysis of, but also provides FA for the ω-O-esterification required for acylCer and bound ω-OH Cer generation. The recent demonstration of a post-natal lethal barrier defect and deficiencies of both acylCer and bound ω-OH Cer in Cgi-58-null mice further supports this conclusion.

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Characterization of "normal" keratinocyte gene expression.

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Genomic methods that study gene expression of keratinocytes in growth-activated or neoplastic skin diseases need a reference of "normal" keratinocytes to define pathologic alterations. Often, mRNAs isolated from cultured epidermal keratinocytes are used to define normal gene expression patterns. In this study, we profiled global gene expression in human epidermal keratinocytes on Affymetrix U133 2.0+ arrays from three different "normal" sources: 1) cultured keratinocytes, 2) FACS sorted keratinocytes from dispase-separated epidermis, and 3) laser-capture microdissected (LCM) epidermis. In all sources, keratin 14 mRNA was expressed at high levels (>800-fold increase compared to dermal fibroblasts). However, major differences were detected in growth-related and differentiation-related genes between cell sources. Keratin 16, associated with regenerative maturation of keratinocytes, was increased by >25-fold in cultured KCs compared to LCM epidermis. Major increases in transforming growth factor alpha and amphiregulin mRNAs were contained in cultured and FACS sorted KCs compared to LCM samples. In contrast, the highest expression of keratin 1 and filaggrin was detected in LCM epidermis. Many other examples of differential expression cytokines, receptors, and growth-regulating genes were present in these samples. Our results suggest that the best definition of "normal" keratinocyte gene expression is obtained via LCM of normal epidermis. Even short term suspension culture of KCs (used for FACS sorting) significantly alters gene expression. Established KCs in culture express some genes, e.g., keratin 16, at levels found in "pathologic" states such as psoriasis. Overall, our results indicate the need to carefully consider "normal" sources of cells to define normal vs. pathologic gene expression programs.

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Activation of Toll-like receptor2 enhances barrier function of tight junctions in normal human keratinocytes

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Toll-like receptors (TLRs) detect external invaders and trigger an innate immune response. In light of their presence together in the epidermis, tight junctions (TJs) and TLRs are presumed to play a coordinated role in protecting the skin against the invasion of foreign substances. The aim of this study is to examine whether TLR activation functionally alters the TJs in cultured keratinocytes. Stimulation of keratinocytes with the ligands for TLR1-5 and 9 augmented the transepithelial electric resistance (TER), suggesting enhanced TJ function. Further detailed studies of underlying TER elevation were conducted using peptidoglycan, the ligand for TLR2 which is located beneath the *stratum corneum* together with TJs. Stimulation of keratinocytes with peptidoglycan elevated the TER values as early as 3 hours. Immunoprecipitation studies revealed that this early response was accompanied by augmentation of occludin-atypical PKC complexes. In addition, immunocytochemical observations showed atypical PKC was concentrated and co-localized with occludin at the plasma membrane in the cells stimulated by peptidoglycan. The topical application of peptidoglycan to the surface of the *stratum corneum* in living-skin-equivalent models increased the amount of occludin-atypical PKC complex at 3 hours after application. These findings suggest that TLR2 activation promotes the membrane translocation of atypical PKC and its accumulation at TJ areas, which results in enhanced TJ function. The TER elevation was also confirmed at 24 hours, but without an accompanying increase in the occludin-atypical PKC complex. On the other hand, claudin-4 was up-regulated in the mRNA and protein levels. Taken together, these findings suggest that the invasion of foreign substances into the epidermis enhances the intercellular permeability barrier of TJs as an innate immune response through two distinct mechanisms: early response by the accumulation of atypical PKC into the TJ areas and later response by *de novo* synthesis of TJ protein.

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Endoplasmic reticulum (ER) stress upregulates human cathelicidin expression via NF-κB, but not via a vitamin D receptor-mediated pathway in keratinocytes

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LL-37 is the carboxyterminal fragment of human cathelicidin protein (hCAP18) and a major antimicrobial peptide in epidermis. LL-37 (hCAP18) expression increases during keratinocyte (KC) differentiation, as well as in response to external stress, *e.g.*, infections, permeability barrier abrogation. Since endoplasmic reticulum (ER) stress may result from these external stressors, we hypothesized that ER stress regulates hCAP18 generation in KC. First, we ascertained that activation of a transcriptional factor, XBP1 (an ER stress marker assessed by quantitative RT-PCR analysis) occurred in both cultured primary human KC (CHK) and HaCaT human KC following exposure to a specific ER stressor, thapsigargin (Tg), an inhibitor of Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase. Concurrently, we found increased hCAP18 mRNA levels by quantitative RT-PCR analysis in Tg-treated cells at Tg levels that do not induce cell death (%200 nM). Western immunoblot analysis also showed increased in hCAP18 protein levels in Tg-treated KC. Activation of NF-κB has been demonstrated to occur in a variety cell types subjected to ER stress. Indeed, using a reporter assay, NF-κB activation occurred in Tg-treated HaCaT KC, while blockade of NF-κB activity by quinazoline, an inhibitor of NF-κB, attenuated the Tg-induced increases in hCAP18 mRNA/protein expression. Although prior studies have established that vitamin D receptor (VDR) activation induces hCAP18 expression at a transcriptional level, our VDR reporter assay revealed that Tg-induced ER stress suppressed both basal VDR and $1,25(OH)_2$ vitamin D-induced-VDR activation in HaCaT KC. Together, these results indicate that ER stress upregulates hCAP18 expression via NF-κB-dependent (but VDR-independent) pathways in HaCaT KC. Since consensus binding sequence(s) for NF-κB have not been shown on the hCAP18 promoter, NF-κB serves as a key intermediary step in the transcriptional upregulation of hCAP18.

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PKCδ and η, MEKK1, MEK6, MEK3 and p38δ are essential mediators of normal keratinocyte differentiation

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Keratinocytes express a host of protein kinase c (PKC) and mitogen-activated protein kinase (MAPK) isoforms that are activated by common stimuli and participate in multiple signaling networks. Because these kinases are highly homologous, respond to common stimuli and have overlapping functions, it has been difficult to assign specific roles in keratinocyte differentiation. Previous studies used pharmacologic inhibitors and dominant-negative kinases, but these methods inactivate related kinases. Thus, it is not clear which kinases are required regulators of keratinocyte differentiation. In the present study we used targeted siRNA-mediated knockdown to study the role of individual kinases. Among the five PKC isoforms (α, β, ε, η, ζ) expressed in keratinocytes, PKCδ knockdown alone eliminates TPA- and calcium-dependent nuclear AP1 factor accumulation, hINV promoter activation and morphological change. PKCη knockdown also reduces some of these responses, but PKCα, β, and ε appear to have no role. The MAPK kinase cascade kinases, MEKK1, MEK6 and MEK3 are also required. Among the three p38 MAPK forms (α, β, δ)expressed in keratinocytes, only p38δ knockdown attenuates response. These findings are remarkable in several ways. First, although four of the PKC isoforms (α, δ, ε and η) expressed in keratinocytes can be activated by TPA, only PKCδ has a functional role. Likewise, it is intriguing that TPA- and calcium-initiated signaling is exclusively funneled through p38δ and not the other p38 MAPK isoforms. These studies are important as they assign specific roles for individual members of the PKC and MAPK kinase families in regulating keratinocyte differentiation.

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Multiple mechanisms regulate expression of the p21^{Cip21} cyclin-dependent kinase inhibitor leading to cessation of proliferation during keratinocyte differentiation

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Cessation of cell proliferation, via activation of p21^{Cip1} cyclin-dependent kinase inhibitor expression, is a key event during keratinocyte differentiation. p21^{Cip1} blocks cell cycle progression via inhibition of cdk2/4/6 in the G1 phase of the cell cycle. However, the mechanisms that regulate p21^{Cip1} level during this process are not well understood. PKCδ is an important regulator of keratinocyte differentiation and in the present study we investigate whether it also suppresses cell proliferation. Overexpression of PKCδ produces a dose-dependent increase in p21^{Cip1} mRNA and protein level, and promoter activity. This PKCδ-dependent increase is inhibited by the PKCδ inhibitor rottlerin, and the general PKC inhibitor BIS-IM. p21 promoter deletion and mutagenesis studies indicate that the PKCδ response element is a cluster of Sp1 sites in the proximal promoter at nucleotides -52/-120. Mutation of these Sp1 binding elements or knockdown of Sp1 eliminates the response. Sulforaphane (SFN) is a dietary agent that suppresses keratinocyte proliferation via a dose-dependent increase in p21 mRNA and promoter activity. The SFN-response element is located in the p21 distal promoter at nucleotides -1989/-2001; however, activation via this element also requires PKCδ and Sp1 proteins and the p21 promoter PKCδ response element. Moreover, co-treatment with PKCδ and SFN results in synergistic activation. These remarkable findings indicate that PKCδ, a key regulator of keratinocyte differentiation also causes cessation of cell proliferation via activation of p21^{Cip1}, that multiple p21^{Cip1} promoter elements mediate activation of p21^{Cip1}, and that Sp1 factors are required for this regulation. These findings identity a previously unknown link between PKCδ activation with p21^{Cip1}-mediated cessation of cell proliferation in keratinocytes.

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TIG3, a retinoid-regulated suppressor of psoriatic phenotype, suppresses keratinocyte cell division by inhibiting daughter centrosome separation in mitosis

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TIG3 is a retinoid-regulated tumor suppressor protein that is expressed in the epidermal suprabasal layers. TIG3 levels are reduced in psoriasis and increased TIG3 expression mediates tissue normalization in retinoid-treated psoriatic epidermis. TIG3 suppresses keratinocyte proliferation and increases differentiation. One mechanism of TIG3 action is activating type I transglutaminase to increase cornified envelope formation. However, the mechanism whereby TIG3 suppresses cell proliferation is not known. To understand this growth cessation effect, we expressed TIG3 in normal human keratinocytes and monitored subcellular localization and the impact on nuclear compaction and mitosis. These studies show that TIG3 co-localizes with the centrosomes in early prophase of mitosis and inhibits daughter centrosome separation and migration. This is associated with abnormal microtubule formation, cessation of mitosis and DNA synthesis, nuclear compaction, failure of the daughter centrosomes to separate. Thus, TIG3 expressing cells contain two centrosomes adjacent a compacted nucleus. In differentiated keratinocytes in raft culture TIG3 is located in a similar peri-centrosomal location. Mutagenesis studies reveal that the TIG3 c-terminal membrane-anchoring domain is required for peri-centrosomal localization. These results are consistent with an important role for TIG3 in suppressing keratinocyte proliferation. We argue that this novel regulatory mechanism is important in limiting keratinocyte proliferation during differentiation and that TIG3 is an important participant in the normalization of retinoid-treated psoriatic epidermis.

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Type I transglutaminase inactivation via misfolding and accumulation in the endoplasmic reticulum is a cause of ichthyosis

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Mutations in the cornified envelope assembly enzyme, type I transglutaminase (TG1), cause autosomal recessive congenital ichthyosis. However, there are a wide range of mutations, and it is not understood how most TG mutants cause disease. Our present studies suggest a new and novel general mechanism whereby these TG mutants cause ichthyosis. When present at physiologically levels, a catalytically active TG1 point mutant, TG1(C377A), accumulates to abnormally high levels in the endoplasmic reticulum (ER) and in aggresome-like structures where it is ubiquitinylated. Aggresomes are repositories for elimination of misfolded proteins. This leads to ER swelling, reduced TG1 activity and a loss of intracellular homeostasis. Accumulation of this mutant in the ER is the result of protein misfolding, as treatment with a chemical chaperone reduces the severity of the response and reduces ER accumulation. A similar accumulation was observed for several TG1 mutants implicated in the pathogenesis of ichthyosis. In contrast, wt-TG1 travels efficiently through the ER and is transferred to the plasma membrane where it accumulates at points of cell-cell contact and associates with E-cadherin. A remarkable conclusion from this work is that TG1 mutants that harbor single amino acid changes are misfolded and as a result cause remarkable changes in intracellular cell homeostasis. Moreover, it suggests that TG1 is normally efficiently shuttled post-synthesis to the plasma membrane, perhaps as a cell protective mechanism. Our findings suggest that faulty TG1 processing and folding and inappropriate accumulation in intracellular organelles is new mechanism causing ichthyosis.

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AP1 factor inactivation in suprabasal epidermis causes increased epidermal hyperproliferation and hyperkeratosis but reduced carcinogen-dependent tumor formation

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AP1 (jun/fos) factors comprise a family of transcriptional regulators *(c-jun, junB, junD, c-fos, FosB, Fra-1 and Fra-2)* that are key controllers of epidermal keratinocyte survival and differentiation, and are important drivers of cancer development. Understanding the role of these factors in epidermis is complicated by the fact that each member is expressed in defined epidermal layers during differentiation, and because AP1 factors regulate competing processes (i.e., proliferation, apoptosis and differentiation). We have proposed that AP1 factors function differently in basal versus suprabasal epidermis. To test this idea, we have inactivated suprabasal AP1 factor function in mouse epidermis by targeted suprabasal expression of dominant-negative c-jun (TAM67) which inactivates function of all AP1 factors. This produces a remarkable epidermal phenotype including a massive increase in basal layer cell proliferation, delayed differentiation, reduced involucrin and loricrin levels, and extensive hyperkeratosis. Moreover, the tissue displays marked changes in AP1 factor signaling including changes in AP1 factor level, distribution and subcellular location. Finally, it is very interesting that in the face of extensive keratinocyte hyperproliferation, susceptibility to carcinogen-dependent tumor induction is markedly attenuated. These findings stand in stark contrast to previous studies which show that basal layer AP1 factor inactivation does not impact epidermal phenotype. These novel observations strongly suggest that AP1 factors have distinct roles in the basal versus suprabasal epidermis and confirm that AP1 factor function is required for normal terminal differentiation.

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The polycomb group proteins are key regulators of keratinocyte survival

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Epigenetic control of keratinocyte gene expression is an area of intense interest. The Polycomb Group (PcG) proteins are epigenetic silencers of gene expression that enhance cell survival. This is achieved via action of two multiprotein PcG complexes - PRC2 and PRC1 that suppress gene expression by increasing histone methylation and ubiquitinylation, and reducing acetylation - leading to a closed chromatin conformation and increased cell survival. We show that PcG proteins have an important role in keratinocytes to enhance survival and protect against challenge with stress agents. This is associated with increased expression of pro-proliferation cell cycle control proteins and inhibition of apoptotic responses. In addition, we show that increased expression of key PcG proteins in immortalized keratinocytes and skin cancer cell lines. We examine the role of two key PcG proteins, Bmi-1 and Ezh2, and the impact of the active cancer preventive agent in green tea, (-)-epigallocatechin-3-gallate (EGCG), on the function of these regulators. EGCG treatment of SCC-13 cells reduces Bmi-1 and Ezh2 level and this is associated with reduced cell survival. The reduction in survival is associated with a global reduction in histone H3-K27-trimethylation (H3 K27-3M), a hallmark of PRC2 complex action. This change in PcG protein expression is associated with reduced expression of key proteins that enhance progression through the cell cycle (cdk1, cdk2, cdk4, cyclin D1, cyclin E, cyclin A, and cyclin B1) and increased expression of proteins that inhibit cell cycle progression (p21 and p27). Apoptosis is also enhanced, as evidenced by increased caspase PARP cleavage. Vector-mediated enhanced Bmi-1 expression reverses these EGCG-dependent changes. These findings suggest that PcG proteins act to enhance keratinocyte survival via mechanisms that impact cell cycle control and apoptosis.

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Skin barrier dysfunction with aberrant lamellar bodies in mice lacking keratinocyte-specific Golgi pH regulator

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Golgi pH regulator (GPHR) is an anion channel critical for acidification and functions of the Golgi apparatus. Golgi is thought to be the origin of the lamellar bodies in the skin. To study the functional roles of GPHR in the skin, we established keratinocyte-specific GPHR knockout mice by utilizing the Cre-loxP system. The skin of these mutant mice appeared hypopigmentated. Histological examination of these mutant mice showed ballooning of the basal cells, follicular dysplasia, and enlargement of the sebaceous glands. In addition, inflammatory cells with melanin deposition were seen in the dermis. Examination by electron microscopy revealed that their keratinocytes demonstrated aberrant and decreased number of lamellar bodies and intracytoplasmic droplets in the basal layer. TEWL of the mutant mice were increased compared to wild type mice. About half of knockout mice died within 1 month. These results suggest that GPHR is essential for the homeostasis of the epidermis including the lamellar body formation and the barrier function.

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Histological differences in skin architecture and matrix protein expression in photo-protected and photo-exposed skin of Caucasians, East Asians and African Americans

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While skin color is the most notable difference among ethnic skins, unfortunately, the current knowledge of physiological and pathological properties of the skin is based mainly on Caucasian skin studies. We therefore evaluated histological differences in the skin of different ethnic group individuals. The study was conducted at Lynchburg, Virginia on 35-45 year old females from Caucasians, East Asians and African Americans (n = 10 per ethnic group). A 2 mm punch biopsy was obtained from the inner upper arm (photo-protected site) and from the dorsal forearm (photo-exposed site) from individuals on a single visit. The histological endpoints assessed included epidermal thickness, rete ridge length, tropoelastin and collagen expression. The findings from this study showed that in photo-protected skin the thickness of the epidermis was similar between Caucasian, East Asian, and African American individuals. Caucasians had a significant increase in epidermal thickness in photo-exposed skin compared to photo-protected skin, and rete ridge length was also significantly decreased in photo-exposed skin of Caucasians . In contrast, rete ridge length was not different in photo-exposed skin of East Asian, and African American individuals. In Caucasian subjects, the expression of tropoelastin, which is the precursor to the elastin molecule, was substantially less than in age-matched East Asian and African American skin. Expression of tropoelastin in photo-exposed skin of East Asian and African American subjects were similar to their photo-protected skin, suggesting that in these ethnic subjects photo-exposure did not effect tropoelastin formation to the same extent as Caucasian subjects. These results suggest that East Asian and African American subjects may have less photo-exposure dependent loss of skin architecture than Caucasian subjects.

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Advanced glycation end products in the human stratum corneum and their relationship to physical properties of the skin

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There is an increase in advanced glycation end products (AGEs) in the human skin during the aging process, with a greater accumulation in photoaged skin. The accumulation of AGEs in the skin is associated with reduced softness and elasticity as well as increased yellow-brown coloration. Previous reports have focused on AGEs as a dermal aging factor; however, little is known about AGEs in the epidermis. The objective of this study was to determine the presence of AGEs in the stratum corneum (SC) of human facial skin and assess their relevance to the physical properties of the skin. Subjects comprised of 40 healthy Japanese women (mean age, 38.1 years; range, 20-56 years). Measurements were taken at the cheek using a Venustron tactile sensor to assess skin softness and viscoelasticity, a Cutometer for skin elasticity, a SPEX SkinSkan spectrofluorimeter to evaluate the amount of AGEs in dermis and quantitative analysis of the three-dimensional surface configuration of the skin. Using tape-stripped SC, the presence of AGEs in the SC was assessed by immunohistochemical staining. Immunohistochemistry revealed the presence of AGEs in the SC of the facial epidermis, and we evaluated the levels of AGEs both visually and by image analysis. No significant correlations were seen between AGEs in the SC and the parameters of subject's age, skin elasticity, amount of AGEs in the skin, skin softness, average distance between skin surface furrows and skin surface isotropy. In contrast, AGEs in the SC closely correlated in a linear fashion with epidermal viscoelasticity and mean surface roughness. In addition, we screened botanical extracts for their ability to reduce the level of AGEs in the SC and found that Chinese milk vetch (Rengesou in Japanese) was effective in this regard. Overall, we suggest that AGEs in the SC may influence epidermal viscoelasticity and mean skin surface roughness.

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Differential expression of ABO antigens in normal and altered skin conditions

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Since its discovery by Karl Landsteiner in 1900, ABO blood group antigens have played a pivotal role in transfusion medicine. Recently, their possible roles in inflammation and cancer have been proposed; however, their role in skin remains elusive. To elucidate their expression and function in skin, semiquantitative immunohistochemical analyses using monoclonal anti-A, B, or, H antibody were performed for the specimens diagnosed as psoriasis, atopic dermatitis, ichthyosis vulgaris, cellulitis, and cutaneous lupus erythematosus, and for 2-MED ultraviolet (UV)-irradiated skin and normal control. In normal skin, A/B antigen was mainly expressed in stratum granulosum, while H antigen was in stratum spinosum. In psoriasis and atopic dermatitis, A or B antigen expression showed an appreciable decrease in stratum granulosum with a slight increase in stratum spinosum, and H antigen was extensively stained in stratum spinosum. Although A/B antigen expression also decreased in ichthyosis vulgaris, it was not evident as psoriasis or atopic dermatitis. A/B antigen expression in cellulitis and cutaneous lupus erythematosus decreased in stratum granulosum, but increased considerably in stratum corneum, stratum spinosum. In UV-irradiated skin, A/B antigen expression in stratum granulosum were decreased, but H antigen expression showed marked increase in stratum spinosum and stratum basale. Real-time RT-PCR revealed that transferase A and B decreased after the UV irradiation, whereas related glycosyltransferase including FUT1, B4GALT1,2,3,4, B3GNT5, B4GALT6 increased at 24h after the irradiation. In conclusion, the expression of ABO blood group antigen showed a differential distribution and intensity according to skin disorders, suggesting that ABO blood groups might be implicated in the pathogenesis of various skin diseases in the aspect of differentiation and inflammation.

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Effects of hypoxia upon differentiation and proliferation of epidermal keratinocytes

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Normal epidermis is mildly hypoxic. Hypoxia is also an important regulator of epidermal response to wounding, UV irradiation and in skin diseases such as cancer and psoriasis. Yet the role of hypoxia in control of keratinocyte turnover and differentiation remains poorly understood. Here we study the effect of hypoxia in primary mouse (PMK) and human (N-TERT and SCC-derived) epidermal keratinocytes. Confluent cultures, stimulated with calcium to enhance differentiation were maintained in normoxic conditions (21% O_2), or at 5% or 1% O_2 in a hypoxic chamber for up to 48h. Expression of basal keratins (K5 and K15) was significantly suppressed by 5% O_2 and completely abolished by 1% hypoxia. In PMK these keratins were moderately downregulated by severe hypoxia (1% O_2). Differentiation markers respond differentially. Keratin 1/10 were suppressed in N-TERT and SCC-13 cells (at 1% O_2 only) while involucrin was not affected in these cells. In contrast, in PMK involucrin expression was nearly abolished by 1% O_2. The overall level of transglutaminase (Tg) was not responsive to hypoxia in any of the cell lines, nevertheless 1% O_2 stimulated proteolysis of an active 67kDa Tg chain in PMK and N-TERT cells. Whilst hypoxia reduced keratinocyte proliferation (PCNA expression and cell counting) FACS study did not reveal cell cycle block or increased apoptosis. Hypoxic cells were metabolically active and maintained membrane integrity (WST-1 and LDH assays). Surprisingly, significant protein downregulation of ARNT and HIF1α, the main components of hypoxia pathways, occurred despite elevated VEGF during hypoxia. Thus, our results revealed significant effects of varying O_2 levels on differentiation and proliferation in epidermal keratinocytes. These effects differ between normal and transformed cells. At the same time, severe hypoxia had no apparent effect on cell viability or basic metabolism implying similarity to senescent phenotype and providing new links between hypoxia, epidermal proliferation/differentiation disorders and carcinogenesis.

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A novel function for TAp63α in the epidermis

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p63 is an essential transcription factor expressed as isoforms that either contain (TA) or lack (ΔN) a transactivation domain. ΔNp63 isoforms are essential for development, orchestrating the conversion and differentiation of simple epithelia to stratified epithelia. Conversely, TAp63 is not required in development, but is induced in wounded skin. To elucidate the *in vivo* role of TAp63α in simple and stratified epithelia, we generated a Tet-inducible mouse model that can ectopically express TAp63α in a tissue-dependent manner. Utilizing lung and uterine Tet-activator lines, we ectopically expressed TAp63α in these simple epithelia. We found that these tissues are not competent to respond to TAp63α, thus no effect was seen. We then utilized a K14 Tet-activator line to express TAp63α in stratified epithelia such as the epidermis. Expression of TAp63α *in utero* has no obvious effect on epidermal stratification. However, treated newborn bigenic mice develop focal skin lesions and die by postnatal day 5. To determine if the lesions were due to cell death, we performed TUNEL and cleaved-caspase 3 staining, revealing massive epidermal apoptosis. Previous studies have implicated TAp63, as well as its homologs, p53 and TAp73, in inducing apoptosis in other organ systems. Therefore, we wanted to determine if p53 and TAp73 were also induced in TAp63α expressing skin. We found transcriptional upregulation of TAp73 and protein stabilization of p53 before apoptosis occurs. Noxa, bax and bcl-2, which are known downstream targets of p53 and TAp73, were also induced in the skin of bigenic mice. ChIP analysis revealed that all three homologs, p53, TAp73, and TAp63α were present on the promotors of both Noxa and Bax. Based on these results, it is unclear whether TAp63α induces apoptosis alone or via the formation of functional complexes with p53 and TAp73. However, our data documents the ability of TAp63α to induce apoptosis in the epidermis *in vivo*. This provides an explanation for its acute induction in response to wounding and extends our knowledge of p63 and the diverse functions of this complex transcription factor.

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Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin

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Caspase-14 is mainly expressed in suprabasal epidermal layers and activated during keratinocyte cornification. Caspase-14 deficient mice display a reduced epidermal barrier function and an increased UVB radiation sensitivity. We found that although profilaggrin, a protein with a pivotal role in skin barrier function, is processed correctly to its functional filaggrin monomeric unit in caspase-14$^{-/-}$ mice, these mice accumulate proteolytic filaggrin fragments in the epidermis. We show here that the accumulation of these filaggrin fragments is due to a defect in filaggrin degradation in the cornified layers of caspase-14$^{-/-}$ skin. Indeed, *in vivo* Raman spectroscopy revealed that this lack of normal filaggrin degradation results in a significant reduction in the levels of natural moisturizing factors, such as urocanic acid and pyrollidone carboxylic acid, in the skin from caspase-14 deficient mice as compared to wild-type mice. In addition, we demonstrate that caspase-14 can directly cleave the filaggrin monomer. Taken together, our data identify caspase-14 as a crucial protease in filaggrin catabolism.

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Investigation of the effect of extracts of South East Asian medicinal plants on the expression of extracellular matrix proteins in human skin and skin cells

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Indigenous people of South East Asia have a rich history of medicinal plant use for treating a variety of human health conditions. As part of a continuing effort at Avon Global R&D to identify medicinal plants with potential skincare benefits, we investigated a number of South East Asian medicinal plants used in Traditional Medicine for efficacy in stimulation of extracellular matrix (ECM)proteins in human skin cells. Two novel plant extracts have been found to increase the expression of collagen and dermatopontin in skin cells as well as to positively impact a variety of other biological targets. *In vitro* and *in vivo* screening results will be presented along with data regarding efficacy in human biopsy testing.

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Insect bites selective upregulate skin's antimicrobial protein expression

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Insect bites by mosquitoes or other bugs are a common problem leading to toxic or allergic reactions, inflammation, itch and pain. Infections occur only occasionally, though stinging disrupts the permeability barrier and may lead to invasion of bacteria, which colonize the skin surface or the insect's poison sting. We ask whether antimicrobial proteins may be responsible for the low rate of infections after insect bites. Expression of the antimicrobial proteins human beta defensin (hBD)-2 and -3, human neutrophil peptide (HNP)-1-3, RNase 7, psoriasin (S100A7) and cathelicidin LL-37 was determined by immuno-histochemistry in skin samples obtained from insect bite lesions compared to healthy skin. In addition H&E and mast cell tryptase stainings were performed. H&E staining revealed a pronounced infiltrate of inflammatory cells, mainly lymphocytes and neutrophils and occasionally eosinophils in the dermis. In the insect bite channel we found an infiltrate with neutrophils. The number of mast cells and mast cell tryptase was only moderately increased. The antimicrobial protein expression was variable: in the epidermis adjacent to the insect bite channel moderately increased staining for hBD-2 and -3, and RNase 7 was found, whereas a pronounced increase of psoriasin was noted. Within the insect bite channel and in the adjacent dermis, but not within the epidermis, we found an intense staining for LL-37 and HNP 1-3, both derived from neutrophils. In bullous insect bite reactions, staining for LL-37 and HNP 1-3 was found within the blister fluid. Staining for HNP 1-3 and psoriasin, but not LL-37, was also found in the epithelia of the blister floor. In summary, we found a pronounced increase expression of psoriasin, LL-37 and HNP 1-3 in insect bites. Psoriasin expression occurred in the epidermis, whereas HNP 1-3 and LL37 was identified within the neutrophils in the epidermis (only HNP1-3) and dermis. Induction of antimicrobial peptides may protect against infections after skin barrier disruption and potentially invasion of bacteria after insect bites.

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IGFBP7 plays a key role in the pathogenesis of psoriasis

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Psoriasis has been shown to result from combined immunological and epidermal defects. We recently demonstrated that IGFBP7 is downregulated in the psoriatic epidermis and regulates three processes typically abnormal in psoriatic keratinocytes: proliferation, apoptosis and differentiation (J Invest Dermatol, 130:378). Since IGFBP7 was found to counteract psoriasis-associated abnormalities in epidermal cells, we hypothesized that IGFBP7 may play a role in the pathogenesis of the disease. To substantiate this hypothesis, we assessed the pathological features of 3D organotypic cell cultures following RNAi-induced IGFBP7 down-regulation. We observed that decreased IGFBP7 expression in the stratifying epidermis was associated with the progressive appearance of histological features characteristic of psoriasis including marked parakeratosis, hyperkeratosis and hypogranulosis. Supporting a role for IGFBP7 in psoriasis, we further demonstrated that down-regulation of IGFBP7 induces ERK1/2 phosphorylation in keratinocytes as seen in psoriasis. In contrast, ERK inhibition was found to prevent cell proliferation induced by decreased expression of IGFBP7. We next treated SCID mice grafted with human skin in which psoriasis had been induced with NK/T cells (J Invest Dermatol, 119:384) for 10 days with (1) PBS (n=5), (2) dexamethasone (n=5; 2 μg/mouse/day) and (3) recombinant IGFBP7 (n=5; 2,4 μg/mouse/day). All mice treated with PBS developed psoriasis. In contrast, dexamethasone cured psoriasis in 5/5 of the mice. Recombinant IGFBP7 reversed the psoriasiform phenotype totally or partially in 3/5 and 1/5 mice respectively. Dexamethasone also prevented leukocyte infiltration in the dermis; in contrast, IGFBP7's effect on the dermal infiltrate was less marked, suggesting that IGFBP7 mainly targets epidermal elements. Taken altogether, our data position IGFBP7 as a key regulator of keratinocyte differentiation and proliferation and strongly substantiate the notion that IGFBP7 contributes to the pathogenesis of psoriasis.

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Regulatory mechanisms of a natural moisturizing factor-generating enzyme, bleomycin hydrolase- its relevance to atopic dermatitis

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Recently, we purified a natural moisturizing factor (NMF)-generating enzyme and identified as bleomycin hydrolase (BH) (J Biol Chem, 2009). In the present study, we investigated regulatory mechanisms of BH expression in cultured keratinocytes. The upstream sequence with 1.2 kb from translation start site was cloned from the human genomic library using a Genome Walker. We identified a critical region for BH regulation between -134 and -216 by deletion analysis. Genome Net search suggested that MZF-1, Sp-1, interferon regulatory factor (IRF)-1/2 and GATA-1 would be involved in the regulation of BH expression. The electrophoretic mobility shift assay showed that MZF-1, SP-1 and IRF-1/2 directly interacted with the cis-elements in the BH promoter region. Interestingly, BH mRNA was down-regulated in the presence of Th2 and Th17 cytokines and up-regulated with Th1 cytokines in the cultured keratinocytes. These results indicate that MZF-1 and Sp-1 binding to the promoter region regulate the homeostatic BH expression, while IRF-1 participates in the cytokine-mediated BH regulation. Immunohistochemical study showed that BH and filaggrin co-localized in the spinous and granular layers of normal human epidermis. In contrast, lesional epidermis with atopic dermatitis (AD), BH expression was markedly down-regulated compared with that of normal skin. BH activities measured with citrulline-MCA as a substrate showed significant decrease to 28% and 29% in the extracts of lesional and non-lesional skin with AD (n=13), compared to the control (n=40). Our results indicate a unique regulatory mechanism of BH in association with the keratinocyte terminal differentiation and immune-modulating cytokines. In addition, a defect in the filaggrin degradation pathway would be associated with the pathogenesis of AD.

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Environmental stimuli up regulate IL-33 in normal human keratinocytes

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IL-33 is an IL-1 family member recently identified as the ligand for ST2L [IL-1 receptor-related protein], that activates mast cells and Th2 effector T-cells. Its function has been studied in the context of Th2-associated inflammation. IL-33 expression was detected in the nucleus of epithelial cells including epidermal keratinocytes and endothelial cells in tissues exposed to the environment. Thus IL-33 may function as "alarmin", an endogenous danger signal to alert the immune system after epithelial damage during trauma or infection. We examined whether IL-33 is up regulated in keratinocytes when exposed to UV irradiation, pro-inflammatory cytokine, and mechanical stretching both at protein and RNA levels. Normal human keratinocytes (NHKs) were exposed to UVB (10, 30, 100, 300 mJ/cm3), UVA (20, 40, 60 kJ/m2), TNF-α (3, 10, 30 ng/ml) or repeated stretching by +20% for 24 hours. Culture supernatant concentration of IL-33 and the level of RNA were evaluated with ELISA and real time PCR, respectively. IL-33 expression was detected at RNA and protein levels without any stimulation. Exposure of NHK to low dose UVB (10 and 30 mJ/cm3) increased IL-33 expression at RNA and protein levels. In contrast, IL-33 was not up regulated by UVA. Low concentration of TNF-α induced expression of IL-33 at RNA level. Mechanical stretching increased IL-33 after 24 hrs. This is the first study showing that low dose UVB, TNF-α and mechanical stretching stimulate IL-33 production from NHK. IL-33 may be one of the causative agents in inflammation provoked by these factors. Furthermore we emphasize the putative role of IL-33 as "alarmin" in skin.

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Endo-β-D-glucuronidase disturbs epidermal proliferation and differentiation

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Recently, we reported that ultraviolet B (UVB) irradiation activated epidermal endo-β-D-glucuronidase (EGase) and induced degradation of heparan sulfate at the dermal-epidermal junction in human skin. UVB irradiation is also known to induce aberrant pathways of epidermal proliferation and differentiation, leading to alterations of the epidermal permeability barrier. However, it has remained unknown whether EGase plays a role in the induction of the aberrant epidermal proliferation and differentiation in skin. In order to explore the involvement of EGase in the induction of abnormal epidermal proliferation and differentiation, we used a skin equivalent model (EFT-400; MatTek, Ashland, MA), which showed abnormality in epidermal differentiation histologically, and examined the effect of a synthetic specific inhibitor of EGase. Epidermal proliferation and differentiation were assessed by analyzing the expression of Ki67 and filaggrin, respectively, by means of immunohistochemistry. The gene expression profile in the epidermis was analyzed using microarrays. In the skin equivalent model EGase was activated during culture. Subsequently, differentiation markers such as filaggrin and loricrin became disorganized and Ki67, an epidermal proliferative cell marker, was greatly decreased, as judged from immunohistochemical findings. On the contrary, in the skin equivalent model in the presence of an EGase specific inhibitor, filaggrin and loricrin was localized uniformly at the granular layer of the epidermis and Ki67 levels were well maintained. To explore differences of gene expressions between the skin equivalent models with and without EGase specific inhibitor, gene expression microarray analysis was performed. Expression of proliferation-related genes and expression of differentiation-related genes were reduced and increased, respectively, by the inhibitor. These results suggest that activation of EGase disturbs proliferation and differentiation in the epidermis. Thus, EGase may be involved in the abnormality of epidermal differentiation and proliferation in UVB-irradiated skin.

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Morphological study of penetration pathways via hair follicles using nano-sized iron oxide

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Nano industry could contact easily in surrounding of our life already along with development of industrial technology. However, nanomaterial is bringing positive effects and negative effects at the same time to our human. In affirmative part, this supplies innovative medical therapy being applied in field such as nanomedicine but, it can have toxicity in *in vivo* and *in vitro* by exposure that do not want in contradictory part. Therefore, we studied possibility that nanoparticle is permeated via skin among various absorption pathways in our body. We treated with a topical iron oxide of 15nm size as a tracers by each for 1 minute, 5 minutes, 1 hour and 24 hours in C57BL/6 mice skin and we observed aspect that iron oxide nanoparticles are permeated on skin using transmission electron microscopy, and morphological analyzed permeation route of tracers. Iron oxide nanoparticles(INPs) observed in mostly upper stratum corneum, hair follicle and sebocyte through pilosebaceous duct. Represented equal permeation aspect from all groups regardless of application time but, according to increase of application time, confirmed that the permeation amount of INPs is increased in sebocyte specially. Also, INPs confirmed that is permeated to upper hair bulb through inner root sheath and hair cuticle interface in hair follicle. But, permeation of INPs could not observed from dermis except hair follicle and sebocyte in all groups. This study expects to become useful basis data regarding transdermal drug delivery research.

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Improving epidermal lipid barrier by enhancing HMG-CoA reductase expression

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Cholesterol is a key component of stratum corneum (SC) extracellular lipids. Cholesterol synthesis requires the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and topical application of statins disrupt barrier lipids by producing defective lamellar bodies and deficient SC lipid lamellae. In these studies, we evaluated the effects of IV09.001, which is designed to upregulate HMG-CoA reductase in epidermal keratinocytes, pre-adipocytes, as well as *ex vivo* skin. Hence, we examined enzyme expression through immunostaining, lipid content through Nile red staining, and production of lamellar bodies through TEM. Normal human keratinocytes (NHK) were treated with IV09.001. Lipid content was then assessed by Nile red, where yellow-gold fluorescence indicated neutral lipids and red indicated more polar lipids. Our results showed an enhancement of lipid content in NHK. In 3T3-L1 induced to differentiate into adipocyte-like cells, concurrent treatment with IV09.001 increased the size of intracellular lipid droplets. In *ex vivo* human skin, topical application of a cream formula containing 1% of IV09.001 noticeably increased the expression of HMG-CoA reductase after 24 and 48 hours, compared to the placebo-treated samples. This activity was accompanied by an enhancement of epidermal lipid content evaluated by Nile red staining. In a separate experiment, we evaluated the ultrastructural profile of the epidermis in *ex vivo* skin treated for 48 hours with 1% of IV09.001. Compared to the untreated control, the treated epidermis revealed increased lamellar body content in the upper layers of the stratum granulosum (SG), as well as at the SG-SC interface. These results suggest great promise in methods that improve skin barrier lipids and function by enhancing HMG-CoA reductase expression, especially in aged skin that may suffer from decline in cholesterol synthesis.

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Enhancing Caspase-14 expression in the epidermis improves protection and repair against UVB irradiation

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Caspase-14 is a member of the cysteinyl aspartate-specific proteases. Its role in epidermal barrier formation has been attributed to the direct processing of (pro)filaggrin into free amino-acids. Moreover, the upregulation of caspase-14 expression by UVB irradiation suggests its implication in the UV-stress response. Using IV08.003, which has been previously characterized as a caspase-14 inducer, we emphasized the role played by caspase-14 in barrier recovery, as well as in UVB repair and protection. Human skin samples were submitted to repeated tape-stripping in order to induce injury in the skin barrier. Skin biopsies were then performed on stripped and adjacent undamaged area, and treated with IV08.003 for 72h. Our results showed better restructuring of the cornified layer in treated biopsies. In order to study protection against UV stress, normal human keratinocytes (NHK) were pre-treated with IV08.003 for 24h, and then irradiated with 50 mJ/cm2 of UVB. Expression of cyclobutane pyrimidine dimers (CPDs) and of 8-Oxo-2′-deoxyguanosine were investigated 1 hour after UVB stress. The level of UVB-induced DNA damage was markedly reduced in IV08.003 pre-treated NHK. UVB stress was also performed on human skin biopsies pre-treated or not with IV08.003. In skin biopsies where caspase-14 was pre-induced, we noticed fewer signs of UV stress as visualized by hematoxylin-eosin staining, as well as less CPDs staining, compared to untreated biopsies. Moreover, the level of active caspase-3 revealed by immunostaining was also reduced in pre-treated biopsies. Interestingly, a reduction of DNA damage markers and active caspase-3 was also observed when NHK and skin biopsies were first irradiated with UVB and then treated with IV08.003, indicating a repair effect. These results strongly suggest that enhancing caspase-14 is a relevant method for protecting the epidermis against UV and facilitating DNA repair after UV damage.

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Genotoxicity evaluation by comet assay following long-term treatment of THP1 cells

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Interest in developing in vitro methods for subacute cytotoxicity assessment has grown in recent years. There has also been increasing interest in the monitoring of product genotoxicity, and in the evaluation of links to DNA alteration. In this study, comet assay was performed on THP-1 cells after treatment with two control substances, over a 28-day period with a frequency of three applications per week. Comparative evaluations were made between H2O2, which is widely used as a positive control for genotoxicity assessment by comet assay, and Paraquat (PQ), which is used as positive control for long-term studies. Comet assays were performed at different time points: at 1h (4°C) with the classical Singh et al. method; after a 24h recovery period (37°C); and finally after a long-term period of treatment (28 days, 37°C). At 1h, the genotoxic effects of H2O2 were confirmed, whereas Paraquat's tail moments were slight. After the recovery period, H2O2 tail moments decreased, suggesting efficient repair system activation at 37°C, while those of PQ remained stable compared to the control. At this stage of the experiment, cell mortality did not exceed 20% for PQ and H2O2. Subacute cytotoxicity assays after 28 days revealed a dramatic decrease in the cell viability rate of doses beyond 1µg/ml and 10µM, for PQ and H2O2 respectively. Surprisingly, H2O2 tail moments remained stable, while those of PQ increased in a dose-dependent manner. It seems that, in the case of THP-1 cells, long-term cytotoxicity results observed after 28 days of culture with H2O2 are not due to single strand breaks. On the other hand, we hypothesized that, after repeated treatment with PQ, repair systems could be overloaded by DNA damage. In conclusion, this assay could be of interest for genotoxic assessment of daily applied products and suggests that PQ is a choice worthy positive control.

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Assessment of THP1 subacute cytotoxicity in order to evaluate long-term innocuity of soluble cosmetic ingredients

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After March 2013, chronic toxicity assays performed on animals will be prohibited in the field of cosmetics. In response to the present lack of validated alternative methods, we developed an *in vitro* model for repeated dose cytotoxicity on THP-1 cells. Cultured in suspension, cells were treated with tested chemicals for 14 days with a frequency of 3 applications per week, and cell viability was determined by MTT assay. We first investigated the long-term effects of chemicals that induce different kinds of cytotoxicity: Paraquat, 3-Nitropropanoic acid (3-NPA), and sodium dodecyl sulfate (SDS). Inspired by an *in vivo* subacute toxicity testing procedure, we determined the no-observed-effect-level (NOEL) from an acute cytotoxicity study (24h). Doses between 1 and 10µg/ml were chosen to perform our subacute cytotoxicity assay. As expected, after 14 days of treatment with Paraquat, cell viability rates dramatically decreased for doses beyond 3µg/ml, while 3-NPA and SDS did not induce more than 44% of cell death. The method was thus validated and Paraquat was confirmed as a positive control for the assay. Moreover, we were greatly interested in setting up an assay with water as a negative control. Results highlighted an important decrease in cell viability rates, which are thought to be caused by an osmotic shock. After a salt equilibration, a subacute cytotoxicity assay was performed with water at 5-10 and 20%. Cell viability was restored with this protocol, allowing us to validate water as a negative control and to use it for testing some of our compounds. Even with a cutoff at 90% of cell viability, all compounds tested at 0.5 to 5µg/ml were classified as safe. Using THP-1 cells as a model for long-term safety testing yielded encouraging results and has the advantage of being inexpensive, fast, and reproducible.

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Survivin, a protein member of the chromosomal passenger complex, helps protect the integrity of somatic stem cells of the epidermis

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The chromosomal passenger complex (CPC) is an assembly of four interacting proteins: survivin, borealin, incenp, and aurora kinase-B. CPC is the key regulatory complex responsible for the correct development of each mitosis step. This process is particularly important in undifferentiated cells that must renew themselves, further differentiate, and specialize. The epidermis needs to continuously generate new cells by proliferation and differentiation of progenitor cells. Both mitosis supervision by the CPC and a correct extracellular environment are physiologically required for the homeostasis of epidermal somatic stem cells (SSCEs). SSCEs are mainly found in the basal layer of the epidermis and are responsible for replenishing and maintaining tissue by compensating for the loss of terminally differentiated cells (corneocytes), especially during aging. In previous studies, we demonstrated the ability of compound IV08.009 to favor a correct basal environment for SSCEs of the epidermis by acting on the expression of basal integrins and keratins. Here we further investigated the role of survivin in the skin. Using IV08.009, we regulated the survivin expression without affecting the clonogenic potential of SSCEs-enriched cellular fractions, and reversed siRNA-induced survivin loss in keratinocytes. In the same way, the clonogenic pattern of cells submitted to surviving-silencing was restored by the compound. Interestingly, immunohistological studies revealed that survivin induction protected *ex-vivo* skin against basal UVB-induced damage. Moreover, comet assay demonstrated IV08.009-confered protection of SSCEs from UVB damage. Finally, the potential role of Exportin-1 (CRM1) was investigated. These studies demonstrate the interest of modulating survivin expression in order to help protect basal cells and SSCEs from UVB stress and suggest a protective activity of SSCEs essential renewing potential.

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Chemical modification and physical stabilization of papain – development of stabilized high molecular weight crosslinked enzyme polymer with skin exfoliation activity

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Epidermal desquamation is the removal of the topmost cornified layer of the epidermis resulting in removal of dead and scaly corneocytes and renewal of the skin surface. As a result of chronobiological aging or extrinsic aging, epidermal desquamation is disturbed resulting in accumulation of dry thickened skin. Exfoliation via physical, chemical or mechanical means are some of the common ways of promoting skin desquamation, but with serious side effects. We have developed a high molecular weight crosslinked papain polymer that retains its protease activity. Papain is a thiol protease isolated from the latex of Carica papaya known for its use in the food, pharmaceutical and cosmetic industry. Some of the major concerns about the usage of papain in the cosmetic industry are its autodegradation, and therefore loss of activity and skin penetration. The newly developed high molecular weight form of papain minimizes its skin penetration and improves its surface activity. Further crosslinking of the polymeric papain with molecular weight crosslinkers minimizes its autodegradation. Additionally, sodium alginate was identified to be a physical stabilizer of the crosslinked enzyme polymer. The stabilized crosslinked polymeric enzyme complex was found to be hypo-allergenic and non-toxic in nature. This novel chemically and physically modified form of papain retained its protease activity at high temperature for several weeks and proved to be a gentle skin exfoliant with moisturizing and anti-aging benefits. In conclusion, the stabilized papain polymer provides a safe and efficient alternative to abrasive and irritating traditional methods of skin exfoliation.

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The stem cell factor/c-kit receptor signaling pathway is implicated in the regulation of epidermal melanocyte migration and homogenization of skin pigmentation

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Stem cell factor (SCF) is the natural agonist ligand of the c-kit receptor which is a member of subfamily III of the Receptor Tyrosine Kinase family. In the skin, this pathway regulates melanocyte homeostasis and their epidermal distribution. SCF binding leads to the dimerization of KIT, its auto-phosphorylation, resulting in the activation of the microphthalmia-associated transcription factor protein (MITF) and the expression of target genes (tyrosinase, etc.). In the present study, we analyzed the localization of SCF and KIT expression in the skin and in several skin-derived cell lines, and evaluated their implication in the pigmentation process. We developed a specific compound (IV09.007) to help us in our approach, and studied its ability to modulate the SCF/KIT signaling pathway. IV09.007 treatment of *ex vivo* skin or cultured cells markedly increased the expression of both SCF and KIT (observed by immunohistological studies and western blotting). It also increased melanin content in the basal layer of the epidermis (Fontana-Masson staining). These effects could contribute to a better protection of the epidermal basal layer. Interestingly, migration of cultured human melanocytes was enhanced in the presence of IV09.007 in the culture medium. The action of the compound on other participants of the SCF/KIT signaling cascade (ERK1, 2; MITF; etc.) was also investigated, and gene expression and microarray studies confirmed at the molecular level the above results and the role of SCF/KIT signaling. Taken together, these studies confirm the importance of the SCF/KIT pathway in the skin and suggest that modulating this pathway could enhance the homogeneity of melanocyte repartition in the skin, and favor a higher production and content of protective melanin.

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The control of epidermal differentiation gene expression by ARNT through EGFR- and HDAC-dependent pathways

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Precise orchestration of gene expression is essential for the formation of various layers of the epidermis and epigenetic mechanisms are potential candidates as a means for this control. EGFR activation by specific ligands is also known to be involved in control of growth and differentiation in normal and cancerous epidermal keratinocytes. Previously we have demonstrated a role for the aryl hydrocarbon receptor nuclear translocator (Arnt or Hif1b), a key factor in hypoxia and toxic responses, in regulating expression of differentiation-associated genes and EGFR ligands in mouse epidermis *in vivo*. In order to examine the potential role of human ARNT in control of epigenetic mechanisms and the EGFR pathway in differentiating keratinocytes we created an *in vitro* model by stable shRNA-mediated suppression of ARNT (0.2-0.4 of normal level) in human N-TERT cells. Expressional studies of ARNT-depleted keratinocytes show a significant upregulation of differentiation markers such as keratins 1 and 10, filaggrin and loricrin. Furthermore, we demonstrate that in some instances this effect is HDAC-dependent and can be modulated by TSA. We show that ARNT-depleted cells have an increase in total HDAC activity and in the protein levels of HDAC1 and HDAC3. We demonstrate that in human keratinocytes ARNT deficiency results in the down-regulation of EGFR ligands such as TGFα, EGF and AREG and in suppression of EGFR phosphorylation. Exposure of keratinocytes to AG1478 (EGFR inhibitor) significantly modulates Arnt-dependent changes in the expression of differentiation markers. These results suggest a significant role for ARNT in the regulation of epidermal differentiation and demonstrate at least two different mechanisms implicated in this regulation – modulation of EGFR pathway and control of HDAC activity. These two Arnt-dependent pathways seem to be linked to each other and our future work will examine the mechanisms of their interaction in control of epidermal differentiation.

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2-photon-imaging of skin: Single- vs. multi-beam fluorescence lifetime imaging detection-systems

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Two-photon-microscopy is a powerful method for imaging of tissues and cells. Through its near infrared excitation of fluorophores in a very small volume of the specimen, phototoxicity and photobleaching are reduced to a minimum. Through FLIM (fluorescence lifetime imaging microscopy), inhomogeneous dye distribution and emission-wavelength limitations may be circumvented. Yet, all 2-photon methodologies are inherently "dark", the low probability of the 2-photon effect therefore requires sensitive detection systems. Most FLIM-detectors are used with a single-beam point-scanning and PMT (photomultiplier tube) detection. We here compare spatial resolution, penetration-depth, accuracy and acquisition-speed of a multiplexed 16-channel time-correlated single photon counter (TCSPC), in single beam mode at a sampling rate of max. 78 Mhz. The alternate approach is a time-gated CCD-camera (Picostar), with a multi-focal beam-system of up to 64 single foci for accelerated scanning. Both systems were used in *ex vivo* porcine skin models stained with fluorescein. We detected minor advantages for the TCSPC system in resolution; this system measures time-parallel events, is easily saturated but insensitive to scattered light. The Picostar system proved advantageous at high signal intensities as it measures locally parallel, although with scattered light sensitivity. For TCSPC, we found an approximate point-spread-function (PSF) of 350 nm in x-y direction, which deteriorated by a factor of 3 for Picostar. In z-direction both systems reached the theoretical maximum of the lense in use, here 1,3 µm. The minimum penetration depth for TCSPC reached 300, for Picostar 200 µm; acquisition rates under our current experimental conditions did not vary greatly. We are currently fine-tuning the detection process to maximize data acquisition and speed for 3-5 dimensional data-stacks from skin samples, including animal models of human disease.

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Zebrafish type XVII collagen/the 180-kDa bullous pemphigoid antigen: Gene structures, expression profiles, and morpholino "knock-down" phenotypes

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The *COL17A1* gene encodes type XVII collagen (also known as the 180-kDa bullous pemphigoid antigen), an integral component of hemidesmosomes, attachment complexes providing integrity to the dermal-epidermal junction. Zebrafish, a facile model system to study skin development, displays fully developed hemidesmosomes at approximately 5 days post-fertilization. We have identified two *COL17A1* orthologues in the zebrafish genome, *col17a1a* and *col17a1b*, which are expressed in the skin and neural system, respectively. The proteins coded by these genes have structural module organizations homologous to the human type XVII collagen. "Knock-down" of the expression of *col17a1a* with a specific morpholino targeting the 5′ UTR of the gene resulted in reduced expression and perturbed assembly of anchoring fibrils, manifesting with a cutaneous phenotype characterized by detachment of the epidermis from the underlying dermis. "Knock-down" of *col17a1b* expression had no gross morphologic changes in the skin but resulted in a reduction of hair cells in the neuromast in the lateral line. Thus, zebrafish has two *COL17A1* orthologues which may have evolved tissue-specific functions during vertebrate development. Collectively, zebrafish provides a model system to study the molecular aspects of skin development and offers a means to test pharmacologic approaches for treatment of the corresponding human diseases.

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A surinam quassia extract boosts lysyl oxidase expression in keratinocytes and improves epidermal differentiation in a skin equivalent model

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Skin aging is characterized by an epidermal thinning, due to a reduction of cell renewal and a disorganization of the differentiated keratinocyte layers. Lysyl oxidase (LOX) is an extracellular enzyme that cross-links elastin and fibrillar collagens in connective tissues that is also found in epithelia where its role remains unclear. In the epidermis, LOX protein is only expressed in differentiated suprabasal layers which decrease with aging. In an attempt to understand the role of LOX in the epidermal differentiation process, we abolished its functions, either by inhibiting its activity or by silencing its gene using a short hairpin RNA (shRNA). These procedures delayed the appearance of early differentiation markers and impaired terminal differentiation in tridimensional culture models made of normal or immortalized human keratinocytes. These data designated LOX as an important target to restore epidermal terminal differentiation in aging skin. Therefore, we screened a plant library and selected a surinam quassia extract as a potent inducer of LOX gene expression. On confluent keratinocyte monolayers this induction was followed by an increase of some early differentiation markers such as K10, involucrin and transglutaminase. In a skin equivalent model made of normal human fibroblasts and keratinocytes, the surinam quassia extract applied throughout the culture increased the same early markers as well as late differentiation markers such as filaggrin and loricrin. This up-regulation was accompanied by an increase in the number of spinous and granulous layers leading to a thicker epidermis. Taken together, these results suggest that a subtle regulation of LOX improves epidermal homeostasis. Moreover, in our reconstructed skin model, the surinam quassia extract appeared to improve epidermal thickness without impairing the normal terminal differentiation process. On the contrary, it increased components of the cornified envelope, filaggrin and loricrin, crucial players for barrier function formation.

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Microarray profiling of gene expression response to modulation of the stem cell factor/c-kit receptor signalisation pathway in human skin keratinocytes

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Stem cell factor (SCF) is the natural ligand of the c-KIT receptor, a member of subfamily III of receptor tyrosine kinase transmembrane proteins. In the skin, the SCF/KIT pathway regulates and allows for communication between melanocytes and surrounding keratinocytes, which contributes to melanocyte homeostasis and their distribution in the epidermis. The binding of SCF to KIT triggers the dimerization of the receptor, its auto-phosphorylation, and the activation of intracellular responses, finally regulating the expression of subsequent genes. In the present study, we examined the histological localization of SCF and KIT expression in the skin, as well as the cytological expression profile of SCF and KIT mRNAs and proteins, through different RT-PCR/qPCR approaches and immunocytology. We also investigated the action of a biological compound (IV09.007) known to modulate the SCF/KIT signaling pathway, by using gene expression profiling, with the help of a thematic, skin-oriented microarray. A custom microarray consisting of 1,300 different genes was employed to assess the expression profile of normal human keratinocytes (the study will be extended to melanocytes) treated with active ingredient IV09.007, versus the untreated condition. Interestingly, we observed the modulation of genes implicated, for example, in DNA repair and replication, inflammatory responses, extra-cellular matrix constitution, oxidative stress resistance, and cell migration. This study highlighted the ubiquity and importance of the SCF/KIT pathway in the skin. Moreover, expression profiling using thematic microarrays was shown to represent a choiceworthy technique in the field of dermatological research for analyzing gene expression in response to the application of a biological compound or any other stimulus targeting a particular pathway.

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***In vitro* percutaneous absorption of salicylic acid from three distinct topical formulations**

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The study compared percutaneous absorption pharmacokinetics of salicylic acid 6% (SA) from 3 distinct formulations over a 48 hour period using the *in vitro* human skin, finite dose model and static Franz Diffusion Cells (FDC). The study tested 3 SA formulations, Gel (Keralyt®), Cream (Salex®) and Foam (Salvax™) on 4 replicate trunk skin sections from 6 different *ex vivo* skin donors. Static 1cm2 FDCs were filled with a magnetically stirred reservoir solution of phosphate-buffered isotonic saline, pH 7.4 ±0.1 with the epidermal surface open to ambient laboratory conditions. Skin surface temperature was maintained at 32± 1°C. Formulations were applied to the skin with a positive displacement pipette (5µL/cm2). Pre-dose and at 1, 2, 4, 8, 12, 24, 32, and 48 hours after dose application, reservoir solution was collected and replaced with fresh solution. After collecting the last reservoir sample, surfaces were washed with 50:50 Ethanol:Water to collect unabsorbed formulation. Skin was tape stripped to recover the stratum corneum and then split into epidermis and dermis. Quantification of SA in all collected samples was done by High Performance Liquid Chromatography. SA did penetrate into and through *ex vivo* human trunk skin *in vitro*. Mass balance was excellent at 91-98%. The penetration profile was characterized by a rise to a peak flux between 5-10 hours after application followed by either a rapid decline (Gel), or a steady-state like level of absorption (Cream and Foam). Total absorption was significantly greater with Gel (87.19+3.38% of dose) than with Cream (23.89+3.36% of dose), which was significantly greater than Foam (2.57+1.28% of dose). Cream demonstrated greater tissue levels of SA than the other 2 formulations. The study demonstrates that potential for systemic absorption of SA can be minimized by altering the physicochemical characteristics of the delivery system.

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The effect of TRF2 telomeric protein modulation in an *in vitro* model of replicative and accelerated senescence

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Telomere shortening is one of the main characteristics of cellular aging. TRF2 plays a central role as a binding platform for other telomere-associated proteins. Our previous studies have shown that positive modulation of TRF2 protein expression was associated with a decrease in the cellular senescence marker beta-galactosidase. In the present study, we further investigated the effect of TRF2 modulation in a model of accelerated, *in vitro* senescence induced by methylglyoxal (MGO). Treatment of cells with MGO at 0.4 mM was shown to increase reactive oxygen species in cells and induce glycation adducts, leading to a senescent phenotype within 72h. Morphological changes were observed by hematoxylin-eosin staining on fibroblasts aged by replicative senescence in parallel to MGO-induced senescence. Microscopic observation of non-treated aged cells showed that their cytoplasm became larger and irregularly shaped, whereas TRF2-induced cells showed fewer signs of aging. Aged phenotype was also associated with modifications of the cytoskeleton protein vimentin, observed in replicative and MGO-induced aging. Treatment with IV08.007 tended to limit the increase in vimentin expression in aged cells. Parallel studies on aged fibroblasts using the senescence-associated beta-galactosidase (SABG) marker revealed, in TRF2-induced cells, a reduction in the number of SABG-positive cells. Moreover, treatment of human skin biopsies with MGO (5-10 mM) induced different kinds of damage to skin structure, as observed by H&E staining. This damage was limited by a concomitant treatment with the TRF2 inducer. Our results confirmed that the TRF2 telomeric protein plays an important role in the appearance of a senescent phenotype, and suggest that modulation of TRF2 expression can be of interest in anti-aging applications.

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UCP2 is associated with differentiation in human epidermal keratinocytes via regulation of ATP production

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UCP2 is one of the molecular species of uncoupling proteins (UCP) which exist in the mitochondrial inner membrane and uncouple oxidative phosphorylation by reducing the electrochemical gradient across the inner membrane. This uncoupling reaction leads to a decrease in ATP production and can convert energy to heat. It has been reported that epidermal keratinocytes express various UCP species, but their function in the epidermis remains obscure. In this study, we examined the distribution of UCP2 in the epidermis to clarify the role of this protein in epidermal differentiation. We found expression of UCP2 was higher in the suprabasal differentiated keratinocytes compared to that in the basal keratinocytes. UCP2 expression increased in keratinocytes induced to differentiate by Ca2+, detachment or ATP2C1 knockdown. In addition, UCP2 up-regulation by adenoviral gene transduction induced keratinocyte differentiation as measured by an increase in K10 keratin expression. These findings suggest that UCP2 expression is positively associated with keratinocyte differentiation. To clarify the involvement of mitochondrial activity in UCP2-induced keratinocyte differentiation, we interfered with mitochondrial ATP production using an inhibitor for ATP synthase, oligomycin. Oligomycin induced keratinocyte differentiation, suggesting that ATP is a key regulatory factor in keratinocyte differentiation. In parallel experiments with UCP2 overexpression, we examined the effect of a chemical protonophore, carbonyl cyanide m-chlorophenylhydrazone, on keratinocyte differentiation. As expected, it also induced keratinocyte differentiation. Taken together, these findings indicate that a minimum level of ATP production is necessary to keep keratinocytes in an undifferentiated state and UCP2 expression controls keratinocyte differentiation by regulating proton accumulation and ATP production.

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A decline of TRPV6 function derives from a decrease of vitamin D activation and accounts for disturbed epidermal calcium gradient followed by skin barrier alteration in aged skin

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Epidermal Ca++ regulates keratinocyte differentiation and plays an important role in forming skin barrier. Because epidermal Ca++ gradient is restored with the recovery of skin barrier after its disruption, a disturbance in the recovery of Ca++ gradient delays skin barrier recovery. TRPV6 as a calcium ion channel, belonging to the superfamily of transient receptor potential channels constitutes an apical Ca++ entry mechanism in active Ca++ transport in the intestine, which has been also found in the epidermis of mammalian skin recently. TRPV6 modulates an influx of Ca++ into keratinocyte, and its expression is directly up-regulated by 1α, 25 dihydroxyvitamin D3. Facial skin of aged humans showed the loss of epidermal Ca++ gradient, and the synthesis of cutaneous vitamin D3 was declined with aging. We hypothesized that this disrupted epidermal Ca++ gradient is caused by a decrease of TRPV6 function due to decreased vitamin D activation with aging. The mRNA expression of TRPV6 and vitamin D receptor (VDR) on fully aged skin (87 weeks old hairless mice) decreased; moreover their mRNA expression was not enough increased after 3-day low-dose (50mJ/day) UVB irradiation compared to young skin. Western blot for TRPV6 and VDR protein expression showed very similar results with mRNA. Topical ketoconazole, an inhibitor of 1α, 25 dihydroxyvitamin D3, decreased TRPV6 expression even after UVB irradiation under immunohistochemical stain. In conclusion, a decline of TRPV6 function could be derived from decreased vitamin D activation and accounts for disturbed epidermal Ca++ gradient and altered skin barrier in aged skin.

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EC-SOD induces apoptosis through COX-2 and galectin-7 in skin

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Extracellular superoxide dismutase (EC-SOD) is an anti-oxidant enzyme, which is located in the extracellular matrix of tissues, and plays an important role in terms of preventing many disease caused by oxidative stress. However, other functions of EC-SOD are not well known, so we generated EC-SOD transgenic mice to investigate the functions of EC-SOD. We found that the epidermis in EC-SOD transgenic mice was thinner than that of wild type mice. In addition, we demonstrated that the thin epidermis of EC-SOD transgenic mice results from the apoptosis of epidermal cells by TUNEL assay. To elucidate which molecules are involved in the EC-SOD-induced apoptosis, we performed two-dimensional electrophoresis; the results showed that the epidermis of EC-SOD transgenic mice produces more galectin-7, which is known to be a pro-apoptotic factor, than that of the wild type. Furthermore, we showed that transfection of EC-SOD expressing plasmids induces production of galectin-7 and galectin-7 expressing plasmids induce pro-apoptotic proteins in keratinocyte cell lines, HaCaT cells, suggesting that EC-SOD induces apoptosis through increased galectin-7 expression. Finally, we demonstrated that EC-SOD-induced expression of galectin-7 results from the production of COX-2. Taken together, our results indicate that over-expression of EC-SOD could induce apoptosis through COX-2 and galectin-7 in the epidermis. These results imply that EC-SOD plays a role as not only an ROS scavenger but also as a pro-apoptotic factor via COX-2/galectin-7 pathways in epidermis.

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Role of podoplanin in human epidermis

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Podoplanin (PDPN)/AT1/aggurus is a transmembranous glycoprotein, a well known marker of lymph vessels. In addition, PDPN-expression is detected not only in lymph vessels but in normal sebaceous basal cells (Yang et al. 2008). Although lack of PDPN-expression in mice induces lymphedema (Schacht et al. 2003), function of PDPN in terms of epidermal homeostasis and diseased condition remains still unclear. Our objective is to analyze PDPN-expression and its function in normal and diseased keratinocytes. Our results showed that 1. PDPN-expression in diseased skin: Immunostaining showed strong expression on the basal layer of psoriatic epidermis and the edge of wounded epidermis, but minimal expression in normal epidermis. 2. Cytokine regulation of PDPN-expression in keratinocytes: IFN-γ, IL-6, IL-22 and TGF-β upregulated PDPN-expression in mRNA and protein levels. JAK inhibitor and Stat-3 inhibitor abolished the PDPN-upregulation induced by these cytokines, suggesting PDPN-induction via JAK-Stat signaling pathways. 3. Adenoviral PDPN-induction induces morphological change in keratinocyte: PDPN-overexpression of keratinocytes induced by adenovirus showed filopodia-like structure similar to TGFβ- or IFNγ-treated keratinocytes. 4. Stably PDPN-expressing HaCaT cell (HaCaT-PDPN) shows hypermotility associated with the activation of matrix matalloprotease (MMP) and cofilin: HaCaT-PDPN showed hypermotility by collagen-I-coated Boyden chamber assay. Activation of MMP-2/9 was detected by gelatin zymography. Active cofilin was increased in HaCaT-PDPN, and dominant-negative cofilin reduced the motility of HaCaT-PDPN. 5. HaCaT-PDPN shows downregulated adhesiveness and proliferation but upregulated Stat-3 activation induced by IL-22: HaCaT-PDPN showed decreased adhesiveness to collagen-I-coated plate and decreased proliferation rate. However, IL-22-induced phosphorylation of Stat-3 was upregulated in HaCaT-PDPN. In conclusion: PDPN might be one of the key molecules of keratinocyte biology which may be related to the pathomechanism of psoriatic and wounded epidermis.

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The histone demethylases LSD1 is differentially regulated the expression of the differentiation markers during the calcium-induced differentiation of human keratinocytes

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The histone demethylases, lysine-specific demethlase 1 (LSD1; KDM1), the first demethylase identified, specifically demethylate lysine 4 of histone H3 and contribute the regulation of gene transcription, and thereby controlling in development and differentiation. Along with histone methyl transferase such as mixed lineage leukemia (MLL), SMYD3 and SET1, which methylate lysine 4 of histone H3, the demethlase control the balances of homeostasis of mammalian tissues by the loss and gain of methylation on the residues. In this study, using the chemical, pargyline capable of inhibiting demthylase activity of LSD1, we examine the ability of the demthylases regulating the expression of the keratinocyte differentiation markers. After inducing the differentiation, at the time of 1 day, the expression of keratin1, early differentiation marker was relatively induced in the pargyline pre-treated NHEK compared to the mock-treated cells. But, at the time of 3 days, the expression levels of keratin 1, 10 and involucrin markers were downregulated in the pargyline-treated NHEK. Without triggering the differentiation by calcium addition, the pre-treatment of pargyline was not changed the expression levels of cyclin D1 and p21, which represent the state of cell proliferation or growth arrest. The effect of pargyline on the expression of differentiation markers was observed in a dose-dependent manner. The effect was showed a maximum at 3 mM concentration, more than the concentration, the effect was dramatically decreased. These results suggest that LSD1 plays some roles in regulating the expression of differentiation markers during the calcium-induced differentiation of NHEK.

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Expression of the homeobox gene, HOPX, is modulated by PKC dependent signaling pathways and is involved in the expression of differentiation markers during the differentiation of human keratinocytes

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Homeodomain only protein X (HOPX), an unusual homeodomain protein was originally identified as a key regulator of cardiac development. We first demonstrated that the expression of HOPX was dependent on the differentiation of human keratinocytes and has an effect on the expression of differentiation markers. HOPX was suppressed in proliferating human keratinocytes and was gradually induced by calcium-triggered differentiation of human keratinocytes. In the epidermis, HOPX is highly expressed in the terminally differentiated suprabasal layers. Among the transcript variants of HOPX, the variant 3 driven by promoter A was the main transcript and it was regulated by cell differentiation in human keratinocytes. The expression of HOPX was induced through the phorbol-12-myristate-13-acetate (PMA)-dependent protein kinase C (PKC) signaling pathway, and not by the demethylating agent, 5-aza-dC (5-aza-2′-deoxycitidine) suggesting the suppression of HOPX is not associated with DNA methylation in human keratinocytes. The RNA interference (RNAi) silencing experiment showed that the knockdown of HOPX expression resulted in the increase of such differentiation markers as involucrin and loricrin. Exogenous expression of HOPX down-regulated the expression of differentiation marker genes in immortalized human keratinocytes (HaCaT). Collectively, HOPX is modulated by cell differentiation in human keratinocytes and this might contribute to homeostasis of keratinocytes by controlling differentiation-dependent genes.

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Ability to Achillea millefolium and Prunus spinosa to increase the expression of MC-2R and MOR-1 in keratinocytes and improve epidermal differentiation

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In the skin, the proopiomelanocortin (POMC) pro-peptide gives rise to α-melanocyte- stimulating hormone (α-MSH), adrenocorticotrophic hormone (ACTH) and β-endorphin, whose respective receptors are MC-1R, MC-2R and μ- opioid receptor 1 (MOR-1). These three POMC-related peptides exert a broad action in skin physiology (skin pigmentation, keratinocyte proliferation and/or differentiation, anti-inflammatory role). In a recent publication, we showed that, in basal keratinocytes, MC-1R, MC-2R and MOR-1 gene and protein expressions decreased with aging whereas POMC gene expression increased, thus providing evidence for a disturbed neurohormonal balance between ligands and receptors that could partly account for the age-related epidermal thinning. In order to restore the balance between POMC-related peptides and their respective receptors in aging skin, we used real time RT-PCR methods to screen 1000 ingredients in their ability to upregulate MC-2R and MOR-1 receptors without changing POMC and MC-1R expressions in cultured keratinocytes. Screening results were then confirmed by measuring the protein expression levels. This way, we observed that two plant extracts of Achillea millefolium and Prunus spinosa were able to upregulate MC-2R and MOR-1 in a dose dependent manner. We showed that Achillea millefolium and Prunus spinosa used at 0.5% stimulated MC-2R gene expression by 5.6 and 2.5-fold respectively and increased MOR-1 gene expression by 24.3 and 2.2-fold respectively. Eventually, we showed that Achillea millefolium and Prunus spinosa did not modify keratinocyte proliferation while improving keratinocyte differentiation, as evidenced both by an increase in involucrin gene expression in cultured basal keratinocytes and by an increased expression of the intermediate markers involucrin and transglutaminase of the spinous layer in our skin equivalent model. These *in vitro* results provide new evidence for the relevance of restoring POMC-derived peptides and their receptors in order to improve epidermal homeostasis.

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Genetic over-expression of protease-activated receptor-2 in mouse epidermis leads to skin inflammation, pruritus and neurotrophin release *in vivo*

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Protease-activated receptor-2 (PAR-2) has been implicated in various inflammatory skin diseases with pruritus such as atopic dermatitis. Recently, we have shown that PAR-2 agonist application into human skin leads to neurogenic inflammation and pruritus, Furhtermore, an increased expression of PAR-2 and kallikreins was observed in keratinocytes of atopic dermatitis patients. The underlying cellular mechanisms of PAR-2 induced inflammation and pruritus in the skin are still not understood. Behavioral and histological analyses, including immunohistochemical staining and immunofluorescence labeling, and quantitative polymerase chain reaction were performed *ex vivo* and *in vitro* studies using C57BL/6 transgenic mice which over-express PAR-2 in keratinocytes. Our results show that PAR-2 over-expressing mice developed dry skin, spontaneous eczematous skin lesions in ears together with increased scratching bouts. The histological analysis of the lesional ear skin specimen has revealed epidermal acanthosis and inflammatory cell infiltration within the papillary dermis. Immunofluorescent labeling of PGP9.5 has shown the elongation of an increased number of sensory nerve fibers in the dermis and into the epidermis. The up-regulated expression of nerve growth factor in ear skin has been demonstrated by quantitative polymerase chain reaction. In sum, our results suggest that an over-expressed level of PAR-2 in the epidermis leads to atopy-like skin inflammation accompanied with neurotrophin release, nerve elongation, and increased scratching. This indicates a possible therapeutic effect of PAR-2 neutralizing antibodies/antagonists for inflammatory skin diseases with pruritus such as atopic dermatitis or prurigo, for example.

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Nummular eczema is highly associated with diminished innervation of the epidermis

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Nummular eczema is a chronic inflammatory condition characterized by extremely pruritic eruptions of scaly "coin-shaped" plaques. It is considered a form of adult onset of atopic eczema. The current study hypothesized the number of immunoreactive nerve fibers is increased in nummular eczema, similar to atopic eczema and other chronic pruritic skin diseases. We studied 22 tissue samples of biopsy proven nummular eczema and 8 control biopsies of disease free skin from similar body areas and age. Nerve fibers were immunolocalised with the pan-neuronal marker PGP9.5. Confocal microscopy and a bespoke image analysis algorithm were used to subsequently analyze the images. Results indicate a significant decrease in total amount of nerve fibers ($p=0.0054$) in nummular eczema versus healthy control (0.35pi vs. 1.33pi). PGP9.5 density (immunostained quantity/distribution area) was 4-fold less in nummular eczema compared to healthy skin ($p=0.0015$). No differences were observed in the papillary dermis between nummular eczema and healthy control. In conclusion, we refute the common notion that chronic pruritic conditions are characterized by increased nerve fiber amount and density in the epidermis. In addition we found that dermal innervation in nummular eczema was unaffected by the pathology. The mechanism for the loss of intraepidermal nerve fibers remains unknown. We postulate that the loss of nerve fibers leads to hypersensitivity of remaining itch selective nerve fibers as the cause of the intense pruritus in this condition. Further studies assessing the receptors expressed on these remaining itch selective free nerve endings are important in helping to elucidate the cause of pruritus, and lead to targets for itch therapy.

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Regulation of epidermal tight junction proteins, claudin-1 and -4, by T-helper 1(Th1)- and Th2-type cytokines

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Tight junction (TJ), especially claudin-1, plays a critical role in epidermal permeability barrier function. Reduced expression of claudin-1 and polymorphisms in the gene region encoding claudin-1 were reported to be associated with atopic dermatitis(AD). T-helper 2(Th2)-mediated inflammation is a characteristic feature of AD, which causes barrier defects. To extend this concept to the level of TJ, in this study, we investigated the role of Th2-type cytokines in the regulation of claudins. We also assessed the effect of Th1-type cytokines on the expression of claudins. In HaCaT keratinocytes which cultured with 1.8 mM Ca2+, claudin-1 and -4 were observed to be expressed at the cellular margins as continuous ring pattern. After 2 h treatment with IL-4, claudin-1 was decreased and showed altered expression pattern with cytoplasm staining distinct from the normal continuous ring pattern. IL-13 also decreased and altered the expression of claudin-1 in HaCaT after 2 h of incubation. We confirmed that IL-4 and-13, at a concentration we used, did not affect the viability of HaCaT using MTT assay. Intradermal injection of IL-4 into the hairless mouse skin slightly decreased the immunoreactivity of claudin-1 compared to that of untreated epidermis, however, claudin-4 expression was not affected. IL-13, when injected, also decreased claudin-1, but not claudin-4. In addition, we observed a significant decrease of claudin-1 expression in mouse skin treated with intradermal injection of another Th2-type cytokine, IL-5. In contrast to Th2-type cytokines, intradermal injection of IL-1α did not affect the immunoreactivity of claudin-1 and -4. Moreover, tumor necrosis factor (TNF)-α seemed to slightly increase claudin-1 expression. From these findings, we suggest that Th2-type cytokines might play an important role in the regulation of claudin-1 in AD patients.

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Caspase-14 participates in the processing of prosaposin during the terminal differentiation

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Caspase-14 (casp-14) is expressed most dominantly in the epidermis and its activation occurs at the onset of cornified cell formation. However, its physiological roles are still obscure. In order to find out interacting molecule(s) with casp-14, we employed a proteomics approach. Protein extracts obtained from cultured keratinocytes, human corneocytes and psoriatic scales were incubated with Sepharose-immobilized active casp-14 or pro-casp-14. Proteins bound to casp-14 were identified with LC/MS/MS analyses. By comparing those lists of proteins, we selected prosaposin as a candidate molecule, which was found only in the extracts from differentiated keratinocytes and corneocytes as an active-casp-14 interacting protein. Saposins are sphingolipid activator proteins and required for epidermal permeability barrier formation. Since we actually verified saposin A sequence in the analyses, we prepared cleavage-site directed antibody to saposin A. This antibody recognized only saposin A, but not prosaposin. We then tested the interaction between casp-14 and prosaposin. Casp-14 showed limited proteolysis on prosaposin, generating saposin A and other intermediate products. Immunohistochemical study showed that prosaposin localized in the living layer of human epidermis and saposin A was detected at the upper granular layer. Proximity ligation assay suggested that saposin A and casp-14 interact together in the granular layer of the epidermis in vivo. In contrast, although prosaposin staining was similar to that of normal human epidermis, saposin A staining was markedly decreased in the lesional skin with atopic dermatitis (AD). Active casp-14 specific antibody also revealed that it was hardly detectable especially in the parakeratotic area of AD in contrast to the strong staining of cornified layer of the normal epidermis. Our results suggest that casp-14 plays an important role for the permeability barrier formation and loss of active casp-14 may be related to the barrier disruption of AD.

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The effect of protease activated receptor-2 on the expression of epidermal tight junction protein, claudin-1

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Skin is now considered to play a critical role in sensitization against allergens, through barrier dysfunctions. Proteolytically active allergens cause barrier dysfunction by regulating corneodesmolysis, inflammation, and lamellar body secretion partially via protease-activated receptor 2 (PAR-2) signaling. Tight junction (TJ) also consists of functionally active epidermal barrier, therefore, we investigated the effects of cockroach allergen, which is known to have serine protease activity, and PAR-2 on the expression of claudin-1. Acute barrier disruption by repeated tape-stripping caused changes in the expression of claudin-1 as fragmented dot-like pattern compared to that of normal epidermis which observed as continuous lines in hairless mouse skin. This barrier disruption-induced breakdown of claudin-1 expression restored at 1 h after tape-stripping, however, cockroach allergen, when topically applied on the barrier disrupted site, delayed the restoration of claudin-1 expression following acute barrier disruption. The intradermal injection of PAR-2 agonist peptide (AP), NH2-SLIGKV, into the mouse skin also decreased the immunoreactivity of claudin-1 and caused breakdown of claudin-1 expression as dot-like pattern. In cultured HaCaT keratinocytes, treatment with cockroach allergen for 2 h significantly decreased claudin-1 compared to that of untreated HaCaT. Redistribution of claudin-1 immunoreactivity into the cytoplasm of HaCaT cells frequently found in allergen-treated group compared to that of untreated group which showed continuous ring-like pattern. AP, SLIGRL-NH2, also slightly down-regulated the expression of claudin-1 in HaCaT after 1 h of treatment. These findings suggest that the allergen protease activity weakens the intercellular permeability barrier of the TJ, and these effects might be partially mediated by PAR-2 signaling.

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EGFR, caspases and PI3K but not apoptosis are involved in Pemphigus vulgaris pathogenesis

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The autoimmune blistering disease Pemphigus Vulgaris (PV) is characterized by autoantibody binding to desmoglein3 (Dsg3), which triggers a variety of signaling pathways shown to be involved in acantholysis. Opposite results were published on the role of EGFR and apoptosis. To clearly demonstrate whether these pathways but also PI3K (activated by Dsg3 or EGFR) mediate acantholysis in PV, primary mouse and human keratinocytes were incubated with specific inhibitors of these pathways with or without AK23, a pathogenic mouse monoclonal Dsg3 antibody. As a read-out we used adhesion assay, monitored keratin retraction and investigated apoptosis by TUNEL assay and PARP cleavage. For the adhesion assay, inhibitors of EGFR and its downstream effectors (JNK, Src and p38), caspase III and VI inhibitors as well as inhibitors of PI3K downstream signaling molecules (GSK3, mTor and c-Myc) reverted AK23-induced cell detachment. In contrast, the Erk pathway was not involved in AK23-dependent events. Furthermore, the PI3K inhibitor (LY294002) effect was time-dependent in mouse keratinocytes, with a long-time incubation reverting the effect of AK23 (consistent with EGFR activation) but a short-time increasing it (consistent with loss of Dsg3 transadhesion). Regarding AK23-mediated keratin retraction, caspase, EGFR, JNK, p38 (in human keratinocytes), Src and GSK3 inhibitors reverted AK23 effect, whereas p38 (in mouse keratinocytes) and ERK inhibitors induced keratin retraction and c-Myc, mTor and PI3K inhibition showed no reversion. Our results above indicated that caspases play a role in PV. To distinguish apoptosis-dependent and -independent roles of the caspases, TUNEL positive cells and PARP cleavage were analyzed in control and AK23-treated cells. No sign of apoptosis was observed. Taken together, these results demonstrate that EGFR, caspases and PI3K but not apoptosis play a role in Pemphigus vulgaris pathogenesis, and that keratin retraction is not a necessary prerequisite for loss of intercellular adhesion in cultured keratinocytes.

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Serglycin in keratinocytes

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We have recently shown that UVB induces serglycin in endothelial cells and fibroblasts. Serglycin is a proteoglycan core protein that is involved in granule integrity in hematopoietic cells, intracellular storage, and may have immunoregulatory functions. However, the presence of serglycin in human keratinocytes (KCs), and possible effect of UVB has not been demonstrated. Sections of UVB-irradiated human skin were examined using immunohistochemistry with anti-serglycin antibody. In addition, cultured KCs were exposed to UVB, with serglycin mRNA levels quantified by real time PCR, and intracellular serglycin labeled using standard immunofluorescence techniques and examined by deconvoluted microscopy. Staining intensity (in pixels) was measured by the ImagePro plus software v5.1. We report positive serglycin staining in the epidermis of skin sections, with increased nuclear staining after UVB exposure. Real time PCR and Western blot analysis both revealed the presence of serglycin mRNA and proteins, respectively, in human epidermis and cultured KCs. Immunofluorescence study of cultured human KCs revealed the presence of serglycin, with staining intensity within the KCs increased after UVB exposure, compared to non-irradiated (sham-treated) cells. Specifically, comparison of serglycin staining intensity in sham-treated versus UVB-treated cells revealed; mean intranuclear intensity of 0.34±0.13 versus 0.99±0.34, ($p<0.05$); mean perinuclear intensity of 0.44±0.23 versus 1.44±0.46, ($p<0.05$); and mean cytoplasmic intensity of 0.18±0.12 versus 0.78±0.32, ($p<0.05$). Exposure of the cultured KCs to UVB did not result in increased expression of serglycin mRNA, suggesting post-transcriptional regulation of serglycin. We have shown the presence of serglycin in human skin and cultured KCs. The role of serglycin in KCs is unknown, but may involve the skin's response to UVB.

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Cavitation effects: Ultrastructural exchanges and enhancement of epidermal permeation by the low-frequency ultrasound in hairless mice

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The effects of transdermal drug delivery(TDD) using low-frequency sonophoresis (LFS) have been demonstrated and many studies about delivery of various low- as well as high-molecular weight molecules via transdermal route using LFS have been reported. The cavitation effect is one of the important mechanisms of skin permeation by LFS. But, morphologic studies to find and evaluate the exact cavities have not been undergone actively. In this study, we used a different type of LFS device from that which have been used previously and examined the ultrastructural morphology of cavities with a transmission electron microscope and scanning electron microscopy. The device used in this study works with the ultrasound probe directly attached to the hairless mouse skin. We applied ultrasound to normal and tape stripping mouse skin. After the LFS, 1um~3um sized cavities were observed in mostly stratum basale, especially in the intracellular spaces. After the combined use of LFS and tape-stripping, 2um~10um sized, continuously extended lacunae were observed in some areas of the stratum corneum. Our results shown that most cavities were observed in stratum granulosum and stratum basale probably due to the ultrastructural property to form the cavities easily. We suggest that these continuous formation and disappearance of cavities may have a role in the formation of a route of skin penetration.

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Serum response factor (SRF) controls transcriptional network regulating epidermal function and hair follicle morphogenesis.

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A transcription factor SRF regulates expression of growth-related, cytoskeletal and muscle-specific genes to control growth, differentiation and cytoskeletal integrity in different cell types. Our previous studies, using mice lacking SRF in the epidermis, show an essential role for SRF in regulation of epidermal differentiation and barrier formation (Hindes A et al., JID, 2008, 128, S1:637). To explore the molecular mechanisms underlying the role of SRF in acquisition of functional epidermal barrier, we carried out transcriptional profiling studies. Our data revealed profound molecular changes in SRF-null versus wild-type control E17.5 epidermis, including alterations in expression of genes involved in epidermal development/differentiation, lipid biogenesis, transcriptional regulation, adhesion, cytoskeletal organization, growth signaling, wound response and inflammation. Loss of SRF led to altered expression and localization of AP1 and C/EBP transcription factors in epidermis. Unexpectedly, many known SRF target genes were found to be markedly upregulated in SRF-null epidermis (i.e. *Junb, Erg2, Krt17, Fgf10*), indicating that SRF may function to repress transcription of a subset of its targets in this tissue. Our data also showed that multiple genes linked to hair follicle morphogenesis, including genes encoding components of Wnt and Shh signaling complexes, were down-regulated in SRF-null epidermis, and engrafted SFR-deficient skin lacked hair. Notably, engrafted SRF-null skin displayed normal epidermal architecture and normalized expression of differentiation markers 14 weeks after transplantation onto immune deficient mice. These data suggest that SRF controls transcriptional network governing epidermal barrier acquisition and hair follicle morphogenesis.

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The effects of change in epidermal calcium gradient on the expression of tight junction protein, claudin-1

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Tight junctions (TJs) are the most apical component of the junctional complex, which are often referred to as barrier and fence function. Epidermal calcium ions have been reported to be required to maintain permeability barrier homeostasis. As acute barrier disruption decreased calcium levels in stratum granulosum, we studied the regulation of tight junctions in murine skin following acute barrier disruption and investigated the effects of change in epidermal calcium gradient on the expression of tight junction protein, claudin-1. The expression of claudin-1 after acute barrier disruption was decreased immediately after tape-stripping and gradually recovered in hairless mouse skin at 30 min after tape-stripping. One hour after treatment, claudin-1 expression seemed to be almost fully recovered. However, when the tape-stripped site was immersed in a calcium containing solution immediately after treatment, the recovery of claudin-1 expression was partially inhibited. Moreover, when barrier-disrupted site was covered with an occlusive membrane, the recovery of claudin-1 expression was delayed. To manipulate epidermal calcium gradient change, we also performed sonophoresis to mouse skin at the energy which did not induce barrier perturbation as our previous study. Claudin-1 expression was decreased after sonophoresis treatment, producing an immediate loss of the calcium gradient without skin barrier disruption. However, sonophoresis of gel containing 1.5 mmol/L Ca2+ did not affect the expression of claudin-1. These results suggest that the change in epidermal calcium ions has an important role in the regulation of epidermal TJs structure, especially claudin-1 on murine skin.

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Upregulation of elastogenesis in the skin and its potential for skin aging benefits

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The elastin fiber network is responsible for the elasticity of our skin allowing it to resume its shape after stretching or contracting. In the dermal connective tissue, the thin elastin fibers represent approximately 5% of dermal compartment by dry weight. Functional elastin fibers are made by properly linking many soluble tropo-elastin protein molecules in a reaction catalyzed by lysyl oxidase, to make a large insoluble, cross-linked array. With aging, the elastic fibers progressively degrade and eventually disappear in the area just below the dermal-epidermal junction. The creation of new, functional elastin requires a comprehensive approach that targets all aspects of elastogenesis. Upregulating the creation of the elastin fiber elements (ie. tropo-elastin, microfibrils) while simultaneously increasing the levels of the cross-linking enzymes can facilitate the production of new elastin fibers in the dermis. We present two natural ingredients, Blackberry Leaf and Dill, which together work to enhance the skin's elastin fiber network. In vitro data shows that Blackberry Leaf inhibits the enzymes responsible for the digestion of elastin while upregulating the production of tropo-elastin, an important elastin fiber building block. Dill extract has been shown to increase the expression of LOXL1, a cross-linking enzyme that assembles elastin-related proteins into functional fibers. In a 12-week, round-robin, double-blind, randomized clinical biopsy study the effects of different formulations containing either Blackberry Leaf or Dill extract alone and the combination of both natural extracts were examined. Subjects applied products to the upper inner arm twice daily. Instrumental assessments were conducted at baseline and week 12. 2-mm skin biopsies were taken from each treatment site at the end of the study. Histological analysis of skin biopsies and instrumental assessments on the treated skin suggest that the combination of Blackberry Leaf and Dill extracts upregulated the creation of new, functional elastin fibers in the upper dermis.

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Chemical and biological compatibility of calcipotriene and clobetasol foam formulations

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VitD analogs such as calcipotriene (CAL) are known to regulate epidermal proliferation and differentiation. The efficacy of topical CAL in the treatment of psoriasis is relatively low compared to that of topical steroids such as clobetasol propionate (CP), which act by reducing inflammation. Because of their complementary modes of action, CAL and CP are often applied concomitantly to treat psoriasis. We studied the compatibility of a CAL 0.005% foam formulation with a CP 0.05% foam formulation by examining: 1) the potential chemical degradation of the drug substances when the products are mixed together; and 2) the activity and irritation potential of CAL foam when the products are applied in combination to reconstructed human epidermis (RHE). To examine chemical compatibility, equal amounts of CAL and CP foams were mixed together and incubated at 40°C. At various timepoints up to 48 hrs, concentrations of CAL and CP were determined by UPLC. The degradation of CAL and CP was <2%, suggesting that the products will be chemically stable when mixed together on the skin surface. To assess the activity and irritation potential of CAL foam in the presence of CP foam, formulations were applied topically alone or in combination to RHE cultures, and gene expression of filaggrin (FLG), evaluated by qRT-PCR, served as a biomarker of CAL activity. Irritation potential was evaluated by measuring IL-1α release and histology. Application of CAL foam caused a marked downregulation of FLG compared to untreated and vehicle controls. CAL+CP-treated cultures caused a similar downregulation of FLG, suggesting that the activity of CAL foam is not negatively affected by co-application with CP foam. There was no increase in IL-1α release nor any evidence of cytotoxicity by histology in the combination group compared to those treated individually. In conclusion, the results demonstrate that CAL and CP foam are compatible both chemically and biologically, supporting their use in combination.

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Epidermal ablation of Dlx3 is linked to a disruption of barrier formation and a skin inflammatory response

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Dlx3, expressed in the differentiated layers of the epidermis, is a homeodomain transcription factor that induces the terminal differentiation of keratinocytes. We have previously shown that the conditional deletion of Dlx3 in the epidermis results in complete alopecia and epidermal hyperplasia, demonstrating that Dlx3 is a crucial regulator of hair and skin development. Here we report that the K14Cre-mediated deletion of Dlx3 expression leads to an epidermal barrier defect at late embryonic development and early postnatal stages. The barrier abnormality was detected in cKO E17.5 embryos by performing skin permeability assays, and immunofluorescence on neonatal skin indicated that expression of epidermal differentiation markers such as filaggrin and loricrin were affected in the cKO mice. Microarray analysis of the skin at P9 and P20 recapitulated the misexpression of genes involved in epidermal differentiation while also suggesting an inflammatory response exhibited by the up-regulation of cytokines and chemokines. Skin inflammation in the cKO mice was confirmed by histological analysis with the infiltration of leukocytes and T cells into the skin at five days after birth. The advancement to a systemic inflammatory response was shown by immunochemical analysis of the serum and the enlargement of the spleen and lymph nodes. The results suggest that Dlx3 regulatory function is necessary for normal skin barrier formation and that epidermal deletion of Dlx3 is linked to the development of an inflammatory response.

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Regulation of skin barrier function and inflammation by patented natural peptides

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Skin barrier function resides principally in the *stratum corneum* (sc) with its key elements: corneocytes, cornified envelope, intercorneocyte lipids and corneodesmosomes. Formation of corneocytes is a result of the finely regulated keratinocyte differentiation process, associated with the sequential formation of differentiation proteins: keratin 1 and 10, early markers which will be aggregated by filaggrin into resistant cytoplasmic network; involucrin and loricrin, late markers forming the cornified envelope. Adequate hydration is essential for optimal skin function, contributing to maintain the sc suppleness. Permeability barrier disruption initiates inflammation by two steps: massive entry of the aggressor and subsequent release of early inflammatory mediators. *In vitro* effects of natural patented peptides on different aspects of skin barrier and inflammation were investigated. Gene expression of K1, K10, loricrin, involucrin, desmoglein-1 and PAR2 were studied in keratinocytes. Hyaluronic acid and Glycosaminoglycans release were measured in keratinocytes. Releases of IL1α, IL8, PGE2 and VEGF were quantified in an inflammation model; NF-κB nuclear translocation was followed by immunofluorescence. Release of histamine by mast cells was measured. Gene expression of K1, K10, loricrin, involucrin and desmoglein-1 were stimulated. Production of hyaluronic acid and GAGs were significantly enhanced. Inhibitory properties were shown against aspecific inflammation (IL1α, IL8, PGE2, VEGF and NF-κB translocation) and neurogenic inflammatio(histamine). Gene expression of PAR2 was reduced. These patented natural peptides might help the skin to elaborate an optimal barrier, maintain optimal hydration and modulate aspecific as well as neurogenic inflammatory reaction. These effects might be achieved by modulation of PAR2, in fact this receptor seems to be involved in many inflammatory dermatitis. Thus, these peptides could be of interest in the management of cutaneous conditions with altered skin barrier and exacerbated inflammation.

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A novel neuroendocrine control of human mitochondrial activity and biogenesis *in situ*: TRH and TSH power mitochondria in human epidermis

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We demonstrate that thyrotropin (TSH) and thyrotropin-releasing hormone (TRH), the key neuroendocrine regulators of thyroid hormone production, also operate as non-classical stimulators of mitochondrial function. Both TRH and TSH potently up regulate mitochondrial activity, efficacy and capacity in normal adult human epidermis in situ. In organ-cultured human scalp skin, treatment with TSH and TRH strongly up-regulate the number of light-microscopically visualized, immunoreactive intracellular dots labelled by a mitochondria-selective antibody raised against MTCO1, which selectively demarcates mitochondria. Electron microscopy confirms on the ultrastructural level that TSH and TRH stimulate mitochondrial proliferation and biogenesis. In epidermal extracts, TSH and TRH enhance mitochondrial complex I and IV activity, as measured by several complementary biochemical assays. Moreover, TRH upregulates transcription of mitochondrial key genes: MTCO1, mitochondrial transcription factor A, heat shock protein 60, peroxisome proliferator-activated receptor gamma coactivator alpha, and brain and muscle ARNT-like protein 1, suggesting complex effects of TRH on mitochondrial energy metabolism and entrainment. A pilot experiment suggests that TSH may increase oxygen consumption of human skin. These studies pioneer the concept of a unsuspected, but potent neuroendocrine control of mitochondrial energy metabolism and biogenesis. In addition, we introduce human skin organ culture as a physiologically and clinically relevant research tool for dissecting the hormonal controls of human mitochondrial biology *in situ*.

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Effects on proliferation, migration and cytoskeleton of keratinocytes and fibroblasts of a secretion of the mollusk cryptomphalus aspersa

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Regenerative properties of skin decrease with age and, thus, it is of pharmacological interest the research for natural products that stimulate the main cell types of skin. In this sense, we have analyzed the effects of the secretion of the mollusk Cryptomphalus aspersa (SCA) on keratinocytes (HaCaT cells) in vitro; particularly on proliferation, migration and on the expression of cell-cell and cell-substrate adhesion proteins. The results obtained how stimulation of the proliferation and migration (wound healing assay) of HaCaT cells, which are related with the SCA concentration employed (25, 50 y 100 g/ml) and the type of treatment (1-7 days). In addition, SCA stimulates rearrangement of the actin cytoskeleton and increases the expression of cell-cell (E-cadherin, -catenin) and cell-substrate (vinculin, 1-integrin); both being determined by immunofluorescence and Western blot analysis. Finally, we have observed that SCA not only promotes cell proliferation, but also seems to improve cell survival by increasing nuclear expression of active -catenin and the focal adhesion kinase. Similar results to those obtained with keratinocytes, related with cell migration and cell-substrate adhesion, have been observed in primary cultures of fibroblasts subjected to SCA.

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Inactivation of autophagy in murine epidermal keratinocytes is associated with hair loss and sebaceous gland abnormalities

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Autophagy is an evolutionarily conserved lysosomal degradation pathway that removes damaged cytoplasmic components and organelles. The role of autophagy in terminal differentiation of keratinocytes and in the homeostasis of the epidermis is not known. We have therefore generated a mouse line which is deficient in one of the essential autophagy-related genes, ATG7, in keratin14 expressing tissues, and investigated the structure and function of their skin. Although autophagy was efficiently abrogated in mutant epidermis, as evidenced by the lack of LC3 II, a marker of autophagosome maturation, these mice were viable, and showed no gross skin or hair abnormalities. Keratin expression was normal, but loricrin expression and keratohyalin granule accumulation were elevated, the latter particularly in older mice. Electron microscopic examination of ear skin indicated differences in lamellar body and mitochondrial turnover, and subtle differences in stratum corneum structure. Nevertheless, transepidermal water loss was similar between the two groups, both in unperturbed skin and after tape stripping. Older mutant mice, as well as some younger ones suffered hair loss on the nape and head. Examination of the dorsal sebaceous glands revealed that these were larger in mutant mice, and produced more sebum than those of controls. An even more profound effect of ATG7 inactivation was on the preputial glands, which showed signs of atrophy, and aberrant sebocyte differentiation. We conclude that ATG7 dependent autophagy is not critical for the structure and function of resting epidermis, but that it contributes to sebaceous gland biology.

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Plakophilin-1 protects keratinocytes from pemphigus vulgaris IgG by promoting desmosome assembly

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Pemphigus vulgaris (PV) IgG bind to the extracellular domain of desmoglein 3 (Dsg3) and disrupt desmosomal cell-cell adhesion. PV IgG binding also causes Dsg3 endocytosis, rearrangement of desmosomal components and retraction of keratin filaments. Cytoplasmic components of the desmosome regulate desmosome assembly and cell adhesion, suggesting that these proteins could be manipulated to blunt the effects of PV IgG. Plakophilin-1 (PKP-1) is a key desmosomal plaque protein that drives desmoplakin recruitment to desmosomes. To investigate the role of PKP-1 in PV and Dsg3 regulation, keratinocytes were infected with adenoviruses carrying full length PKP-1 or empty vector (EV) control and treated with normal human (NH) IgG or PV IgG. Remarkably, PKP-1 expression prevented mislocalization of Dsg3 and other desmosomal components in PV IgG treated cells. Furthermore, PKP-1 also prevented the loss of keratinocyte adhesion caused by PV IgG. To determine the mechanism by which PKP-1 prevents loss of keratinocyte adhesion strength, the ability of PKP-1 to block Dsg3 endocytosis in PV IgG treated cells was examined using a series of pulse-labeling and time lapse fluorescence microscopy approaches. Surprisingly, PKP-1 had no affect on Dsg3 endocytosis. Rather, PKP-1 enhanced the recruitment of newly synthesized and/or recycled Dsg3 to cell-cell borders. To further understand how PKP-1 modulates Dsg3 trafficking, the Dsg3 cytoplasmic tail was fused to the non-adhesive IL2-receptor (IL2R) extracellular domain. Interestingly, PKP-1 caused clustering of the IL2R-Dsg3 fusion protein in a manner that required the plakoglobin binding domain of the Dsg3 tail. Co-immunoprecipitation experiments indicated that PKP-1 forms biochemical complexes with the Dsg3 tail that include both plakoglobin and desmoplakin. Collectively, these findings indicate the PKP-1 prevents loss of adhesion in PV IgG treated keratinocytes by driving the clustering and desmosomal assembly of newly synthesized or recycled Dsg3 through interactions with the Dsg3 cytoplasmic tail.

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CD133 enriches for murine epidermal stem cell *in vivo*.

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The maintenance, repair and renewal of epidermis are thought to depend on a pool of dedicated epidermal stem cells. Like for many somatic tissues, the isolation of a nearly pure population of stem cells has been a primary goal in skin biology. More work is needed to define markers for effective isolation of epidermal stem cells. Here we used a quantitative transplantation assay to assess the long-term repopulating ability of murine epidermal stem cells. We first characterized this transplantation assay by immunostaining of long-term epidermal repopulation units derived from cells injected subcutaneously (involucrin, filaggrin, laminin, CD34, K19, K14). Lineage-tracing studies using Vybrant® DiI/DiO indicated that the epidermal repopulation units were clonal proliferations. We isolated a discrete population of fresh primary murine neonatal keratinocytes on the basis of the putative stem cell marker CD133. We showed that the $CD133^+$ population was enriched for long-term repopulating murine epidermal stem cells compared to total cells (4-fold) while the $\alpha6^{hi}CD71^{lo}$ population was not. In addition, the $CD133^-$ population was depleted of stem cells compared to total cells (0.14-fold). Evidence for self-renewal capacity was demonstrated by serial transplantation of long-term epidermal repopulation units derived from the $CD133^+$ cells and the production of a range of cellular phenotypes similar to the original unsorted population. $CD133^+$ cells also exhibited nuclear expression of the stem cell associated antigen Bmi-1. Thus, murine keratinocytes within the $CD133^+$ population regenerate epidermis for the long-term, are self-renewing, and exhibit nuclear Bmi-1 expression, defining them as epidermal stem cells.

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Assessment of ammonium-ions of the skin surface and its relation to skin surface-pH: A potential basis for further investigation of the hydrolipid film of the skin surface

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Determination of ammonium-ions (NH_4^+) of the skin surface as part of the pH-dependent ammonia (NH_3)-NH_4^+ system may reveal new insights into the hydrolipid film of the skin surface. The aim of the present study is to assess the relation between NH_4^+ and skin surface-pH (pH). Since cigarette smoke contains large amounts of NH_3 smoking status is particularly considered. Overall, 40 woman aged 18-30 (20 non-smokers (NSM) and 20 smokers (SM)) were included. NH_4^+ from the forearm (FA) and from the forehead (FH) was measured using the Berthelot's reaction. PH was assessed using a glass electrode. Site-dependent differences of the parameters were analysed using t-test/Wilcoxon-test as well as correlation analysis. The relation between NH_4^+ and pH was assessed using correlation analysis. Analyses were performed for all volunteers together (ALL) and for NSM and SM separately. For ALL NH_4^+ and pH were higher on the FA than on the FH. However, the differences were not significant ($p>0,05$). The correlation analysis between FA and FH revealed significant positive correlations for NH_4^+ and pH ($p<0,001$). Also, a significant inverse relation between NH_4^+ and pH was assessed on the FA and FH ($p<0,001$). With regard to smoking status a tendency towards higher NH_4^+ in SM were registered (FA:$p=0,090$; FH:$p=0,136$) accompanied by a slight tendency towards lower pH values in smokers. All further results revealed close similarity to ALL. The strong inverse relation between NH_4^+ and pH of the skin surfac suggests that sweat gland activity contributing to the hydrolipid film of the skin surface may be an important source for NH_4^+ and H^+-ions. Moreover, sweat gland activity as fundamental mechanism that is modified locally may explain the strong correlations between FA and FH. Smoking appears to affect NH_4^+ directly or via pH-change. Studies with additional parameters are required to further evaluate the interrelationship of NH_4^+, pH of the skin surface and sweat gland activity.

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Novel mechanisms for keratinocyte specification

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Human embryonic stem (hES) cell replacement therapies have great potential for the treatment of skin diseases and disorders. However, differentiation protocols to induce keratinocyte differentiation from hES cells are inefficient, yielding only 20% of keratinocytes. These protocols involve the use of bone morphogenic protein-4 (BMP4) to induce ectoderm specification, which involves expression of keratins 8 and 18 (K8/18). Addition of serum induces keratinocyte specification, indicated by K14 expression. This process mimics the *in vivo* development of the epidermis which also requires BMP signaling and a switch from K8/K18 expression to K14 expression. We aim to uncover the mechanisms that drive the earliest stages of keratinocyte specification in order to improve hES cell based protocols used for skin disease therapies. To this end, we have investigated the process of keratinocyte specification using both hES cells and murine skin development. We find that the keratinocyte specification process involves a novel intermediate state of development between ectoderm and keratinocyte specification. We will present data defining mRNA expression profiles of specific cell populations during keratinocyte specification. These data illuminate a molecular signature for the development of skin from the ectoderm. In addition, these data highlight precise mechanisms of skin differentiation that could be exploited for efficient generation of differentiated keratinocytes for therapeutic purposes.

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A novel neuroendocrine control of keratin expression: Cannabinoid receptor (CB) 1-mediated signaling inhibits the expression of keratins K6 and K16 in human keratinocytes *in situ* and *in vitro*

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Endo- and exocannabinoids reportedly exert inflammation- and proliferation-inhibitory effects via CBs. Since human keratinocytes have been shown to express CBs, it is interesting to ask whether CB agonists might serve as a potential new therapy for hyperproliferative inflammatory skin diseases. Since psoriasis is characterized by enhanced epidermal expression of the hyperproliferation-associated keratins, K6 and K16, we have explored the hypothesis that CB stimulation may modulate their expression. Organ cultured human scalp skin or HaCaT keratinocytes were treated for 24h with arachidonoyl-chloro-ethanolamide (ACEA), a selective CB1 agonist. By quantitative immunohistomorphometry, ACEA significantly decreased K6 and K16 immunoreactivity (IR) in suprabasal epidermal keratinocytes in situ. This was partially reversed by the CB1 specific antagonist AM-251. K6 IR by HaCat cells was also decreased by ACEA. In line with these results, Q-PCR of ACEA-treated HaCaT cells demonstrated significant inhibition of *KRT6* gene transcription. In order to obtain indications how these effects of CB1 agonists are related to its anti-proliferative effects, double-immunostaining for K6 and Ki-67 was performed. The downregulation of K6 IR was accompanied by decreased Ki-67 expression, suggesting that, at least in part, the observed keratin expression are related to decreased proliferation of basal epidermal cells. However, the CB1 agonists also slightly downregulated K6 IR in non-proliferating epidermal compartments. Therefore, CB1-mediated signalling likely also directly down-regulates K6 expression. These results suggest a novel neuroendocrine control of human keratin expression *in situ* and *in vitro* via CB1-mediated signaling.

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ALDH$^+$CD44$^+$ and $\alpha6^{hi}$CD71^{lo} keratinocyte populations are enriched for human epidermal stem cell *in vivo*.

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The maintenance, repair and renewal of human epidermis is thought to depend on a pool of dedicated epidermal stem cells and the isolation of a nearly pure population of stem cells is a primary goal in skin biology. Here we used subcutaneous injections of human keratinocytes *in vivo* to assess the defining stem cell property of long-term repopulating ability. We characterized the epidermal repopulation units produced in this assay by immunostaining for involucrin, filaggrin, laminin, CD34, K19, and K14. Lineage-tracing studies using Vybrant® DiI/DiO indicated that the epidermal repopulation units produced (at the doses used) were clonal proliferations. We isolated a discrete population of fresh primary human neonatal keratinocytes on the basis of the putative stem cell markers ALDH and CD44. We showed that the ALDH$^+$CD44$^+$ population of human neonatal keratinocytes was enriched for long-term repopulating epidermal stem cells compared to total cells 12.8-fold and the $\alpha6^{hi}$CD71^{lo} population was enriched 6.2-fold. The enrichment for the ALDH$^+$CD44$^+$ population was marginally greater than that for $\alpha6^{hi}$CD71^{lo} ($p= 0.046$). In addition, the ALDH$^-$CD44$^-$and $\alpha6^{lo}$CD71^{hi} populations were depleted of epidermal stem cells compared to total cells. ALDH$^+$CD44$^+$ and $\alpha6^{hi}$CD71^{lo} populations of keratinocytes were also enriched in the stem cell associated antigen Bmi-1. This assay provides a functional assay of epidermal stem cells which can be used to pursue an almost pure population of human epidermal stem cells.

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Ozone-induced lipid peroxidation and inhibition by antioxidants

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Tropospheric ozone is a growing worldwide environmental problem. Increased air pollution, UV exposure, and elevated temperatures all contribute to the formation of ozone (O_3). Many studies, including those by the U.S. Environmental Protection Agency, have shown that average ozone levels in places such as Los Angeles can reach concentrations greater than 0.3 ppm. In order to determine the effect of ozone on epidermal biomolecules, we exposed phosphatidylcholine liposomes to environmentally relevant levels of ozone for three hours and induced significant levels of lipid peroxides as determined by the increase in thiobarbituric acid reacting materials (TBARM). Using this technique, 0.1% of an extract of white tea, which has been shown to possess antioxidant properties, protected liposomes against ozone-induced lipid peroxidation by 55.7%. Additionally, a commercially prepared ascorbyl tocopherol derivative at 0.1% also inhibited oxidation by 45.4%. In conclusion, these data demonstrate the susceptibility of an epidermal biomolecule to ozone and provide a model system to assess protective molecules against this form of oxidative stress.

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Involucrin–claudin-6 tail deletion mutant (CΔ206) transgenic mice: A model of delayed epidermal permeability barrier formation and repair

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The mammalian epidermal permeability barrier (EPB) is formed during development and is essential for survival as it maintains thermoregulation and hydration, and provides a defense against infection. Using transgenic mouse technology, we have demonstrated the importance of claudin (Cldn)-containing tight junctions (TJs) in epidermal differentiation and, in particular, that epidermal suprabasal overexpression of Cldn6 results in an EPB-deficient phenotype that phenocopies the dysfunctional EPB of premature human infants. In this study, we used the same approach to target a Cldn6 tail deletion mutant to the epidermis of mice [involucrin (Inv)-Cldn6-CΔ206 transgenic mice]. The Inv-Cldn6-CΔ206 transgenic mice displayed a developmental delay in EPB formation, as shown by the expression of keratins and Cldns, and by X-Gal penetration assays. Transepidermal water loss measurements and immunolocalization studies indicated that the epidermal differentiation program was also perturbed in postnatal Inv-Cldn6-CΔ206 transgenic mice resulting in a delayed maturation. Notably, however, expression/localization of epidermal differentiation and maturation markers, including Cldns, indicated that the transgenic epidermis matured and normalized by postnatal day 10, which is 3 days after the wild-type epidermis. Our results suggest that activation of the extracellular signal-regulated kinase 1/2 (Erk1/2) pathway and Cldn1 phosphorylation are associated with the repair and maturation of the skin barrier processes. These studies provide additional support for the crucial role of Cldns in epidermal differentiation, maturation and the formation of the EPB, and describe a novel animal model for evaluating postnatal epidermal maturation and therapies that may accelerate the process.

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Analysis by capillary electrophoresis of epidermal tryptase-like activity of stratum corneum layers obtained by tape stripping.

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Cathepsins and serine proteases in the skin play a significant role in the pathophysiological mechanism of several dermatological conditions and in the induction of itch. Tape stripping is a useful and practical method to study corneocyte function. Capillary electrophoresis (CE) was used to measure tryptase-like activity of corneocytes adhered to adhesive tapes. Tape discs with an inner diameter of 6 mm were applied to the skin and were submerged directly in a phosphate buffer containing the appropriate substrate peptide. After 2 hours of incubation, the split peptides were separated and detected directly by capillary electrophoresis at 214 nm in a borate buffer. The esterase activity on N-benzoyl-tyrosine ethylester and also the amidase activity on succinyl-Ala-Ala-Pro-Phe-p-nitroanilide were detected by CE. The esterase activity was much higher than that of the amidase. The protease activity was found to decrease with the depth of the stratum corneum layers analyzed. Scratching the skin prior to tape stripping yielded a three-fold increase in protease activity. The main advantages of CE are its simplicity, speed and the use of colorimetric non-expensive substrates. In conclusion, capillary electrophoresis is a suitable technique to study tryptase –like activity of corneocytes adhered to tapes which can aid in the study of itch and of the damage to the skin barrier function.

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Permeability barrier function under physiological and stress conditions: Evidence for impaired barrier integrity in filaggrin-related eczema

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Loss-of-function mutations in the human gene encoding filaggrin *(FLG)* have been shown to represent a strong genetic factor for atopic dermatitis (AD). The association between these mutations and AD has been replicated in a number of studies however so far there is only limited functional data on the permeability barrier function in filaggrin-related eczema. We investigated the epidermal barrier integrity in AD patients harbouring at least one of the prevalent or rare European *FLG* loss-of-function variants R501X, 2282del4, 3702delG, R2447X and S3247X in comparison to non-filaggrin AD patients and healthy controls by repeated measurements of transepidermal water loss (TEWL), stratum corneum hydration and erythema under physiological and stress conditions. While no significant differences in TEWL between the patients with non-filaggrin AD and the control group were observed (7.05±0.78 g/m^2/h, respectively 5.04±0.78 g/m^2/h, $p>0.05$), under basal conditions the *FLG* null mutation carriers had significantly increased TEWL compared to both non-filaggrin AD and the control group (10.22±1.08 g/m^2/h; $p<0.05$, respectively $p<0.001$) and additionally, significant reduction in stratum corneum hydration (capacitance) between the patients with filaggrin-related AD and controls was observed (27.75±2.30 AU, respectively 35.28±1.67 AU, $p<0.05$). Furthermore, under compromised conditions following controlled skin irritation, the patients with filaggrin-related AD displayed enhanced response and distinct reactivity pattern compared to non-filaggrin AD and controls. Our findings provide first functional data on impaired permeability barrier integrity under compromised conditions in AD patients carrying at least one *FLG* loss-of-function mutation and support further the pathophysiologic link between the null variants and the skin barrier abnormality in filaggrin-related eczema.

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The regulation of NHE1 by cytokines and calcium ions related to the epidermal permeability barrier homeostasis

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The 'acid mantle' of the stratum corneum seems to be important for both permeability barrier formation and cutaneous antimicrobial defense. However, the origin of the acidic pH, measurable on the skin surface, remains still unfolding. The Na+/H+ antiporter (NHE1) is an essential endogenous pathway responsible for stratum corneum (SC) acidification. The nonselective inhibitor of NHE1, was effective for increasing the skin surface pH and disrupted the normal skin barrier function. Recent studies have shown the co-regulation of permeability and antimicrobial barriers. Our previous study showed that the negative effect of the amiloride on the expression of antimicrobial peprides, which is co- related to various epidermal barrier functions, in human keratinocytes. We examined the decreased expression of NHE1 in chronic atopic dermatitis skin lesion and normal expression. The inhibition of NHE1 induced by type cytokines. It can be suggested the possibility of type II cytokines and increased skin pH of atopic dermatitis related to the NHE1. During the barrier recovery, the expression of NHE1 was regulated by the extracellular and intracellular calcium ion levels. Using various calcium ion controller, we confirmed that the expression of NHE1 in keratinocyte and murine skin was mediated by the calcium ion levels.

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Traction force microscopy reveals the mechanical heterogeneity of stratum-corneum

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The biomechanical properties of the stratum corneum are essential for the barrier function of skin, and change with environmental conditions and chemical treatments. While the composition of stratum corneum is highly heterogeneous, previous studies have assumed that it is mechanically homogeneous. To define the physical properties of the stratum corneum, we are developing a new traction force microscopy method to image the spatial distribution of forces in skin over time. We find that the response of stratum corneum to changes in humidity is highly heterogeneous. We observe non-uniform deformation of the stratum corneum which imposes spatially varying stresses on the underlying epidermis. These data provide the first measurement of local forces within a tissue sample and lay the foundation for future studies on the regulation of the barrier function due to mechanical cues.

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Identification of Sirt4 in normal human epidermal keratinocytes

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Sirtuin 4 (Sirt4) is a member of the conserved family of sirtuin proteins which are associated with metabolism and longevity. Unlike most sirtuins that exhibit deacetylase activity, Sirt4 is an NAD^+-dependent ADP-ribosyl transferase residing in mitochondria that inhibits glutamate dehydrogenase (GDH) activity. GDH inhibition can prevent glutamine from entering the TCA cycle and is, thus, an important factor in ATP production and amino acid-stimulated insulin secretion. Here we report on the first identification of Sirt4 in normal human epidermal keratinocytes (NHEK). Sirt4 transcripts were detected using real time RT-PCR and its expression was studied over time under nutritional duress. Additionally, mitochondria were isolated from NHEK and the presence of Sirt4 was then detected by western blot analysis. In conclusion, Sirt4 is an important metabolic regulator that has been observed in NHEK and its function may have implications for the maintenance of a healthy skin.

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Loricrin deficient mice as a model for atopic dermatitis

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Atopic dermatitis (AD) is a chronic, reoccurring skin disease that causes dry, itchy, inflamed skin, affecting 15-30% of children in industrialized countries. Genome wide association screens have identified linkage between AD and a region on chromosome 1q21 containing the epidermal differentiation complex (EDC), a conserved cluster of epidermal differentiation genes that include both loricrin (*LOR*) and filaggrin (*FLG*). The proteins generated from the EDC are responsible for the structural integrity of the cornified envelope (CE), making them important for barrier formation. Several groups have identified *FLG* as a major genetic risk factor associated with AD, and mice lacking *Flg* phenocopy the human disease. However, AD patients with no known *FLG* mutations still maintain linkage to the EDC, suggesting mutations in other EDC genes may also result in AD. Published microarray analysis on affected AD skin showed a significant downregulation of *LOR*, suggesting a link between *LOR* and AD. *LOR* is the most prevalent protein found in the CE, constituting >70% of its mass. Despite this high percentage, *Lor* $^{-/-}$ mice do not display an overt phenotype. However, tape stripping and ultra structural analysis on *Lor* $^{-/-}$ epidermis revealed a marked fragility in the CE. Based on this, we reason that mutations in *LOR* may result in pathologies resembling AD. Utilizing the *Lor* $^{-/-}$ mouse as a model, we sought to determine if *Lor* $^{-/-}$ mice were susceptible to cutaneous allergen priming, similar to AD patients and mice deficient in *Flg*. Here we report that *Lor* $^{-/-}$ mice, upon topical application of an allergen, produce allergen-specific antibodies, and show an increase of dendritic cells at the site of allergen administration. Additionally, treated *Lor* $^{-/-}$ mice develop an acanthotic epidermis with hyperkeratotic foci. Thus, mutations in *LOR* may account for a percentage of AD cases where *FLG* is not mutated. These data underscore the importance of barrier acquisition and maintenance, and the role barrier plays in preventing sensitization to allergens.

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The effect of truncating the c-terminal domain of the Cldn6 tail in an aging phenotype of transgenic mice

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Formed during development by a series of cell commitment, mesenchymal-epithelial cell interactions, and terminal differentiation, the mammalian epidermis forms a robust barrier to protect against microorganism invasion and UV irradiation, inhibit water loss, regulate body temperature and is a part of the host defense system. These important functions decline in efficiency with aging, leading to an inefficient epidermal injury response, for reasons that are not yet understood. The importance of Claudins (Cldns) in epidermal differentiation and barrier function has been confirmed by experiments in which Cldn expression has been perturbed in epidermal cells. Involucrin-Cldn6 (Inv-Cldn6) transgenic mice also suffer skin barrier dysfunction, the severity/lethality of which is dependent upon the level of Cldn6 overexpression. These data suggest the importance of the cytoplasmic tail portion of Cldn molecules during epidermal differentiation. The cytoplasmic tails of different Cldns are divergent in sequence and a number of putative functional protein domains are present. To address the activities of the functional domains in more detail, we used the involucrin promoter (Inv) to target a deletion of half of the cytoplasmic tail to the differentiative compartment of the epidermis. The transgenic mice possess subtle epidermal differentiation abnormalities at birth that normalize by one month of age. However, with aging, normal hydration levels were not maintained in the skin. Immunohistochemistry revealed perturbations in the expression and localization of multiple Cldns, as well as various classical markers of epidermal differentiation in the aging skin. These results support the utility of this mouse model for studying intrinsic changes in the aging epidermis.

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Differentiation of human induced pluripotent stem (iPS) cells into keratinocytes

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Embryonic stem (ES) cells have an unlimited proliferative capacity and extensive differentiation capability. They are an attractive and long-term source for regeneration therapies with a potential role to treat several human diseases. However, the clinical use of ES cells has significant ethical and biological obstacles related to their derivation from embryos and immunological rejection, respectively. These disadvantages can be overcome by the use of induced pluripotent stem (iPS) cells, since iPS cells are generated from individual somatic cells by exogenous expression of defined transcription factors and have characteristics similar to ES cells. In recent years, patient-derived iPS cells have been generated to study disease mechanisms and develop iPS cell-based therapies. Generation of iPS cell-based therapies for skin diseases requires successful differentiation of iPS cells into skin components, including keratinocytes. However, this methodology has not yet been established. The aim of this study was to determine the condition under which iPS cells can be differentiated into functional keratinocytes *in vitro* with high efficiency. We established human iPS cell lines from several types of somatic cells, including fibroblasts and keratinocytes. The pluripotent proprieties of the iPS cell lines were demonstrated by gene and protein expression of several stem cell markers (OCT4, SOX2, SSEA3 and TRA-1-60 & 81) and differentiation capability into all three germ layers *in vitro* via embryoid body formation, and *in vivo* via teratoma formation. Moreover, the iPS cell lines derived from both fibroblasts and keratinocytes could be differentiated into keratinocytes using conditions adapted from ES cell differentiation (retinoic acid and BMP4). Keratinocytes were identified based on morphology and expression of p63, keratin 14 and desmoglein 3. These data provide preliminary evidence for successful production of keratinocytes from human iPS cells, thereby establishing a basis for development of disease models and therapies for skin diseases.

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Outside-in signaling through non-junctional Dsg3: A key switch in keratinocyte proliferation and differentiation

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Biochemical fractionation studies revealed that monoclonal Dsg3 antibodies (AK23) which induce a pemphigus vulgaris (PV) phenotype mainly bind to non-junctional or Triton-X100 soluble Dsg3. Supported by in vivo studies, non-junctional Dsg3 is present at the plasma membrane in interdesmosomal areas where the initial loss of cell-cell adhesion is observed in response to PV antibody binding. The concurrent alteration of a variety of signaling molecules, increasingly reported throughout recent years, let us to hypothesize that non-junctional Dsg3 acts as a genuine signaling receptor. Consistent with this concept, here we show that Dsg3 is recruited into signaling platforms known as lipid rafts at onset of differentiation in cultured keratinocytes. Furthermore, pSrc followed by phosphoinositid-3 kinase (PI3K), one of the key signaling molecules in the switch from proliferation to keratinocyte differentiation, associate with non-junctional Dsg3 in confluent keratinocyte cultures. This is followed by activation of the survival kinase Akt, inhibition of GSK3 and as one prominent event, c-Myc suppression. Suppression of the proto-oncogene c-Myc marks the exit of keratinocytes from the cell cycle and onset of differentiation. The physiological relevance of this signaling cascade is underscored by function disrupting Dsg3 antibodies AK23. Within minutes, these antibodies impair pSrc and then PI3K recruitment to Dsg3, reduce Akt and GSK3 phosphorylation, increase c-Myc and support proliferation in cultured keratinocytes as well as in PV patients. In support of a role of Dsg3 in cell cycle exit, the cell cycle regulator cyclin D1 is roughly six fold increased in Dsg3-/- keratinocytes and the proliferation index two times in Dsg3-/- mouse epidermis. Together these results attribute an important outside-in signaling function to non-junctional Dsg3 at cell cycle exit. They further suggest that this function is impaired in PV leading to sustained proliferation with the concomitant consequence of desmosome disassembly and acantholysis.

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"Bathing suit ichthyosis" — a unique phenotypic variant of lamellar ichthyosis observed in a Jamaican female

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"Bathing suit ichthyosis" is a unique clinical phenotype of lamellar ichthyosis which was originally described in South African blacks. This type of autosomal recessive congenital ichthyosis is caused by mutations of the Transglutaminase-1 (TGM-1) gene. Patients with this condition typically present at birth with a collodion membrane and subsequently develop ichthyotic dark brown plate-like plaques on the trunk, axillae, proximal extremities, scalp and neck with sparing of the central face. These lesions typically exhibit a sharp transition from affected to unaffected skin and help to define the characteristic features of this aptly-named "bathing suit" distribution. More recently, individual cases of this condition have been reported in patients of Japanese, French, German, Dutch, Moroccan, and Turkish descent. We present the case of a 16-year-old Jamaican female who was born as a collodion baby and has had persistent brown ichthyotic plaques involving the trunk, scalp and neck in the pattern characteristic of "bathing suit ichthyosis". Both of her parents are from Jamaica, but they are uncertain of their families' original ancestry. There is no family history of anyone with autosomal recessive congenital ichthyosis or any history of collodion baby or any other ichthyosiform skin condition. Our patient is undergoing genetic testing, and the results of this study are still pending at this time. Interestingly, she relays that the severity of her skin condition improves and worsens with cooler or warmer weather, respectively. This observation further supports the theory that the TGM-1 mutations may cause a temperature-sensitive phenotype which would provide an explanation for this distinct clinical appearance. Overall, the patient's condition has remained fairly stable over the years.

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An unexpected role of transcription factor Ovol2 in epidermal integrity

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The Ovo gene family encodes evolutionary conserved zinc-finger transcription factors that reside downstream of key developmental signaling pathways such as Wg/Wnt and BMP/TGF-β. Ovol1 is predominantly expressed in suprabasal epidermal cells and has been shown to regulate cell cycle exit of developmental epidermal progenitor cells, whereas *in vitro* studies suggest a role for Ovol2 in suppressing transient amplification and terminal differentiation but promoting long-term proliferation of culture keratinocytes at least in part by repressing c-Myc and Notch1. Ovol2 is expressed at low levels in the basal layer of developing epidermis and its function *in vivo* has yet to be studied. In this work, we use a well-established Tet-off binary transgenic mouse system to explore the function of Ovol2 when it is overexpressed in mouse skin epidermis. Constitutive Ovol2 overexpression in the basal layer results in thin, blistered skin and lethality in neonatal mice. The transgenic epidermis is characterized by splits within the basal layer and between the basal and spinous layers, increased apoptosis in blistering areas, reduced thickness of the spinous compartment, and severely compromised expression of basal markers including c-Myc and Keratin 5. Current investigation focuses on elucidating the ultrastructural, cellular and molecular bases of these defects.

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The role of Pecanex, a novel member of Notch pathway, in epidermal and hair follicle morphogenesis

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Pecanex is a highly conserved transmembrane protein with homologs found in multiple organisms ranging from flies (pcx) to humans (Pcnx, Pcnx2 and Pcnx3). pcx was described several years ago in Drosophila as a maternal-effect neurogenic gene, such that when the gene is not expressed maternally, the embryos display hyperneuralization, indicating that its function is to promote epidermal cell fate. Such a phenotype also implicates pcx as a new component of the Notch signaling pathway. In order to identify its function, we performed experiments in two model organisms: mouse and Drosophila. To define its role during hair follicle and epidermal morphogenesis, we analyzed Pcnx expression in developing and postnatal mouse epidermis, and found that expression peaked at E14.0-E14.5 and anagen phase. Immunofluorescence analysis with a Pcnx antibody reveled protein expression in the inner root sheath in mouse and human hair follicle. We also detected Pcnx cDNA expression in human keratinocytes and dermal fibroblasts, which correlates with Pcnx antibody detection in mouse epidermis. We next used Drosophila genetics to place pcx in the context of the Notch signaling pathway and to determine genetic interactions. Drosophila pcx is widely expressed during embryogenesis, in both the imaginal discs and ovaries. We confirmed the hyperneuralization phenotype of embryos laid by homozygous mutant females, and we placed pcx function upstream of Notch gamma-secretase cleavage. Finally, we observed extra bristles in the maxillary palps of pcx mutants, a previously undescribed phenotype. The extra bristle phenotype suggests a role of pcx in appendage development. Collectively, these data suggest that pcnx plays an important role in morphogenesis processes, and moreover, that its function is related to the Notch signaling pathway.

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Heat-inactivated fetal bovine serum inhibits human keratinocyte differentiation

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Transepithelial electric resistance (TEER) is a widely accepted method to monitor the integrity of tight junctions (TJs). We and others, have noted that although TJ formation can be induced in primary human foreskin keratinocytes (KC) 2 to 3 days after exposure to high Ca^{2+} concentrations [1.8 mM $CaCl_2$ in Dulbecco's Modified Eagle Medium (DMEM) containing 10% heat-inactivated fetal bovine serum (HI-FBS)], the TEER is fairly low (Mean ± SEM; 154.1 ± 16.8 ohms x cm^2, n=4) by comparison to what is reported for intestinal epithelial cell lines. We hypothesized that the media we use in our *in vitro* cell culture may not be optimum for KC differentiation and/or TJ formation. To address this we grew KC in DMEM with or without 10% HI-FBS and measured TEER, as well as protein expression of the TJ protein claudin-1 and an early differentiation marker, cytokeratin 10 (CK10), and the late differentiation markers, filaggrin and loricrin, by Western blot or immunofluorescence. When KC were grown without HI-FBS, TEER peaked after 5 to 6 days and was significantly greater (Mean ± SEM; 2105 ± 220 ohms x cm^2, n=4) than the TEER observed in KC grown in DMEM with HI-FBS (154 ± 17 ohms x cm^2, n=4). We have also observed a similar effect on TEER when adding 10% HI-human serum from a healthy nonatopic donor. The expression of CK10 was detected by 24 hr by Western blotting, while filaggrin and loricrin positive cells were observed at day 8 by immunofluorescence staining when cells were grown in DMEM without HI-FBS. In contrast, KC grown with HI-FBS had little to no expression of CK10 at 24 hr and very few filaggrin or loricrin positive cells at day 8. Claudin-1 expression reflected TEER values observed in DMEM ± HI-FBS. These results strongly suggest that some constituent of HI-FBS (and human serum) inhibits KC barrier formation. Therefore, we conclude that the *in vitro* study of barrier functions in human KC would be best done in DMEM media without FBS.

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Epidermal gene expression in atopic dermatitis compared to psoriasis and nonatopic controls.

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Atopic Dermatitis (AD), a Th2 polarized disease and psoriasis (PS), a Th1/Th17 disease, are the most common inflammatory skin diseases. Keratinocyte impairment is a key feature of both. The aim of this study was to functionally characterize the gene expression signature for AD and correlate gene expression with clinical severity (serum total IgE, total eosinophil counts and EASI/PASI scores). For these studies we obtained nonlesional epidermis, overcoming possible bias introduced by the inflammation observed at lesional sites or the heterogenous cell populations that would confound the study of skin biopsy samples. Expression profiles were generated from AD, PS and NC samples (n=5, 4, & 5; respectively) using Illumina HumanRef-8 v1 Expression BeadChips. Gene expression values were median scaled and log (base 2) transformed, and then subject to group comparisons (Welch t-test, Benjamini-Hochberg FDR), and examination for correlation with clinical variables. 108 genes were differentially expressed in AD vs NC and 91 in AD vs PS. The DAVID bioinformatics resource (http://david.abcc.ncifcrf.gov/) tool was used for functional analysis of gene lists and revealed 22 functional gene groups dysregulated in AD vs NC and 36 groups in PS vs NC. The majority of these groups were associated with keratinocyte differentiation and cytoskeleton structure (e.g. ectoderm development, FDR = $9.5x10^{-7}$ (AD/NC) and $1.2x10^{-7}$ (AD/PS); epidermal development, $7.5x10^{-5}$ and $9.6x10^{-6}$; keratinocyte differentiation, $6x10^{-4}$ and $1.1x10^{-5}$). Expression of the tight junction gene, claudin-1 correlated with total IgE, total eosinophil count and EASI/PASI scores ($R \geq -0.739$). We conclude that the epidermis from nonlesional sites may provide information on the pathogenesis of atopic dermatitis.

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Inositol polyphosphate-5-phosphatase (INPP5A) expression is associated with increased differentiation and decreased proliferative capacity of normal human keratinocytes

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We have recently identified INPP5A as a novel gene frequently deleted in squamous cell carcinomas (SCC). Reduction of INPP5A protein levels is seen at the earliest stage of SCC development, in actinic keratosis. As the increased rate of cellular growth is the hallmark of malignant transformation, we asked whether the observed loss of INPP5A in SCC may indicate a more general association between INPP5A levels and the proliferative capacity of normal human keratinocytes. To this end, we evaluated INPP5A expression patterns in normal skin and in primary human keratinocytes in an *in vitro* differentiation model. Immunohistologic evaluation of INPP5A levels in normal skin demonstrated cytoplasmic pattern of expression with low INPP5A levels in the basilar keratinocytes and a significant increase in the INPP5A levels in the upper epidermal layers, suggesting an association of INPP5A levels with a decrease in the proliferative capacity and an increase in cellular differentiation state. To more precisely map INPP5A changes in the process of keratinocyte differentiation, we used confluence-induced differentiation model. In this model, rapidly proliferating keratinocytes demonstrate low levels of INPP5A both on RNA and protein level. In contrast, induction of differentiation is accompanied by marked increase in INPP5A levels. Expression levels of several genes with known behavior in the processes of proliferatrion and differentiation were used as controls (i.e. keratins, 1, 10, 5 and 14). Importantly, forced expression of INPP5A in SCC cell lines *in vitro* halted cellular growth and ultimately lead to cell death. Taken together, our data suggest a possible regulatory role for INPP5A in controlling the balance of keratinocyte proliferation and differentiation. Loss of this balance may play important role in pathogenesis of cutaneous SCC tumors.

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Improved dermal responsiveness and reproducibility in the human 3D *in vitro* skin model AccuSkin

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Alternative human full thickness skin models are needed to accommodate varied testing needs, since no single model has been found that yields ideal readouts for all testable parameters. AccuSkin is unique from most other models because innate cell mobility and cell cohesion are harnessed in a single step process yielding a model produced with fewer manipulations resulting in the potential for higher reproducibility and uniformity. The process has been refined to yield a very consistent product. Exogenous collagen is not required to form the epidermal/dermal tissue layers, rather the process creates a healthy layer of fibroblasts poised to synthesize collagen and other dermal components in response to test compounds. For epidermal testing other current models lack self-sustaining basal cells and so terminally differentiate within a few weeks, limiting the test windows to only short treatments. A special formulation of AccuSkin can contain keratinocyte basal cell colonies that allow epidermal renewal. The cultured basal cells were shown to be keratin 5/14 and laminin-332 positive by indirect immunofluorescence and prolong the lifespan potential of AccuSkin. Although originally designed for 96 well formats, the flexibility of the process allows accommodation of custom dimensions.

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Gene suppression in skin through topical delivery of polyvalent siRNA-gold nanoparticles

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The applicability of gene suppression *in vivo* to combat neoplastic, genetic, and inflammatory skin disease has been hampered by the limited ability of siRNA and DNA to traverse the epidermal barrier. We have developed functionalized polyvalent siRNA-coated gold nanoparticles (siRNA-Au NPs) that are taken up by all primary cells and cell lines tested, are stable against degradation, have low toxicity, bind specifically to targets, and silence genes at the point mutation level. We hypothesized that siRNA-Au NPs would penetrate cultured keratinocytes (KCs) and skin after topical delivery, leading to gene suppression. DNA- and siRNA-Au NPs were taken up by ~100% of normal human KCs within 2 h without ancillary reagents and suppressed survivin gene function by more than 90%, leading to cell apoptosis at concentrations that caused no toxicity. Based on mass spectroscopy to measure gold particles, uptake of siRNA-Au NPs by cultured KCs is 10-20-fold more than uptake by 40 other cell types, suggesting that polyvalent Au-NPs may penetrate KC membranes *in vivo*. Both DNA- and siRNA-Au NPs penetrated mouse stratum corneum by 2 h, reached most epidermal cells, including basal cells, by 6-8 h, and were distributed throughout the epidermis and dermis by 24 h after a single application. Franz cell studies also demonstrated siRNA-Au NP penetration through human epidermis within 24 h after application. Topical application of siRNA-Au NPs targeting green fluorescent protein (GFP) in mice ubiquitously expressing the GFP transgene knocked down fluorescence in skin by almost 50% in comparison with scrambled siRNA-Au NPs and vehicle controls. No clinical or histologic evidence of toxicity in skin or viscera was observed after up to 1 month of topical siRNA-Au NP delivery. Topical application of polyvalent siRNA-Au NPs is a promising new tool for both laboratory-based gene knockdown in skin and as a potential clinical treatment for a wide array of skin disorders in which deleterious gene products are overexpressed.

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Different immunosuppressive strategies are required to control immune responses to antigenic fibroblasts and keratinocytes: Implications for cutaneous gene/cell therapy

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Keratinocytes and fibroblasts are both potential targets for skin gene/cell therapy for genodermatoses. Immune rejection of genetically modified cells, however, presents a major impediment to effective therapy. Using *ex vivo* approaches to gene transfer, we have previously shown that expression of an antigen by either cell type was sufficient to prime T cells and induce immune rejection of transplanted cells. The nature of these responses and the kinetics of immune rejection, however, were significantly different for these two cell types suggesting different immunosuppressive strategies to control these responses. In this study, we explored the potential of local expression of immunosuppressive factors to protect transgenic skin cells from rejection. Primary cultures of mouse keratinocytes or fibroblasts were transduced with a bicistronic vector encoding green fluorescent protein (GFP; as a model antigen) and either CD40lg, IDO, CTLA4Ig or PD-L1, and transplanted onto the back of a syngeneic mouse to regenerate skin. Surface GFP expression in live mice was monitored weekly to assess the fate of transgenic cells. Coexpression of transgene with either CD40lg or CTLA4Ig in fibroblasts resulted in long-term survival of transgenic fibroblasts (>20 weeks) despite the presence of systemic transgene-specific immune responses. Similar treatment did not, however, protect keratinocytes from immune rejection. Long-term protection of transgenic keratinocytes was achieved through transient blockade of CD40/CD154 interactions by administration of anti-CD154 antibody during the first two weeks of cell transplantation. Although neither of these strategies induced long-lived antigen-specific tolerance, they were sufficient to prevent rejection of genetically modified cells. These results thus indicate that different strategies are required to restrict rejection of neoantigen-expressing keratinocytes and fibroblasts and neither requires induction of antigen-specific tolerance.

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Regulating the expression of genes responsible for the synthesis and maintenance of dermal hyaluronan and barrier lipids via topical treatment of salicin- an *in vitro* analysis

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Willow bark extracts have been used for hundreds of years as antipyretic agents in treating rheumatic disorders. More recent work, specifically of an extract of white willow bark known as salicin ([2-(Hydroxymethyl)phenyl β-D-glucopyranoside]), has focused on its capability as an anti-inflammatory agent. While salicin's anti-inflammatory mechanisms have been explored, little has been done to investigate other benefits and capabilities of this extract. In topical human clinical testing, salicin containing formulations have been observed to increase the hydration of skin. Hydration is regulated by many different components involved in various biological processes in the skin. These include the synthesis and maintenance of components such as barrier lipids and dermal hyaluronan (HA). This research sought to explore the *in vitro* effect of salicin in influencing the expression of the genes involved in skin hydration. Salicin at 0.5% (in water) was applied topically to human equivalent skin cultures containing normal human epidermal keratinocytes and normal human dermal fibroblast for 24 and 48 hours at 37°C. Taqman RT-PCR experiments were then conducted for both treated and untreated cultures. Data analysis was carried out according to RQ analysis method using RQ Manager and StatMiner (v3.1) software programs. The results showed that salicin at 0.5%, when applied topically to human equivalent skin cultures for 24 and/or 48 hours significantly increases the expression of genes responsible for the synthesis of dermal HA and barrier lipids. These include HAS1, HAS2, PPARD, ARDP and SCD5. In addition, salicin also decreases the expression of genes known to negatively regulate the maintenance of HA and barrier lipids, such as, HYAL, HPSE, SPTLC2 and GBF1. Based on these findings, it can be concluded that salicin regulates the expression of key genes responsible for the synthesis and maintenance of dermal HA and barrier lipids.

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Salicin reduces the expression of genes associated with skin inflammation- An *in vitro* analysis

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Salicin ([2-(Hydroxymethyl)phenyl β-D-glucopyranoside]) is an extract from white willow bark. It has been researched to be a potent anti-inflammatory agent when taken orally. Recent studies involving salicin in finished cosmetic formulations has led to the hypothesis that this extract may be able to reduce "skin sensitivity" and inflammation when applied topically. Although consumers frequently report "sensitive skin", the mechanism is not understood. It is believed to involve inflammatory pathways. Increased "skin sensitivity" may be a result of the activation of inflammatory mediators that regulate how the body responds to certain irritants. Reducing the presence of such mediators may therefore reduce some of the effects of inflammation, such as "sensitivity". This research explores the *in vitro* effect of salicin in reducing the expression of genes associated with the production of inflammatory mediators. A 0.5% salicin solution was applied to human equivalent skin cultures containing normal human epidermal keratinocytes and normal human dermal fibroblast for 24 and 48 hours at 37°C. RNA extracted after 24 hours from both treated and untreated samples were used to conduct DNA microarray experiments and RNA after 24 and 48 hours were used for Taqman RT-PCR experiments. Statistical analysis of both microarray and RT-PCR data showed that salicin at 0.5%, when applied to human equivalent skin, reduces the expression of genes for mediators known to be involved in skin inflammation. These include F2RL1, LTB4R, NGF, PTAFR, ERBB2, HRH1, PTGS2, PTGER3, IL18 and S100A12. The findings from this study indicate a possible role of salicin in addressing consumer reported "skin sensitivity" and inflammation when applied topically.

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***LIPH*-prevalent founder mutations lead to loss of P2Y5 activation ability of PA-PLA1α in autosomal recessive hypotrichosis**

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Autosomal recessive hypotrichosis (ARH) is characterized by sparse hair on the scalp without other abnormalities. Three genes, *DSG4*, *LIPH* and *P2RY5*, have been reported to underlie ARH. We performed a mutation search for the three candidate genes in four independent Japanese ARH families and identified two *LIPH* mutations: c.736T>A (p.Cys246Ser) in all four families, and c.742C>A (p.His248Asn) in three of the four families. Out of 200 unrelated control alleles, we detected c.736T>A in three alleles and c.742C>A in one allele. Haplotype analysis revealed each of the two mutant alleles is derived from a respective founder. These results suggest the *LIPH* mutations are prevalent founder mutations for ARH in the Japanese population. *LIPH* encodes PA-PLA1α(LIPH), a membrane-associated phosphatidic acid-preferring phospholipase A1α. Two residues, altered by these mutations, are conserved among PA-PLA1α of diverse species. Cys^{246} forms intramolecular disulfide bonds on the lid domain, a crucial structure for substrate recognition, and His^{248} is one amino-acid of the catalytic triad. Both p.Cys246Ser- and p.His248Asn-PA-PLA1α mutants showed complete abolition of hydrolytic activity and had no P2Y5 activation ability. These results suggest defective activation of P2Y5 due to reduced 2-acyl lysophosphatidic acid production by the mutant PA-PLA1α is involved in the pathogenesis of ARH.

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Combination therapy of Rapamycin and Imatinib syngergistically inhibits tuberous sclerosis tumors *in vivo*

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Tuberous sclerosis (TS) is a common autosomal dominant disorder characterized by benign and malignant tumors of the skin, lung, brain, and kidneys. Mutations in one of two genes, tsc1 or tsc2 results in activation of mammalian target of rapamycin (mTOR). Because of this, clinical trials have been performed in patients using rapamycin monotherapy, resulting in temporary regression of tumors, followed by regrowth once therapy had stopped. This finding implicated involvement of additional pathways. We have previously implicated platelet derived growth factor-BB in TS related tumorigenesis, thus providing a rationale for combination mTOR/PDGF blockade using rapamycin and glivec. We used a well established model of cutaneous tumorigenesis in TS, tsc2ang1 cells, derived from a skin tumor from a mouse heterozygous for tsc2. Treatment of tsc2ang1 cells with glivec alone or rapamycin alone led to an increased amount of PDGFR beta protein, but the levels were decreased upon combination treatment. Similarly, phosphorylation of tyr-1009 of PDGFRb was decreased upon combination treatment with rapamycin and glivec. Gene array analysis of tsc2ang1 cells treated with combination of glivec and rapamycin indicated specific perturbation of the Aurora kinase A and B signaling pathways, with combination therapy resulting in an imbalance between Aurora kinase A and B, with upregulation of Aurora kinase A protein and downregulation of Aurora kinase B protein. In vivo combination of rapamycin and glivec was well tolerated in mice, and a synergistic downregulation of tumor growth was noted in mice receiving the combination therapy. Our findings provide a rationale for the combination use of rapamycin and glivec, both FDA approved drugs, for the treatment of TS.

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Genomewide association study of generalized vitiligo identifies specific and shared autoimmunity susceptibility genes

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Generalized vitiligo is an autoimmune disease in which melanocyte loss results in patchy depigmentation of skin and hair, and is associated with elevated risk of other autoimmune diseases. To identify generalized vitiligo susceptibility loci, we organized VitGene, a multi-center consortium, and conducted a genomewide association study. We genotyped 579,146 single-nucleotide polymorphisms (SNPs) in 1514 generalized vitiligo cases of European-derived white (CEU) ancestry and then compared genotypes of cases with those of 2813 "public" CEU controls. We then tested 56 SNPs in two replication sets, one comprising 677 independent CEU cases and 1106 CEU controls, and the other comprising 183 CEU simplex generalized vitiligo trios and 332 CEU multiplex families. We detected significant association of generalized vitiligo with SNPs at 13 loci, several previously associated with other autoimmune diseases [*HLA* class I ($P = 9.05 \times 10^{-23}$; OR 1.58), *HLA* class II ($P = 4.50 \times 10^{-34}$; OR 1.62), *PTPN22* ($P = 1.31 \times 10^{-7}$; OR 1.39), *LPP* ($P = 1.01 \times 10^{-11}$; OR 1.31), *CCR6* ($P = 3.94 \times 10^{-7}$; OR 1.23), *IL2RA* ($P = 2.78 \times 10^{-9}$; OR 1.27), *SH2B3* ($P = 2.03 \times 10^{-8}$; OR 1.25), *UBASH3A* ($P = 1.26 \times 10^{-9}$; OR 1.27), *C1QTNF6)* ($P = 2.21 \times 10^{-16}$; OR 1.38)] and three novel immune-related loci [*RERE* ($P = 7.07 \times 10^{-15}$; OR 1.36), *FOXP1* ($P = 1.04 \times 10^{-8}$; OR 1.33), *GZMB*) ($P = 3.44 \times 10^{-8}$; OR 1.28)]. The only significant non-immune-related vitiligo susceptibility locus is *TYR* ($P = 1.60 \times 10^{-18}$; OR 1.53), encoding tyrosinase, a key melanocyte enzyme and a major vitiligo autoantigen. Moreover, we detected epistasis between *HLA-A*02* and the major allele of *TYR* variant R402Q. Together, these loci account for approximatly 9% of the total genetic risk for generalized vitiligo. We also identified an important locus for vitiligo age-of-onset in the *HLA* class I region, close to *BTNL2*. Our findings underscore the autoimmune basis of generalized vitiligo and suggest a possible inverse relationship between susceptibility to vitiligo and melanoma, possibly reflecting genetically-based variation in immune surveillance.

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Redirecting adult human skin keratinocytes into smooth muscle cells via temporary reprogramming

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Recently, stem cell biology was revolutionized by the reprogramming of differentiated skin cells into induced pluripotent stem (iPS) cells. Although groundbreaking, this technology is fraught with problems that will limit its use for human therapy. A major obstacle is the viral delivery system itself, which permanently intercalates the factors into the cellular genome. Further, the factors introduced have lead to the tumor formation in previous mouse models. Our laboratory has taken a different approach to produce human cells for tissue regeneration. We temporarily reprogram human skin keratinocytes, and then briefly expose these cells to a program of directed differentiation. These partially dedifferentiated cells temporarily reactivate a few endogenous embryonic genes. We have redirected such cells to differentiate into smooth muscle cells by exposure to specific smooth muscle differentiating factors. These redirected cells not only expressed smooth muscle markers as demonstrated by RT-PCR and Western blot analysis, but also functioned as real smooth muscle cells in a standard gel contraction assay. Thus, skin keratinocytes appear to be susceptible to directed differentiation after only a short reprogramming regime.

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Increased lysyl hydroxylase2(long) mRNA in scleroderma skin correlates with formation of pyridinoline collagen crosslinks

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Collagens are a major component of the extracellular matrix. The level of collagen synthesis is significantly increased in fibrotic conditions such as scleroderma (SSc), which encompasses both the localized form, morphea (skin thickening), and systemic sclerosis (affecting other organs). The trifunctional pyridinoline (Pyr) crosslinks, associated with the over-accumulation of collagen in scleroderma, are derived from telopeptide (non-helical) collagen lysyl residues that have been hydroxylated by a specific telopeptide lysyl hydroxylase (LH). This has been reported to be the alternatively-spliced long form of LH2. In this study, using qRT-PCR, we have shown that levels of LH2(long) mRNA in clinically affected skin from SSc patients (n=6) were >10-fold higher than in non-affected skin from the same patients. The ratio of LH2(long)mRNA to LH2(short)mRNA, measured in a single RT-PCR reaction, was >3-fold higher in affected skin compared to non-affected skin from SSc patients (n=6). Although Pyr crosslinks are not usually detectable in normal skin, they could be measured in affected skin from SSc patients (n=7), accompanied by an increase in the precursor bifunctional crosslinks. Although these bifunctional crosslinks were increased in long term cultures of cells from affected skin, Pyr crosslinks were not always detected. Using qRT-PCR, levels of LH2(long) mRNA measured in early passage fibroblasts (<5) cultured from skin biopsies from SSc patients, showed a lesser increase than in the skin itself, with a 2 to 4-fold increase in LH2(long)mRNA from affected skin compared with non-affected skin. Results from this study show that the increase of LH2(long) mRNA in SSc skin clearly correlates with formation of Pyr crosslinks, but indicate that explanted SSc fibroblasts may show an incomplete reflection of the SSc phenotype.

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Filaggrin mutation study and clinical characterization of patients with Ichthyosis Vulgaris and Atopic Dermatitis in Kyushu area, the most southern part of Japan

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Filaggrin is a key protein involved in skin barrier function. Mutations in the gene encoding filaggrin (FLG) have been shown to be responsible for ichthyosis vulgaris (IV) and atopic dermatitis (AD), initially in Europeans. To date, about 40 different FLG mutations have been reported in IV and/or AD, and population-specific mutations in Europeans and Asians have been detected. It was proposed that background information is needed to detect prevalent FLG mutations for each geographical population. To further investigate genetic background, we screened 8 FLG mutations described previously in Japan (p.Arg501X, c.3321delA, p.Ser1695X, p.Glu1701X, p.Ser2554X, p.Ser2889X, p.Ser3296X, and p.Lys4022X) in additional IV and/or AD patients from Kyushu, the most southern part of Japan. We found FLG mutations in 7 out of 16 patients diagnosed as IV. 12 (22%) of 55 AD patients were shown to carry one of these mutations. This was of slightly lower frequency than that (27%) reported previously from Hokkaido Univ. located in the most northern part of Japan for AD (Nemoto-Hasebe I et al. Br J Dermatol 161:1387-90, 2009). Frequencies for c.3321delA (5.5%) and p.Lys4022X (5.5%) in our AD patients were slightly higher than those reported previously for AD patients. On the other hand, frequencies for p.Ser2554X (3.6%) and p.Ser2889X (5.5%) were slightly lower, and p.Ser3269X was not detected. We also compared these FLG mutations with several clinical parameters, including disease severity, concomitant atopic diathesis and serum IgE level, and found clear correlation between them. Our data confirmed for the first time the relationship between FLG mutations and IV/AD in Kyushu. The slightly different frequency in FLG mutations in our study from those reported previously in Japan indicates that Kyushu population may have additional FLG mutations reported in other South Asian countries.

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Distribution of mitochondrial DNA deletions in Csb m/m and Csa -/- mice of different ages

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Genotoxic stress can lead to cancer or aging. Defects in the repair mechanism nucleotide excision repair (NER) either lead to skin tumors in Xeroderma pigmentosum (XP) or premature aging and neurodegeneration in Cockayne syndrome (CS). Aging-associated reduction of subcutaneous fat is a hallmark of physiological aging, premature aging of CS patients as well as many other progeroid syndromes. Accumulated mutations of mitochondrial (mt)DNA are also linked to chronological aging, premature skin aging and neurodegeneration. Less is known about the age dependent distribution of mtDNA large scale deletions in multiple organs of prematurely aging mice. We investigated spleen, liver, skin (epidermis, dermis and subcutaneous tissue), quadriceps muscle, heart and brain of mice of different age groups and different genotypes (Csbm/m, Xpa-/- and wildtype) for the relative amount of large mitochondrial deletions (mtD17 and mtD1). We found an age dependent increase of mtD17 in many organs in all mice. Csbm/m mice accumulated more mtDNA deletions than Xpa-/- and wildtyp mice of the same age group. For further investigation we microdissected subcutaneous fat of Csa-/- and Csbm/m, Xpa-/- and wildtype mice of different age groups and analyzed the relative amount of mtD17 and the mtD1 deletion. Aged Csa-/- and Csbm/m mice show a strong increase of mtD17 and mtD1 in age-dependently reduced subcutaneous fat tissue which was not observed in other DNA repair deficient mice that do not show prematurely reduced subcutaneous fat. MtDNA deletions were increased in the epidermis and dermis of these animals but at much lower levels compared to subcutaneous fat. Sequence analysis and restriction enzyme digest confirmed the identity of PCR results. These data show that mtDNA mutations age-dependently increase in practically all tissues of progeroid animals with the highest levels in subcutaneous fat, indicating that these mutations may be involved in age dependent loss of subcutaneous tissue.

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Connecting base excision repair (BER) and nucleotide excision repair (NER) within mitochondria: Cockayne syndrome A and B interact in complexes with mitochondrial (mt)DNA repair associated proteins 8-oxo-guanosine glycosylase (hOGG)-1 as well as mt single strand binding protein (SSBP)-1

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DNA damage induced by ultraviolet (UV)-B radiation predominantly gets repaired by the nucleotide excision repair (NER) mechanism, while UV-A induced oxidative DNA damage is preferentially repaired by base excision repair (BER). Defective NER either causes skin cancer prone Xeroderma pigmentosum (XP) or premature aging in Cockayne syndrome (CS). While mitochondrial BER is well characterized, mitochondria are thought to be free of NER. Mutations of mitochondrial (mt)DNA have been linked to aging. We previously showed localization of CSA and CSB proteins in mitochondria, their enrichment upon oxidative stress, premature induction of oxidatively induced mtDNA mutations in CSA and CSB cells as well as binding of CSA and CSB proteins to mtDNA. However, it has been unclear if CSA or CSB interact with other mitochondrial proteins. We provide evidence that CSA and CSB are associated to complexes of the DNA repair associated mitochondrial single strand binding protein (mtSSBP)-1 and the BER-associated 8-oxo-guanosine glycosylase (hOGG)-1 in oxidatively stressed mitochondria by Coimmunoprecipitation. These data present a link between mtCSA/CSB proteins and protection from large scale deletions of the mtDNA via a previously unreported interaction of two hitherto separate repair mechanisms NER and BER.

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A survey of c-Kit mutations in adult-onset and childhood-onset mastocytosis patients

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Autoactivated KIT-V560G and KIT-D816V are gain of function mutations associated with the pathogenesis of mastocytosis. Thus, mutated KIT is a target for treating mastosytosis and other Kit-associated disorders. Previous studies from our laboratory have shown the effectiveness of therapies targeting Kit is dependent on the KIT mutation locus. However, to date detection of KIT mutations have been largely focused on the 560 and 816 loci, and the existence of other mutations in KIT have not been well investigated in this patient group. Thus, we prospectively characterized KIT mutations in 26 mastocytosis patients with either adult-onset (n=22) or childhood onset (n=4) disease. Total mRNA was isolated from skin biopsies and treated with DNase I to prevent DNA contamination. Full-length KIT c-DNA was amplified using long-and-accurate PCR with nested primer sets targeting the 5'- and 3'-end untranslated regions. The KIT sequence was analyzed with region specific primers. The KIT-D816V activating mutation was identified in 16 adults (73% of adults)and the KIT-V560G activating mutation was detected in 2 adults (9% of adults). In addition, we have discovered KIT-M541L in 3 other adults (14% of adults), a Kit with alternative splicing that results in the deletion of exons 3 to 13 in the skin lesion of one adult patient and a truncated KIT with nonsense mutation KIT-Q920X in another adult mastocytosis patient. Three of four childhood-onset mastocytosis patients were found to express unique KIT mutations: one in the transmenbrane region (KIT M541L), two in the extracellular region (a Kit with insertion of two amino acids phe-ala at 502-503) and (a KIT with E414D+D419del). The results of this study confirm that the D816V mutation is the most common KIT abnormality in adult-onset mastocytosis patients, but that other gain of function mutations also exist in this patient group. Surprisingly, 3 or 4 childhood-onset mastocytosis patients also had KIT mutations that could potentially play a pathophysiologic role in their disease and will need to be further characterized for their effects on KIT function.

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Identification and characterization of zebrafish orthologues of *SAMD9* – the gene mutated in familial tumoral calcinosis

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Normophosphatemic familial tumoral calcinosis (NFTC) is an autosomal recessive disorder characterized by calcium deposition in skin and mucosae and associated with pain and skin infections. NFTC is associated with mutations in the Sterile Alpha Motif Domain Containing 9 Gene *(SAMD9)*. The human genome, in addition to *SAMD9*, contains a *SAMD9-Like (SAMD9L)* gene within the same locus in chromosome 7. The *Samd9* gene, however, has been deleted from the mouse genome through genomic reorganization during evolution. Thus, generation of a *Samd9* knockout mouse is not feasible. The zebrafish *(Danio rerio)* has essentially the same complement of genes as mammals. By searching the NCBI zebrafish database, we identified one putative gene for *samd9* and one for *samd9L*, with 32% and 28% sequence homology with the corresponding human genes. The human *SAMD9* orthologue in zebrafish was identified by morpholino-mediated knockdown coupled with rescue experiments with human mRNA. Two morpholinos were designed to the *samd9* gene, one at the 5'UTR to block translation and one at the splice site at the exon 1/intron 1 junction to prevent pre-mRNA splicing. These morpholinos decreased *samd9* expression by 74-79% and induced a similar knockdown phenotype: pericardial edema, curled tail, and premature death at 4-5 days post-fertilization. The knockdown phenotype was partially rescued with full-length human *SAMD9* mRNA; however, there was no rescue with human *SAMD9L* mRNA coinjection. A morpholino targeting the 5'UTR in the *samd9L* gene was not associated with phenotypic changes of the injected embryos. These results suggest that *samd9* is the *SAMD9* orthologue in zebrafish, making zebrafish a potential model system for studying the role of *SAMD9* in the pathogenesis of NFTC.

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The *abca12* gene is required for normal zebrafish skin development - a model system for harlequin ichthyosis

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Harlequin ichthyosis (HI), characterized by hyperkeratosis and impaired barrier function of the stratum corneum, is caused by mutations in the *ABCA12* gene, which encodes a lipid transporter in epidermal keratinocytes critical for skin development. The zebrafish *(Danio rerio) abca12* has 49.3% sequence identity, at the amino acid level, with human *ABCA12*. To study the function of *abca12* during zebrafish development, the mRNA expression levels during early development were measured by RT-PCR. The results showed *abca12* expression beginning after fertilization and increasing until achieving a constant level of expression at 1 day post-fertilization (dpf). A morpholino was designed to target a splice site at the exon 4/intron 4 junction to block *abca12* pre-mRNA processing. Injection of the morpholino into 1-4 cell zebrafish embryos was performed, followed by observations of the visible and ultrastructural phenotype and assaying of mRNA expression. The morpholino decreased *abca12* expression by 88% and induced a visible phenotype in 3 dpf larvae, consisting of altered skin surface contour and disorganization of the melanophore distribution, coupled with pericardial edema and enlargement of the yolk sac. It was also associated with premature death at around 6 dpf. Scanning electron microscopy of the skin surface revealed perturbed microridge formation with development of spicules in the center of the keratinocytes. Transmission electron microscopy confirmed maldistribution of the pigmented granules within the epidermis. Microinjecting zebrafish embryos with full-length human *ABCA12* mRNA together with the morpholino partially rescued the "knock-down" phenotype. These results, which suggest that *ABCA12* is an essential gene for normal zebrafish skin development, provide novel insight into the function of *ABCA12*, the gene mutated in HI.

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The rate of wound healing is increased in psoriatic skin

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Given that more than 2% of the world population has psoriasis, we hypothesized that carrying the genes causing psoriasis may confer a survival advantage. Psoriatic inflammation, both clinically and biochemically, reflects multiple aspects of the inflammatory and proliferative stages of wound healing. The overlap between psoriatic inflammation and the pathophysiologic mechanisms of a healing wound suggested that these individuals may be able to heal their skin wounds more rapidly. To test this hypothesis we measured the rate of wound healing in psoriatic plaques and compared this to normal skin of unaffected individuals with no personal or family history of psoriasis. Wounds were created using a 3 mm punch biopsy on the upper buttocks and were allowed to heal by secondary intention. The wounds were photographed and the non-epithelialized area was assessed using the NIH ImageJ analysis program. By day 6, the non-healed area in the involved psoriatic skin was significantly smaller than that of the normal skin controls (mean size of psoriatic wounds 3.5 +/- 0.38 mm^2 vs. 6.5 +/-0.32 mm^2 for normal wounds, p= 0.0001). This increased rate of wound healing taken together with the published finding of expression of antimicrobial peptides in the psoriatic plaque suggests that involved psoriatic skin is primed to deal with the consequences of skin injury and required wound healing. Before the availability of antibiotics, the psoriatic phenotype may have resulted in a significant survival advantage. Further comparison and evaluation of genes involved in wound healing and psoriatic inflammation may identify new clinical targets and therapeutic strategies to treat psoriasis, as well as, improve wound healing.

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Novel mutation of COL7A1 gene in a family with epidermolysis bullosa pruriginosa

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Epidermolysis bullosa pruriginosa (EBPr) is an unusual variant of dystrophic epidermolysis bullosa, characterized by intense itching and skin fragility, resulting in profound hypertropic scarring. While skin fragility is attributed to mutations in the COL7A1 gene, leading to impaired function of anchoring fibrils composed of type VII collagen, the cause of the severe pruritus remains unclear. We describe a 14-year old patient with EBPr who had originally been diagnosed with hypertropic lichen planus, along with a severe neurodermatitis component. His scratching behavior was so severe that he was treated for obsessive compulsive disorder with fluoxetine, sertraline, and quetiapine. Further investigations revealed a novel COL7A1 gene mutation (IVS55+G>C), also demonstrated in the patient's mother and younger sister. We believe this to be the first reported case of EBPr in the U.S. We highlight the diagnostic challenges of EBPr and discuss current treatment options.

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DNMT1 maintains epidermal progenitor function

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Progenitor cells maintain self-renewing tissues throughout life by sustaining their capacity for proliferation while suppressing cell cycle exit and terminal differentiation. DNA methylation provides a potential epigenetic mechanism for the cellular memory needed to preserve the somatic progenitor state through repeated cell divisions. DNA methyltransferase 1 (DNMT1) maintains DNA methylation patterns after cellular replication. Although dispensable for embryonic stem cell maintenance, a clear role for DNMT1 in maintaining the progenitor state in constantly replenished somatic tissues, such as mammalian epidermis, is unknown. Here we show that DNMT1 is essential for epidermal progenitor cell function. DNMT1 protein was found enriched in undifferentiated cells, where it was required to retain proliferative stamina and suppress differentiation. In tissue, DNMT1 depletion led to exit from the progenitor cell compartment, premature differentiation and eventual tissue loss. Genome-wide analysis revealed that a significant portion of epidermal differentiation gene promoters were methylated in self-renewing conditions but were subsequently demethylated during differentiation. Furthermore, we show that UHRF1, a component of the DNA methylation machinery that targets DNMT1 to hemi-methylated DNA, is also necessary to suppress premature differentiation and sustain proliferation. In contrast, Gadd45A and B, which promote active DNA demethylation, are required for full epidermal differentiation gene induction. These data demonstrate that proteins involved in the dynamic regulation of DNA methylation patterns are required for progenitor maintenance and self-renewal in mammalian somatic tissue.

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Follow up study idendifies six novel susceptibility loci for psoriasis in Chinese Han population

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Psoriasis is a chronic inflammatory hyperproliferative skin disease influenced by multiple genetic factors. The exact etiology of psoriasis is not fully clear so far. In the past several years, genome-wide association study (GWAS) plus linkage and association studies have identified at least 20 established susceptibility loci involved in the pathogenesis of psoriasis. Previously, we have completed a first large GWAS of psoriasis in Chinese Han population, in addition to validating the established susceptibility loci major histocompatibility complex (MHC) and IL12B, we identified a novel susceptibility locus within the late cornified envelope (LCE) gene cluster. To further explore additional susceptibility loci for psoriasis and investigate disease heterogeneity, we performed a follow-up study of psoriasis within multiple populations including 6634 cases and 10868 controls in Chinese populations, 539 cases and 824 controls in Chinese Uygur, and 823 cases and 1840 controls of European population to search additional risk loci for psoriasis. We identified 6 new susceptibility loci (Pcombined Chinese Han ≤ 3.78 × 10-8) and validated one previously reported one for psoriasis (P<4.55 × 10-18) in Chinese Han. Comparision with previous GWAS highlighted the heterogeneity of psoriasis susceptibility between Chinese and European populations. Our study not only enriched the genetic factors involved the development of psoriasis but also throwed novel biological implications for understanding on disease pathogenesis.

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Follow up study identifies two novel susceptibility loci for systemic lupus erythematosus in Chinese Han population

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by dysregulated immune responses mediated by T and B cells, leading to increased production of pathogenic autoantibodies, against several self antigens. The etiology of SLE might include genetic and environmental factors. In past two years, six genome wide association study (GWAS) of SLE have been published, which identified more than 30 susceptibility genes or loci for SLE and confirmed some of genes found by candidate gene study. In 2009, we have completed a GWAS of SLE in Chinese Han population, and identified 9 new susceptibility loci, as well as confirmed 8 reported ones in previous study. Our study was the first GWAS of SLE in non-European ancestral population. To investigate additional potential genetic variants for SLE, we re-analyzed the GWAS data and selected 72 SNPs, which were genotyped in 3,152 cases and 7,050 controls of Chinese Han using the Sequenom MassArray system. Two new susceptibility loci were validated and surpassed genome-wide significance in combined analysis with GWAS data, which located at 16q11 (P=1.352×10-9,) and 2p23 (P=3.91×10-8). This study should expand the catalog of genetic factors for SLE and throw new insights into the pathogenesis to the molecular level.

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A genome-wide association study of vitiligo identifies two independent associations within MHC region and one novel susceptibility locus on 6q27

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Vitiligo is a common depigmentary disorder resulting from selective destruction of melanocytes in the skin and hair, with diverse prevalence rates ranging from 0.1 to 2.9% in different geographic regions and ethnic groups.The pathogenesis of vitilogo is still unclear. We conducted a genome-wide association study of generalized vitiligo in Chinese Han population by genotyping 1,117 cases and 1,429 controls using Illumina Human 610-Quad BeadChips. We took the most promising SNPs for replication in Chinese Han (5,910 cases and 9,916 controls) and Chinese Uygur (713 cases and 824 controls). We identified two independent associations within the MHC region: one within the HLA class III region (Pcombined= 1.48×10-48) and the other within the HLA class I region (Pcombined= 2.21×10-33). We also identified one novel locus at 6q27 (Pcombined=9.72×10-17), which contains three potential susceptibility genes biologiacally. Our study should help us gain a better understanding on the genetic background of vitiligo and provide new insights into the genetic basis of vitiligo.

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Meta-GWAS of psoriasis reveals genetic heterogeneity between Chinese and European populations and identifies two new susceptibility loci

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Genome-wide association studies (GWAS) of common disease have so far been conducted mainly in populations of European descent, limiting our understanding of population differences in genetic susceptibility to common diseases. By analyzing two genome-wide datasets from populations of Chinese origins (1589 psoriatic patients and 1702 controls) and two datasets representing populations of European origins (2074 psoriatic patients and 2450 controls), we revealed significant genetic heterogeneity between the two populations at the previously identified susceptibility loci, although many associations were found to be the same across different populations. Furthermore, by performing a joint analysis of the combined GWAS dataset (consisting of a total of 3663 patients and 4152 controls), and following-up 54 top SNPs emerging from the joint analysis in additional 7167 patients and 8655 controls of Chinese, we identified two novel susceptibility loci in 14q and 17q. The ethnic difference of genetic risk factors between Chinese and European populations indicates the complexity of the genetic basis of psoriasis and highlights the importance for performing GWAS in diverse populations.

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A double-blind, randomized, vehicle-controlled proof of concept study to evaluate the safety, local tolerability, pharmacokinetics and pharmacodynamics of multiple topical administrations Of LDE225 (a specific smoothened inhibitor) on skin basal cell carcinomas in Gorlin Syndrome patients

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Purpose: We conducted a proof of concept study evaluating LDE225, a novel smoothened (SMO) inhibitor, for the topical treatment of Basal Cell Carcinomas (BCCs) in Naevoid Basal Cell Carcinoma Syndrome (NBCCS) patients. Methods: A total of 8 NBCCS patients, presenting 27 BCCs, were treated bid with 0.75% LDE225 cream or vehicle for 4 weeks. Results: LDE225 was well tolerated and showed no skin irritation. Plasma LDE225 concentrations after 4 weeks were below detection level (0.05 ng/mL) in 4/8 patients (highest plasma level detected was 0.11 ng/mL). Mean LDE225 skin concentrations were 737 ng/g (BCC) and 605 ng/g (uninvolved skin). LDE225-treated BCCs (n=13) showed complete clinical response in 3, partial response in 9 and no response in 1 BCC. Except for one partial response, the vehicle produced no clinical response in any of the 14 treated BCCs. Mean volume reductions of 49.8% were observed in the LDE225-treated BCCs *vs.* 9.1% with vehicle; mean surface area reductions were 40.8% and 10.5%, respectively (3D digital photography). Biomarker analysis showed that, except for one patient, Gli 1, Gli 2, Ptch 1 and Ptch 2 mRNA level reductions correlated with the clinical outcome. Conclusions: This is the first demonstration that inhibiting the hedgehog pathway with a topical SMO inhibitor is effective in the treatment of BCCs in NBCCS patients. Since the use of other currently available topicals for treatment of BCCs is limited by skin irritation, treatment with LDE225 cream in NBCCS patients may offer a significant point of differentiation.

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Pemphigus vulgaris: A genome-wide association study

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Pemphigus vulgaris (PV) is a rare autoimmune blistering disease. It is prevalent among Ashkenazi Jews and is in part genetically determined. The low prevalence of the disease represents a significant obstacle to a genome wide approach to the mapping of susceptibility genes. We reasoned that the study of a genetically homogeneous cohort may filter out irrelevant signals and therefore genotyped 300K SNPs in a case-control study of 102 PV patients of Jewish Ashkenazi descent (4 grand-parents originating from Eastern Europe countries) and 400 matched control subjects. Results for 99.5% of the SNPs that passed quality-control filters were combined with the use of Cochran-Mantel-Haenszel stratified analysis. The top 1% of the SNPs were marked as indicative of an association and their concentration along the genome was scored via the hypergeometric distribution of associated vs. non-associated SNPs within an examined window. SNPs showing a significant association were validated in our initial set of 502 subjects and in an additional 100 healthy matched controls using PCR-RFLP. The highest scoring area was found to map to the MHC locus on 6p21.3 (Bonferroni corrected p-value = 10e-30), confirming the results of previous candidate gene-based studies. We also observed association between PV and a genomic segment on 20p12 (Bonferroni corrected p-value = 0.000012). This later region spans the PLCB1 gene, encoding a phospholipase possibly involved in the pathogenesis of acantholysis in PV. Resequencing of the critical PV-associated PLCB1 region in PV patients revealed two strongly associated SNPs (rs708910 and rs1047383) which are part of recognition sequences for 4 miRNAs predicted to regulate the expression of PLCB1. In conclusion, this first genome wide association study in PV suggests novel biologically relevant risk loci for the disease and underscores the advantages of genetically homogeneous populations for the mapping of rare traits.

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Clericuzio-type poikiloderma with neutropenia resulting from a homozygous internal deletion mutation, c.179delC, in the *C16orf57* gene

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Clericuzio-type poikiloderma with neutropenia (CPN; OMIM604173) is an autosomal recessive disorder characterized by poikiloderma, palmoplantar keratoderma, pachyonychia, fragile carious teeth and lachrymal duct obstruction, as well as neutropenia that manifests with infections, particularly chest. CPN shows some clinical overlap with Rothmund-Thomson syndrome (RTS; OMIM268400) but CPN does not result from mutations in the RTS gene, *RECQL4*. We investigated the molecular basis of CPN in a consanguineous Moroccan family with 3 affected offspring. Genome-wide linkage using a 10k single nucleotide polymorphism (SNP) array identified 5 areas of possible homozygosity, including a ~15.3cM region on 16q12.2-q21 (maximum lod score 2.53). This interval contains ~100 genes/putative genes but a recent publication has proposed *C16orf57* as a candidate gene for CPN, with identification of a homozygous splice site mutation in a consanguineous Italian pedigree with CPN and compound heterozygous splice site mutations in an Italian individual with atypical RTS associated with myelodysplasia (Volpi et al., Am J Hum Genet 2010;86:72-6). Direct sequencing of genomic DNA from the affected individuals in our family identified a novel homozygous deletion (c.179delC, p.Pro60fsX54) in the *C16orf57* gene; both clinically normal parents were heterozygous carriers of this frameshift mutation. Little is known about *C16orf57* or the functions of its encoded protein, although there may be a link to RECQL4 via SMAD4-mediated signaling. This observation provides initial data to link the phenotypes of CPN and RTS but identification of *C16orf57* as the CPN gene now allows for molecular distinction between the two inherited poikilodermatous syndromes as well as better anticipation of myeloid abnormalities in susceptible patients.

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Association of single nucleotide polymorphisms in the IL-12 and IL-12 receptor genes and gene-gene interactions with atopic dermatitis in Koreans

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The acute skin lesions of atopic dermatitis (AD) are associated with Th2 cells; however, the chronic lesions are associated with Th1 cells via IL-12. We evaluated the associations of single nucleotide polymorphisms (SNPs) and haplotype in the IL-12 and IL-12 receptor genes, and determined the gene-gene interactions between the SNPs of these genes and the SNPs of the IL-18 gene. We genotyped 24 SNPs from 4 IL-12/IL-12R genes for 1,089 case-control samples (631 AD patients and 458 controls). We measured the serum IL-12 concentrations in 89 individuals (79 AD and 10 controls) by ELISA. We analyzed the SNPs and haplotypes in each gene and also searched for the gene-gene interactions. The rs582504 (IVS-798A/T) SNP and the haplotype TA (rs582054 and rs2243151) in IL-12A gene, and the rs438421 (IVS12+1266T/C) SNP and the haplotype CCA (rs375947, rs438421, and rs1870063) in the IL-12RB1 gene were significantly associated with AD phenotype. We showed that the rs438421 polymorphism in IL-12RB1 (TT) gene and the rs2066446 polymorphism in IL-12RB2 (AA) gene had a significant interaction to develop the ADe phenotype (allergic type of AD), and those individuals with the risk alleles, TT/AA/CC (IL-12RB1/IL-12RB2/IL-18), have more than a 10-fold increased risk to develop ADe. This study provides evidence for a significant interaction between IL-12RB1 and IL-12RB2 genes that contribute to a 4-fold increased risk for developing ADe. In addition to the IL-12R interaction, we suggest that IL-18 gene can significantly interact with the IL-12R gene to develop ADe. In addition to the interaction, the SNPs and haplotypes in the IL-12A and IL-12RB1 genes are independently and significantly associated with the AD phenotype, and especially with the ADe phenotype. This data may contribute to our understanding of AD genetic interactions and account for the additional risk of certain patients to develop AD.

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Association of single nucleotide polymorphisms and haplotype in SPINK5 gene with atopic dermatitis in Koreans

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Clinical studies, including twin studies, support the concept that the risk of atopic dermatitis (AD) may be mediated through skin-specific genes, rather than simply through systemic immune or atopy risk genes. The SPINK5 gene is expressed on epithelial surfaces and may provide protection against other allergenic serine proteases. Mutations in the SPINK5 gene result in Netherton syndrome, a disorder characterized by AD, ichthyosis, and elevated serum IgE levels. We genotyped 21 SNPs from the SPINK5 gene for 1,090 case-control samples (631 AD patients and 459 normal controls) and analyzed the SNPs and haplotypes in this gene and also searched for gene-gene interactions between SPINK5 and the DEFB1 gene that we previously reported. Six SNPs (rs17718511, rs17860502, KN0001820, rs60978485, rs17718737, and rs1422985) and the haplotype TAA (rs60978485, rs6892205, rs2303064) in the SPINK5 gene showed significant different allelic or genotypic distributions between the AD group and the control group. We also found that four SNPs (rs17718511, rs17860502, rs60978485, rs17718737) and the haplotype TAA in the SPINK5 gene showed associations with the susceptibility of the allergic type of AD (ADe). In addition to this finding, we speculate that the SNPs from DEFB1 and SPINK5 affect the individual susceptibility to development of ADe in an additive manner. This study provides evidence for a significant interaction between allergens and the SPINK5 gene that may contribute to ADe susceptibility.

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Molecular, clinical and functional characterization of a large pedigree with generalized peeling skin disease

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Generalized peeling skin disease (PSD) is an unusual autosomal recessive ichthyosiform erythroderma characterized by lifelong patchy peeling of skin on the the entire body. Up to date only 12 to 15 individuals have been reported. Affected individuals show first symptoms at birth or shortly thereafter, which encompass erythematous, peeling lesions and pruritus as well as hypohidrosis and periodic mild to moderated onychodystrophy. We have studied a large consanguineous pedigree with four affected individuals with PSD with severe pruritus and a history of urticaria and angioedema. High IgE levels were accompanied by severe atopic manifestations, e.g. food allergies. Due to the phenotypic similarities of PSD with Netherton syndrome, as well as the acral type of peeling skin syndrome, we performed mutation analyses in the genes *SPINK5* and *TGM5*, respectively. No mutation was found. We have therefore recruited the 5-generation pedigree and carried out a whole-genome linkage scan using chip-based SNP analysis, which identified a 3.3 cM candidate region with a maximum lod score of 5.4, corresponding to 5.7 Mb in length. All four patients originated from the same Roma tribe but from two different core families. For candidate gene identification we then searched for identical homozygous regions between all four patients using SNPs and microsatellites. The largest homozygous interval was 3.0 Mb in length and contained 195 genes. Findings in a functional candidate gene from the region are now being refined. In parallel we developed a 3D skin model with keratinocytes from one of the patients to further analyse the biochemical and physiological characteristics in an in-vitro setting. These disease models demonstrated a profound epidermal barrier defect accounting for the predisposition to atopic diseases.

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Magnesium carbonate-containing phosphate binder prevents connective tissue mineralization in *Abcc6*$^{-/-}$ mice - potential for treatment of pseudoxanthoma elasticum

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Pseudoxanthoma elasticum (PXE) is a heritable disorder characterized by ectopic mineralization of connective tissues primarily in the skin, eyes, and the cardiovascular system. PXE is caused by mutations in the *ABCC6* gene. There is currently no effective or specific treatment. In this study, we tested oral phosphate binders for treatment of a mouse model of PXE which we have developed by targeted ablation of the corresponding mouse gene *(Abcc6*$^{-/-}$*)*. This knock-out mouse model recapitulates features of PXE and the connective tissue capsule surrounding vibrissae in the muzzle skin serves as an early biomarker of the mineralization process. Treatment of these mice with magnesium carbonate-enriched diet (magnesium concentration being 5-fold higher) completely prevented mineralization of the vibrissae up to six months of age. This diet also prevented the progression of mineralization when the mice were placed on experimental diet at three months of age and followed up to six months of age. Treatment with magnesium carbonate had no effect on serum calcium and phosphorus levels. In contrast, concentration of calcium in the urine was increased over ten-fold while the concentration of phosphorus was markedly decreased being essentially undetectable after long term (> 4 month) treatment. Computerized axial tomography scan of bones in mice placed on magnesium carbonate-enriched diet showed no differences in the bone density and there was a small increase in the calcium and phosphate content of the femurs by chemical assay, in comparison to mice on control diet. Similar experiments with another diet supplemented with lanthanum carbonate did not interfere with the mineralization process in *Abcc6*$^{-/-}$ mice. These results suggest that magnesium carbonate may offer a potential treatment modality for PXE, a currently intractable disease, as well as for other conditions characterized by ectopic mineralization of connective tissues.

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Mutation analysis of *ABCC6* in a family with pseudoxanthoma elasticum - presymptomatic testing with prognostic implications

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Pseudoxanthoma elasticum (PXE) is a autosomal recessive disorder characterized by ectopic mineralization of extracellular matrix of connective tissues. The disease primarily affects the skin, the eyes, and the cardiovascular system, with considerable mortality and occasional morbidity. PXE is caused by mutations in the *ABCC6* gene which encodes a transmembrane transporter expressed primarily in the liver and the kidneys. Over 300 distinct mutations have been identified in the *ABCC6* gene. The utility of mutation detection in PXE is emphasized by the fact that the onset of the disease is delayed, and definitive diagnosis in patients with PXE is often not established until late teens or twenties, or even later in life. In families with history of an affected individual with known *ABCC6* mutations, genotyping can be used to determine if other members of the family are at risk for developing PXE by presymptomatic DNA testing. In this study, we utilized a streamlined mutation analysis in a family with history of PXE. A previously known, homozygous mutation p.R1221H was identified in the proband, a 19-year old female who had been diagnosed for PXE on the basis of characteristic cutaneous findings and the presence of angioid streaks at the age of 14 years. The proband's parents are clinically normal. The proband's younger brother, 13 years of age, was concerned of the potential of developing PXE later in his life. Analysis of the *ABCC6* gene revealed that the proband's brother and parents were heterozygous carriers for p.R1221H. On the basis of these analysis, we concluded, therefore, that the younger brother was not at risk for developing PXE, thus alleviating concerns of his future health status with respect to PXE.

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Angioid streaks in pseudoxanthoma elasticum – role of p.R1268Q in the *ABCC6* gene

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder characterized by ectopic mineralization in the eye, the skin, and the cardiovascular system, resulting in decreased vision, skin lesions, and vascular disease, with considerable intra- and inter-familial phenotypic variability. PXE is caused by mutations in the *ABCC6* gene. Over 300 distinct mutations have been identified in this gene. Careful examination of the mutation database in the context of phenotypic variability in PXE has not revealed any clear cut genotype-phenotype correlations. It has been suggested that p.R1268Q mutation may be associated with angioid streaks, the eye disease in patients with PXE. We have reviewed our PXE database, consisting of a total of 121 individuals with PXE, for the presence of this missense mutation. We identified a total of 10 individuals who were either heterozygous or homozygous for p.R1268Q. All these individuals had characteristic skin findings consistent with PXE, in most cases this diagnosis was confirmed by skin biopsy. All individuals who had the p.R1268Q mutation also showed angioid streaks, associated with other ocular findings. The findings suggest that this sequence variant may contribute to the development and/or severity of angioid streaks in patients with PXE.

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Correction of a mutant keratin 6a gene in HEK293 cells using small oligonucleotides

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Missense mutations in the Keratin 6a gene are responsible for some cases of Pachyonychia Congenita. To test potential therapies that could correct such genomic mutations in this and other dominant negative diseases, we generated cell lines containing a keratin 6a-EYFP chimeric construct with a stop codon in the first helix-coding domain. Correction of the stop codon in K6a should lead to expression of keratin 6a-EYFP, which can be detected by FACS analysis or microscopy of the endogenous keratin filament network. Short homologous oligonucleotides ("donors") are known to correct mutations at a low frequency. Triplex-forming oligonucleotides (TFOs) that bind near a target mutation, have been shown to increase the frequency of donor-directed correction. We designed a peptide nucleic acid (PNA) triplex-forming clamp and used a gel retardation assay to demonstrate binding to duplex DNA in the vicinity of the stop codon in our mutant K6a-YFP reporter. Electroporation of "donor" oligonucleotides that correct the TAG stop codon to an AAG lysine, increase the number of fluorescent cells over baseline, assayed by FACS analysis. Double- and single-stranded donors were effective, with single-stranded antisense donors giving more fluorescent cells than sense donors. Colonies of cells with filamentous fluorescence can be seen in a field of non-fluorescent mutant cells after correction of the stop codon. Tests of the ability to the PNA to increase frequency of correction are underway.

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Mutations in the Zinc transporter gene SLC39A13 do not account for the phenotype of Ehlers-Danlos syndrome type VIB in cell lines from 15 patients

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The Ehlers-Danlos syndrome (EDS) constitutes a group of heritable connective tissue disorders that are clinically characterized by fragile and hyperextensible skin and joint hypermobility. Of the 6 major subtypes, the autosomal recessive kyphoscoliosis type (EDS VIA) is attributed to mutations in the lysyl hydroxylase1 (LH1) gene, which codes for a post-translational modifying enzyme (LH) important in collagen biosynthesis. A class of patients (EDS VIB) exists who have a similar clinical phenotype to EDS VIA but with normal LH activity. Our earlier studies on 4 EDS VIB patients showed no linkage to mutations in the genes for LH1, 2 and 3 (Walker et al, Am J Med Genet, 131A:155, 2004). However, based on recent reports that 3 families with EDS VI-like phenotypes were linked to homozygous mutations in the Zinc transporter gene SLC39A13, we screened 15 of our cell strains from EDS VIB patients for mutations in this gene. To accomplish this, we amplified each of the 9 exons in the SLC39A13 gene by PCR of genomic DNA isolated from their skin fibroblasts. Although sequence analysis of the gel-purified PCR fragments identified an identical heterozygous base change predicted to code for a T242A in exon 2 of cell strains 1248 and 1253, no other mutation could be detected in the exons of these cell strains to account for their EDS VIB phenotype. In exon 5 of SLC39A13, a silent 765G→A was detected as either a homozygous or compound heterozygous base change in 7 out of our 15 cell strains, but not in strains 1248 and 1253. Sequence analysis identified three other polymorphic markers in introns 1 and 2 in the SLC39A13 gene. These included, in intron 1, a change of 8105G→A in 6 cell strains and, in intron 2, an identical polymorphism was detected at base 8537 in the same 6 cell strains. A different polymorphism of an 8542C→T in intron 2 was detected in 7 cell strains. This study indicates that mutations in the SLC39A13 gene do not account for the EDS VIB phenotype in these cell lines and that other genes may be involved.

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A splicing enhancer element in exon 13A of the lysyl hydroxylase2 (LH2) gene promotes expression of the fibrosis-associated, alternatively-spliced long form of LH2

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Collagen synthesis increases significantly in fibrotic conditions such as scleroderma. This over-accumulation of collagen is associated with an increase of irreversible pyridinoline (Pyr) crosslinks that impart tensile strength to collagen. As a prerequisite to Pyr crosslink formation, lysyl residues in the collagen telopeptide are hydroxylated by the long form of the alternatively-spliced, post-translational modifying enzyme lysyl hydroxylase2 (LH2). As LH2(long), which includes the alternatively-spliced 63bp exon 13A, is overexpressed in fibrotic disease, the regulation of LH2 splicing is important to control. In this study we have examined exon 13A for the presence of exonic cis elements that may regulate LH2 splicing. Using an LH2 minigene we mutated the full exon 13A, with the exception of the end sequences to ensure maintenance of the integrity of the splice sites, by substitution of a 57 nucleotide sequence (between nts +4 to +60). Following transfection of this mutated construct into mouse embryonic fibroblasts (MEFs), RT-PCR showed a consistent and significant decrease in exon 13A inclusion in LH2(long). This indicated the presence of one or more splicing enhancer elements in exon 13A. To identify the exact location of the enhancer element in the minigene, smaller 7 nucleotide sequential mutations were introduced into exon 13A, and the mutant constructs were transfected into MEFs and analyzed by RT-PCR. Results from these experiments identified a sequence between nts +11 and +17 of exon 13A that was as effective in dramatically reducing exon inclusion as that resulting from substitution of the entire exon 13A. Identification of this 7bp enhancer element may offer an effective therapeutic target for manipulation of the alternate splicing of LH2.

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Global transcriptional analysis in psoriasis skin and blood highlights several genomic hotspots for differentially expressed genes

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The genetic basis of psoriasis has been extensively investigated but a comprehensive list with the exact locations of susceptibility genes has not been generated. The merging of transcriptional and genetic data may be a strategy to focus efforts to pinpoint disease associated loci. In this study we aimed to: 1) define differentially expressed genes (DEGs) between lesional and nonlesional psoriatic skin, and between psoriatic and nonpsoriatic blood, and 2) evaluate the distribution of DEGs on the genome to identify the overlap with previously reported candidate susceptibility loci and illuminate the chromosomal regions with over-represented DEG frequency (genomic 'hotspots'). Gene expression in 18 skin (9 lesional and 9 nonlesional) and 13 blood (8 psoriatic and 5 nonpsoriatic) samples were examined using Affymetrix HG-U95A microarrays. Paired (skin) and unpaired (blood) t tests and Benjamini-Hochberg method was used to evaluate differential gene expression. At 5% false discovery rate (FDR) 1529 (1148 upregulated, 381 downregulated) DEGs distinguish lesional from nonlesional skin. Comparing psoriatic vs nonpsoriatic blood we identify 153 (41 upregulated, 112 downregulated) DEGs at 5% FDR. Within the pool of transcriptionally dysregulated genes, a subset of sequences may owe their over- or under-expression to genetic alterations. We found that 159 (11.8%) skin and 18 (10.4%) blood DEGs map to one or more PSORS loci. These sequences may offer prioritized targets for genetic fine mapping at PSORS sites. Furthermore, using the genome tool in dChip (http://biosun1.harvard.edu/complab/dchip/), we identified 20 genomic 'hotspots' for skin corresponding to 2 confirmed susceptibility loci at PSORS4 and PSORS11, 4 suggested psoriasis susceptibility loci, and 14 novel chromosomal regions. Two genomic 'hotspots' were found for the blood DEGs, overlapping with 2 novel skin 'hotspots' at 6p25 and 11q12-13. Novel DEG 'hotspots' identified in our study may provide new targets for future susceptibility loci studies in psoriasis.

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Topical application of type VII collagen for wound healing and treatment of DEB

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Patients with dystrophic epidermolysis bullosa (DEB) have incurable skin fragility, blistering and multiple skin wounds due to mutations in the gene that encodes for type VII collagen (C7) that holds the epidermal and dermal layers of human skin together. The intradermal injection of gene-corrected DEB fibroblasts, recombinant C7 protein, or lentiviral vectors expressing C7 is potential therapy for DEB. In this study, we sought to determine if topically applied C7 could be used to treat DEB and enhance wound healing. To accomplish this, we first made a 1 square - centimeter full-thickness wound on the back of athymic nude mice and applied 20-40 micrograms of recombinant human C7 to the skin wounds. Skin biopsies from the wounded areas were obtained every week two weeks after topical application and subjected to immunostaining using an antibody specific for human C7. Surprisingly, the topically applied human C7 stably incorporated into the newly formed BMZ of the mouse's skin. In contrast, there was no human C7 expression in vehicle treated wounds. Time course observations and histological analysis revealed that wounds treated with C7, when compared with control wounds (vehicle or BSA), demonstrated accelerated wound healing, increased epidermal and dermal regeneration, reduced contraction, and more highly organized collagen fiber deposition, consistent with less scar formation. C7-treated wounds also had increased re-epithelialization due to C7's ability of enhancing keratinocyte migration. Lastly, we directly compared the effect of topical C7 and topical recombinant human PDGF (0.01% Regranax), a FDA-approved agent that promotes healing of diabetic skin ulcers. The C7-treated wounds demonstrated remarkable enhanced wound closure compared with PDGF-treated wounds. These data demonstrate that topically applied C7 can deliver C7 to the skin BMZ and promote wound healing. The strategy of using topically applied C7 may be particularly useful for severe DEB patients with multiple skin wounds, as well as patients with chronic skin wounds.

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Polymorphisms in the obesity related FTO gene are associated with psoriasis

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Psoriasis is associated with increased body mass index (BMI). Variants in the FTO gene are associated with obesity. To study if single nucleotide polymorphisms (SNPs) in the obesity related region of the FTO gene associate with psoriasis, we searched for obesity related SNPs in our psoriasis genome wide association study (GWAS) with 1,359 cases and 1,400 controls. Ten SNPs were found within the haplotype block extending from 52355kb to 52407kb in the first intron of the FTO gene which carries the obesity related SNPs. Trend test was used to compare the allele frequency in cases and controls. Based on Bonferroni correction for 10 SNPs, p-value<0.005 was considered significant. Four SNPs significantly associated with psoriasis (rs4784323, p<10-3, OR=0.82; rs8050136, p=0.002, OR=1.19; rs4783819, p<10-3, OR=0.83; rs9941349, p=0.003, OR=1.18). This prompted an analysis on 444 psoriasis patients from Utah where the BMI was known. We used a random sample of 828 Utah subjects as controls. These subjects had previously been genotyped for rs9939609, the major obesity related SNP in the haplotype block noted above. Genotype for rs9939609 was imputed in psoriasis cases. In the original GWAS cohort, rs9939609 associated with psoriasis (p=0.004 OR=1.17). In the multiple logistic regression in the subset with known BMI, only BMI remained significantly associated with psoriasis (p=0.008 OR=1.03 for BMI and p=0.3 for rs9939609). Of note, rs9939609 associated with obesity in our psoriasis cohort (logistic regression in BMI<25 vs BMI≥30, p=0.005 OR=1.63) Our results suggest that psoriasis patients are more likely than controls to carry obesity related genetic variations. This is consistent with the previously reported finding of an increased BMI in psoriasis patients. We were not able to replicate the association of psoriasis and FTO variations after adjustment for BMI. Replication of the results in another set of psoriasis cases and controls is pending.

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Altered DNA methylome in patients with atopic dermatitis

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Epigenetic mechanisms play key roles in normal development and functions of immune system. Aberrant DNA methylation, one of epigenetic mechanisms, causes human diseases, such as cancers and skin diseases. Atopic dermatitis (AD) is a chronic and allergic inflammatory skin disease known to be associated with immune cells, particularly, CD4+ T cells. In this study, we applied genome-wide association (GWA) study with integrated DNA methylome and transcriptome to identify differentially methylated genes that may be responsible for causing AD. Genome-wide DNA methylation of human unstimulated naïve CD4+ T cells isolated from PBMC cells from AD patients or healthy individuals was mapped by high-throughput sequencing after methylated-CpG island recovery assay (MIRA), and genome-wide gene expression of the same CD4+ T cells was investigated by a microarray with affymetrix human gene 1.0 ST. Differential pattern analysis was also used to scrutinize association of DNA methylation and gene expression in the genome level of patients with AD compared to healthy individuals. Although the alteration of DNA methylation was not shown strong inverse correlation with changes of gene expression, DNA methylation patterns at the promoter of genes differed in patients with AD: a number of genes were shifted to hypermethylation or hypomethylation from DNA methylation patterns of healthy individuals. In patients with AD altered DNA methylome was supported by lower levels of transcript of de novo DNA methyltransferases (DNMTs), DNMT3a and DNMT3b. In addition, repetitive element regions in the genome of patients with AD were hypomethylated, which indicates that genome stability was changed. These results imply that epigenomic change and genome instability are related to AD associated with immune cells.

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Delineation of the mode of action of SAMD9, a protein deficient in the normophosphatemic subtype of familial tumoral calcinosis

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Dystrophic calcification accompanies disorders as common as autoimmune diseases and cancer. To get insight into the pathogenesis of this poorly understood process, we studied the function of SAMD9. SAMD9 is a protein of unknown function, recently shown to be deficient in an hereditary form of dytrophic calcification known as normophosphatemic familial tumoral calcinosis (NFTC). Consistent with the fact that in NFTC, severe inflammatory manifestations precede cutaneous calcinosis, SAMD9 was found to be tightly regulated by interferon-gamma (IFNG) in various cell cultures. In addition, the SAMD9 promoter was found to respond to IFNG in a luciferase reporter assay. Of interest, we identified a critical 25 bp fragment upstream to SAMD9 transcription initiation site responsible for most of the gene expression. Bioinformatic analysis suggested that SAMD9 function involves interaction with additional proteins. Using an RSS assay and confirmatory immunoprecipation, we demonstrated that SAMD9 interacts with RGL2. To study the biological importance of this interaction, we assessed the effect of RNAi-mediated downregulation of this pair of proteins in the presence and absence of IFNG using microarrays and validation with qRT-PCR. We observed that downregulation of any of the two protein partners caused increased expression of EGR1, a transcription factor with a known role in the regulation of tissue calcification, inflammation and cell migration. Interestingly, both SAMD9 and RGL2 were found to be required for proper assembly of the actin cytoskeleton. Supporting the physiological relevance of these data, EGR1 levels were also upregulated in a fibroblast cell line derived from an NFTC patient. In conclusion, our data indicate that SAMD9, an IFNG-responsive protein interacts with RGL2 to diminish the expression of EGR-1, a protein of direct relevance to the pathogenesis of ectopic calcification and inflammation.

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Tolerance induction towards type XVII collagen

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The induction and maintenance of tolerance towards a neo-antigen is critical to the success of gene therapy in autosomal recessive genetic diseases. One such disease is junctional Epidermolysis bullosa, in which patients lack type XVII collagen in the dermo-epidermal basement membrane zone. Hence, the aim of this study was to investigate novel protocols that prevent autoimmunity towards type XVII collagen. Our approach involved induction of tolerance by targeting dendritic cells (DC), which are able to induce antigen-specific regulatory T cells. As a model antigen we used the immunodominant NC16A domain of human type XVII collagen. We fused the NC16A domain to the single chain Fv antibody specific for murine DEC205 (DEC-NC16A), which is expressed on DEC205+ DCs. NC16A fused to a single chain isotype control, NC16A without DEC205, as well as DEC205 alone served as control groups. After transfection of murine skin (BALB/c), DEC-NC16A resulted in reduced production of NC16A specific IgG. Moreover, reduced secretion of effector cytokines from skin draining lymph nodes was detected in an antigen-specific ELISPOT. Thus, successful re-stimulation of these T cells with NC16A resulting in IL-2 secretion excluded general unresponsiveness. We conclude from these data that immunoregulatory mechanisms controlled by DCs are able to suppress antigen-specific responses.

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Cylindrospiradenomas may arise from immunoprivileged hair follicle stem cells and are vulnerable to anti-inflammatory treatment

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Cylindrospiradenomas (CSPA) are benign skin appendage tumors that can develop at multiple sites of hair follicle (HF) bearing skin as a result of Brooke-Spiegler Syndrome (BSS). The autosomal-dominant BSS is associated with mutations in the CYLD gene that encodes a deubiquitinase which inhibits NF-kB signalling resulting in an anti-inflammatory and anti-proliferative effect. Following up our previous hypothesis that these tumors arise from epithelial hair follicle stem cells (eHFSC) or their progeny (Massoumi et al. JID 2005), we first immunostained CSPA sections from three BSS patients for the human eHFSC markers keratin 15 (K15) and the immune privilege marker CD200. Interestingly, multiple K15 and CD200 positive, but beta1-integrin-negative cells were found lining the tubular tumor structures, while most of the epithelial tumor nodules were K15-negative but brightly beta1-integrin-positive. This suggests that CSPA nodules in BSS are derived from immunoprivileged HFSC-like cells and share some characteristics with highly proliferative, undifferentiated basal layer keratinocytes. Interestingly, BSS patients also show extensive T cell infiltrates in tumors and tumor-free regions of the scalp and strong, ectopic expression of MHC class II molecules on the HF's ORS. This suggests that inflammatory processes precede or accompany tumor formation and growth. To test whether anti-inflammatory agents inhibit tumor growth, we established a serum-free assay normally used for HF organ culture that allows the maintenance of CSPA fragments for up to 6 days *in vitro*. Addition of Na-salicylate resulted in increased cell death in treated compared to untreated CSPA fragments. Thus, administration of anti-inflammatory agents may offer a pharmacological alternative to surgical cylindro(spiradeno)ma management.

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A milieu of regulatory elements in the epidermal differentiation complex (EDC) syntenic block: Implications for atopic dermatitis and psoriasis

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Two common inflammatory skin disorders with impaired barrier, atopic dermatitis (eczema) and psoriasis, share distinct genetic linkage to the Epidermal Differentiation Complex (EDC) locus on chromosome 1q21. The EDC is comprised of tandemly arrayed gene families (FLG-like, LCE, SPRR, and S100) that encode proteins involved in skin epidermal cell differentiation. Association of loss-of-function mutations in filaggrin (FLG) and a copy number variation within the LCE genes in atopic dermatitis and psoriasis, respectively, demonstrate a role for EDC genes in the pathogenesis of these diseases. To date, little is known about the potentially complex regulatory landscape within the EDC. Here we report a computational approach to identify conserved noncoding elements (CNEs) in the EDC queried for regulatory function. We provide compelling evidence for evolutionarily conserved regulatory milieu in the EDC by identification of coordinate expression of EDC genes during mouse embryonic skin development and a striking degree of synteny and linearity in the EDC locus across a wide range of mammalian eutherian (placental) and metatherian (marsupial) genomes. CNEs identified by comparative genomics exhibit dynamic regulatory activity (enhancer or repressor) in cell-based reporter assays under differentiating or proliferating conditions. Using DNaseI and transgenic mouse asays, we further demonstrate epidermal-specific, developmental in vivo enhancer activities in CNEs, including one within the psoriasis-associated deletion, LCE3C_LCE3B-del. Together, our multidisciplinary study features a network of regulatory elements coordinating developmental EDC gene expression as an unexplored resource for genetic variants in skin diseases.

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A large mutational study in Pachyonychia Congenita

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The International Pachyonychia Congenita Research Registry gathers clinical and molecular details from pachyonychia congenita (PC) patients. PC, a rare autosomal dominant keratin disorder characterized by severe focal palmoplantar keratoderma, hypertrophic nail dystrophy and oral leukokeratosis is caused by mutations in keratin genes; KRT6A, KRT6B, KRT16 or KRT17. Mutations in KRT6C cause focal palmoplantar keratoderma with very mild/no nail changes. Genetic analysis identified mutations in 153/171 families, 47% KRT6A, 24% KRT16, 12% KRT17, 3.5% KRT6B, and 0.6% KRT6C; 2.9% had GJB6 mutations and in 10%, no mutation was detected. The majority of mutations were missense or small insertion/deletion mutations. In this cohort, 57 different keratin mutations were identified. 31 mutations were identified in KRT6A, 8 recurrent, with codon N171 the most common site (49%) for mutation either as missense substitution or deletion mutation. 15 different mutations were found in KRT16, 5 recurrent; the most common, K16p.L132P, occurred in 29% of families. Of 8 different KRT17 mutations, 2 were recurrent with K17p.N92S, occurring in 55% of families with KRT17 mutations. Only 2 different mutations were found in KRT6B and one mutation in KRT6C. As the number of mutations increase, some genotype-phenotype correlations can be made, eg. the K16 mutations p.N125S and p.R127C (both now identified in several families) are associated with a form of PC with very mild or no nail changes. From the detailed clinical information collected, a number of atypical PC families were identified. For example, five families with varying degrees of alopecia in addition to the typical PC features indicated Clouston syndrome; mutations were subsequently identified in GJB6. Many of the remaining 10% of families with no mutation appear atypical of PC; these are currently being investigated for mutations in other genes that may also result in a PC-like phenotype.

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The mouse *Samd9L* gene: Developmental and tissue-specific expression

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Normophosphatemic familial tumoral calcinosis (NFTC) is an autosomal recessive disease characterized by progressive buildup of dermal, particularly periarticular, calcium-phosphate deposits, despite normal plasma calcium and phosphate levels. NFTC has been shown to harbor mutations in the Sterile Alpha Motif Domain containing 9 gene *(SAMD9)* on chromosome 7. Very little is known about the function of *SAMD9* and its paralogous gene, *SAMD9L*, which resides in head-to-tail orientation in the same chromosomal locus. During evolution, however, *SAMD9* homolog is deleted in mouse, and it has been postulated that mouse *Samd9L* may substitute for its function. This study examined the developmental and tissue-specific expression of mouse *Samd9L*. qRT-PCR analysis of *Samd9L* revealed near-ubiquitous expression, with the highest level in the kidney, a major organ subject to calcium-phosphate regulation. Immunofluorescent staining of adult mouse kidney sections confirmed cytoplasmic localization in both proximal and distal tubules. The expression of *Samd9L* in the mouse embryos was first noted at 12.5 dpf, and the expression in the kidney progressively increased with age when the mice at the age of newborn, 2, 4 and 40 weeks were examined. Tissue-specific expression was also analyzed both by *in situ* X-gal staining and quantitative enzymatic activity assay in a transgenic *Samd9L*$^{-/-}$ mouse model in which the LacZ gene replaced exon 2 in the *Samd9L* gene. Beta-galactosidase enzymatic activity was observed clearly in the kidney and spleen. These findings assist in understanding the function of *Samd9L* and its paralogous genes, and in elucidation of the mechanisms responsible for aberrant extraosseous calcification in NFTC.

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Mutation-specific siRNA therapy for epidermolysis bullosa simplex.

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Epidermolysis bullosa simplex (EBS) is the most common hereditary blistering disorder, affecting ~1 in 12,000 individuals. The disease is primarily caused by dominant-negative point mutations in either the keratin K5 or K14 genes. Short interfering RNA (siRNA) technology has the potential to treat dominant genetic skin disorders such as EBS by specifically inhibiting the expression of the dominant-negative mutant allele, thereby allowing the wild-type allele to function normally. Typical siRNA molecules are of low molecular weight (19 nucleotides complementary to the target plus a 2 nucleotide overhang) and therefore have potential for delivery to the epidermis by topical formulation chemistry. Here, we have developed potent, allele-specific siRNA inhibitors aimed at two different mutations in the K5 gene. A luciferase reporter gene system was developed to assay wild-type K5 versus the K5 mutations S181P (severe generalised Dowling-Meara EBS) and N193K (site-restricted Weber-Cockayne EBS). For each mutation, all 19 possible allele-specific siRNA molecules were synthesised and tested for their ability to inhibit wild-type versus mutant gene expression over a standardised concentration range. For each mutation, two or more siRNAs were identified from the siRNA sequence walk that potently inhibited the mutant allele with negligible effect on wild-type K5. These lead inhibitors were further tested using epitope-tagged K5 expression constructs, where western blot analysis confirmed that they potently and specifically inhibit mutant K5. In addition, the cellular protein aggregation phenotype, which is a hallmark of EBS, was reversed in cultured cells treated with mutant-specific siRNA. Coupled with non-invasive delivery systems that are in development for topical application of siRNA, these molecules have the potential to treat EBS involving K5 mutations.

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Microarray analysis of CoffeeBerry® extract effects on human epidermis

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CoffeeBerry® extract (CBE) is a safe and natural extract derived from the fruit of the coffee plant, *Coffea arabica*, which contains potent polyphenols such as chlorogenic acid, condensed proanthocyanidins, quicinic acid, caffeic acid and ferulic acid. We have recently shown that topical use of CBE may reduce photo-aging by restoring skin integrity and protecting against epidermal DNA damage caused by sunlight exposure. To elucidate the underlying molecular mechanisms of these effects, a gene array analysis using the Agilent microarray platform was conducted to identify molecular pathways modulated by CBE in human epidermis. Briefly, duplicate reconstructed human epidermis (RHE) cultures were topically treated with vehicle or 1.5% CBE formulation one hour before UVB (150 mJ/cm^2) exposure. Untreated and sham-irradiated controls were included in parallel. At 2 and 24 hrs post-irradiation, cultures were harvested and RNA isolated. Comparative gene expression profiles were generated between CBE- and vehicle-treated groups, as well as between CBE-treated and untreated cultures, with and without UVB irradiation. In sham-irradiated RHE, CBE treatment modulated 19 genes at 2 hrs and 4,286 genes at 24 hrs, including genes involved in inflammation and DNA repair. Interestingly among these genes, early growth response (EGR) types 1, 2 and 3, which are transcription factors involved in differentiation, mitogenesis, morphogenesis and biological rhythm control, were all significantly upregulated by 3-4-fold. EGR type 1, also believed to be a tumor suppressor gene, was upregulated at 2 hrs, while types 2 and 3 were upregulated at 24hrs. In UVB-irradiated RHE, CBE treatment modulated 12 genes at 2 hrs and 23 genes at 24 hrs. EGR type 1 was also upregulated by 1.7-fold at 2 hrs post-UVB irradiation when compared with the vehicle control, suggesting that the involvement of this transcription factor in the restoration of skin integrity. Additional gene ontology analysis will further elucidate the molecular pathways associated with these changes in gene expression.

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Aminoglycosides restore type VII collagen function by overcoming premature stop mutations: implications for DEB therapy

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Dystrophic Epidermolysis Bullosa (DEB) is caused by mutations in the gene encoding type VII collagen (C7) in which 25% of DEB patients have premature stop codon (PTC) mutations. Therefore, a therapeutic approach to suppress PTC mutations could be of considerable benefit to these patients. Interestingly, aminoglycosides are able to suppress PTC mutations and restore synthesis of a full-length protein and have been used successfully in a number of genetic disorders. In this study, we evaluated the feasibility of using aminoglycosides for the potential treatment of DEB on two RDEB keratinocyte cell lines, one homozygous for a Q251X PTC mutation and the other heterozygous for R578X/R906 PTC mutations. Incubation of these cell lines with various concentrations of aminoglycosides including geneticin (G418), gentamicin, and pyromomycin resulted in the synthesis and secretion of a 290 kDa full-length C7 in a dose-dependent manner. G418 was the most potent agent for inducing read through of PTCs in both cell lines. Importantly, aminoglycoside treated cells demonstrated an enhanced cell-substratum adhesion and reversal of the hypermotility phenotype characteristic of RDEB cells. To further evaluate the general utility of aminoglycosides for DEB harboring different PTC mutations, we generated 30 PTC mutations associated with RDEB via site directed mutagenesis and transfected these mutant constructs into 293 cells. Treatment of these transfected cells with G418 or gentamicin induced read through and expression of full length C7 in 24 of the 30 (80%) RDEB mutant transfected cells. Surprisingly, most mutant constructs also produced truncated proteins in the absence of aminoglycoside treatment. These data demonstrate that aminoglycosides can suppress PTC mutations and restore C7 expression and function in DEB. Therefore, aminoglycosides or their derivatives may be a non-invasive, novel therapy for DEB and other inherited skin diseases caused by PTC mutations.

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Linking alopecia areata to atopy: Evaluation of transcriptionally regulated genes in patient subsets suggests a shared basis for genetic susceptibility

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Alopecia areata (AA) is a genetically determined autoimmune disorder of the hair follicle affecting 1.7% of the US population. Previous studies have shown that diseases of atopy affect up to 60% of AA patients. To gain further insight into the genetic links between alopecia areata and atopy we examined global gene expression patterns in the blood of AA patients (n = 9) as compared to healthy controls (n = 5) and mapped the top 200 differentially expressed genes (DEGs) ($p<0.001$) to identify chromosomal 'hotspots' with over-represented numbers of DEGs using the algorithmic "Genome" feature within dChip software (http://biosun1.harvard.edu/complab/dchip/>http://biosun1.harvard.edu/complab/dchip/). This analysis revealed three significant AA-associated genomic 'hotspots' at 1q21-32, 11q12-14, and 16p13-13.3. Interestingly, chromosome 1q and 11q regions have been previously described as susceptibility loci for atopic dermatitis. We next examined global gene expression patterns in the blood of AA patients with (n = 3) and without (n=3) a history of atopy and identified 158 up-regulated genes and 182 down-regulated genes differentiating the 2 patient subgroups. Ontologic analysis of these DEGs reveals an up-regulation of genes involved in inflammation and apoptosis and a down-regulation of genes involved in lipid metabolism in AA patients with a history of atopy. Again, the top 200 DEGs were mapped to reveal 5 statistically significant 'hotspots' (3p21-22, 9p13.3, 12q24, 15q14-21.2, and 17q21) that may harbor loci relevant to phenotypic variation. Utilizing transcriptional analysis as a guide to facilitate the search for disease susceptibility genes offers a novel strategy to enhance our understanding of the genetic basis of complex disease and reveal the genetic underpinnings of phenotypic heterogeneity within clinical entities.

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A comparison of Type VII collagen levels, mmp1 promoter polymorphism and clinical severity in a Mexican cohort of patients with recessive dystrophic epidermolysis bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB) is a severe inherited skin blistering disease caused by mutations in the gene encoding type VII collagen, COL7A1. Recently it has been proposed that a polymorphism in the promoter of the MMP1 gene, which encodes the matrix metalloproteinase MMP1, is a modifier of clinical severity in this disease. The purpose of this study was to correlate clinical severity, the amount of type VII collagen measured at the basement membrane and the MMP1 promoter single nucleotide polymorphism (SNP) rs1799750 in 13 Mexican patients with recessive dystrophic epidermolysis bullosa (RDEB). Clinical severity was scored according to recent guidelines (Fine et al, JAAD. 2008). We analysed six patients with severe generalized RDEB (RDEB-sev gen) and 7 patients with generalized other RDEB (RDEB-O). We compared the amount of fluorescent immunostaining localized to the basement membrane in skin sections from each patient with normal control skin using a monoclonal antibody specific for type VII collagen (LH7.2) and confocal microscopy. The status of the MMP1 SNP was analysed by PCR and restriction enzyme digestion. We found no correlation with levels of type VII collagen and clinical severity and no correlation with MMP1 promoter polymorphism status and clinical severity. However, analysis of 4 patients with the same COL7A1 genotype (2470insG/2470insG), suggests that MMP1 SNP does influence type VII collagen levels. This data indicates that MMP1 SNP can influence type VII collagen levels but other modifying factors not directly related to the amount of type VII collagen is influencing clinical severity in patients with RDEB.

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Genome-wide association study in alopecia areata implicates both innate and adaptive immunity

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Alopecia Areata (AA) is a highly prevalent autoimmune disease in which a collapse of hair follicle immune privilege and subsequent autoimmune attack leads to disfiguring hair loss. We undertook a genome-wide association study (GWAS) in a discovery sample of 250 unrelated cases and 1049 controls, and replicated our findings in an independent sample of 804 cases and 2229 controls. Joint analysis of the datasets identified 139 SNPs that are significantly associated with AA ($p\leq 5x10-7$). We identified association with several key components of Treg activation and proliferation, as well as the HLA gene cluster on chromosome 6p (pmin=1.38x10-35). We also found evidence for genes expressed in the hair follicle itself, including PRDX5 and STX17. Unexpectedly, a region of strong association resided on a haplotype block containing genes which encode activating ligands of the natural killer cell receptor, NKG2D. We discovered that these ligands are expressed in lesional scalp from AA patients is markedly upregulated in the hair follicle dermal sheath during active disease. Taken together, we have defined the genetic underpinnings of AA for the first time, placing AA within the context of shared pathways among autoimmune diseases, and implicating a novel disease mechanism, the upregulation of NKG2D ligands, in triggering autoimmunity.

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A review of the clinical findings in 231 patients with Pachyonychia congenita

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Pachyonychia congenita (PC) is a group of autosomal dominant keratin disorders caused by a mutation in keratin 6a, 6b, 16, or 17. We report the clinical characteristics of the largest cohort of PC subjects with confirmed genetic testing. Our subjects include 134 distinct families with 92 individuals having spontaneous mutations and 143 from families with PC. No autosomal recessive pattern of inheritance was found. While the onset of clinical symptoms varied among our cohort most PC patients reported nail thickening, plantar keratoderma and plantar pain by age 10. Each of the four major PC keratins are represented by our cohort: K6a (n=107) K16 (n= 66), K6b (n=20), and K17 (n=38). The most frequently reported findings were: thickened toenails (225/231, 97%) Plantar keratoderma 223/231, 97%) and plantar pain (208/223). Interestingly, fingernail thickening was less common, reported in 202/231 (87%) of subjects. We noted significant clinical differences between the subjects with different mutations. Among K6a subjects 107/107 (100%) reported thickened fingernails, while 10/20 (50%) of K6b subjects reported thickened fingernails. Cysts were found in 32/38 (92%) of K17 subjects, while 7/66 (11%) of K16 subjects reported cysts. Natal teeth was reported in 32/38 (84%) of K17 subjects and 3/107 (3%) and 0/66 (0%) of K6a and K16 subjects respectively. Regarding some of the historically reported PC associations we report the following findings: Corneal lesions were reported in 5/231 subjects (2%). Hoarseness was found in 66/231 (29%). Learning problems were reported by 23/231 (10%), while 19/231 (8%) reported being enrolled in "gifted," or with an "above average," IQ. Our findings clarify the clinical symptoms associated with PC, and demonstrate the clinical variance between some of the keratin mutations. A clear understanding of the clinical features of PC will improve the diagnostic accuracy of clinicians, correct spurious associations of unrelated findings, and improve patient care and prognostic counseling.

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Hairless and the polyamine putrescine form a negative feedback loop in keratinocytes

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Hairless (Hr) is a transcriptional corepressor that is crucial to hair cycling and keratinocyte differentiation/proliferation. Hr mutant mice are phenotypically similar to mice with excessive putrescine accumulation, particularly those lines overexpressing ornithine decarboxylase (ODC) and spermidine/spermine N1-acetyltransferase (SSAT) which are both rate-limiting enzymes for putrescine accumulation. All three are devoid of hair, with enlarged dermal cysts replacing hair follicles, and have very little subcutaneous fat. This morphological similarity is reinforced by the fact that when DFMO, an ODC inhibitor, is given to Hr mutant mice their phenotype is reversed. We have previously shown that the connection between Hr and ODC extends to the molecular level since Hr mutant keratinocytes have a higher basal level of ODC transcription. While ODC transcriptional regulation can occur through a variety of mechanisms, here we show that Hr negatively regulates ODC through the myc binding sites present in the ODC promoter. Negative regulation of myc target transcription occurs via binding of the mad family of proteins to the myc binding sites. We also show that overexpression of Hr increases mad2 transcription and we postulate that an Hr-Mad2 pathway controls ODC transcription in keratinocytes. We found evidence for a negative feedback mechanism between Hr and polyamine levels, since exogenous putrescine applied to cultured keratinocytes downregulates Hr transcription. Further support for negative feedback is seen in SSAT overexpressing mice that have higher levels of epidermal putrescine and lower levels of Hr. We postulate that a balance of Hr and putrescine is necessary for proper epidermal differentiation and proliferation and perturbation of the balance can lead to hair loss and increased susceptibility to UVB carcinogenesis.

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Requirement of the VDR coactivator SRC3 in the induction of innate immune genes and permeability barrier formation

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Steroid receptor coactivator (SRC3) is a coactivator that facilitates transcription of nuclear hormone receptors. Using GST-VDR affinity beads, SRC3 was identified as a VDR coactivator in differentiated keratinocytes, whereas a different coactivator complex, Mediator/DRIP, was isolated from undifferentiated keratinocytes. The function of SRC3 was investigated by silencing technology in human keratinocytes. The SRC3 silencing blocked the induction of cathelicidin (CAMP) regulated by 1,25(OH)2D3 and resulted in abnormal epidermis specific sphingolipid production and barrier formation. The In vivo role of SRC3 was examined by generation of keratinocyte specific SRC3 null mice. The function of SRC3 was evaluated using a skin wound model and acute barrier disruption. SRC3 null mice showed blunted expression of CAMP, CD14 and TLR2 after wounding, accompanied by impaired inflammatory gene induction. SRC3 null mice also showed impaired barrier recovery after acute barrier disruption by tape stripping with decreased trans-epidermal water loss, decreased number of lamellar bodies and impaired lipid bilayer formation. Tape stripping induced CAMP expression was also decreased. These results demonstrate that keratinocytes utilize SRC3 enable VDR regulation of permeability barrier formation and innate immune response.

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Whole-genome transcriptional profiling for the evaluation of psoriasis-like mouse models

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Although psoriasis is a uniquely human disease, numerous animal models have been identified or created that mirror many aspects of psoriasis. However, the comparisons of these psoriasis-like animal models to psoriasis have so far been limited to specific clinical features and a few histologic and biologic markers. By using whole-genome transcriptional profiling to compare expression patterns in human psoriatic plaques with those obtained from skin lesions from the KC-Tie2 mouse (AJP 174:1443) and the imiquimod-induced psoriasis-like model (JI 182:5836), we developed a mechanism to globally evaluate points of resemblance and dissimilarities of these models to human psoriasis. Similar to psoriasis; IL17A, IL23A and IL-22 were upregulated by QRT-PCR in both the imiquimod and KC-Tie2 model. We found a significant global correspondence between expression patterns associated with human psoriasis and those associated with the KC-Tie2 mouse phenotype ($p<.001$). KEGG pathway and Gene Ontology (GO) analysis showed shared pathways ($p<.05$ and $p<.001$ respectively) involved in cellular proliferation and cytokine-mediated signaling, whereas dissimilar gene expression patterns were observed in relation to bacterial and viral defense responses, neutrophil chemotaxis and fatty acid metabolism. We are currently extending this analytical method to the imiquimod model. Based on points of resemblance and dissimilarity of these models to the human disease we anticipate that the quantitative approach used in this study can be useful, and provide guidance to scientists in choosing the most appropriate mouse model to use when testing specific mechanistic and therapeutic hypotheses. Furthermore, it can help guide development of new psoriasis mouse models by identifying combinations of genetic manipulations that complement each other, resulting in psoriasis mouse models with stronger biochemical similarities to the human disease.

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Tumour necrosis factor alpha promoter -308g/a (tnfα -308g/a) polymorphism in Mexican patients with Alopecia areata

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Background: Alopecia areata (AA) is a chronic inflammatory condition characterised by hair loss, most frequently from the scalp. Its aetiopathogenesis is currently unknown, but inflammatory traits and associations with autoimmune diseases suggest that AA shares a similar origin. The tumour necrosis factor alpha (TNFα) gene, located on chromosome 6 within the major histocompatibility complex (MHC) class III gene, may carry previously described polymorphisms – particularly in the promoter region, such as TNFα -308G/A – known to be risk factors in a wide variety of inflammatory pathologies. In Mexican populations, this polymorphism has been associated with augmented TNFα production and, thus, renders carriers more susceptible to developing autoimmune diseases; however, as yet it has not been associated with AA. Objectives: To assess a possible association between the presence of TNFα -308G/A and AA. Patients/Methods: Blood samples were taken from 51 patients affected by AA and 103 control subjects without AA, all from the northeastern Mexican population. Genomic DNA was isolated using the phenol-chloroform method and samples subjected to PCR-RFLP in order to detect the TNFα -308G/A polymorphism. Results: TNFα -308G/A (p= 0.011, OR= 3.89, 95% CI= 1.15-13.77), and the heterozygous genotype (p= 0.0093, OR=4.2, 95% CI=1.19-15.48) both confer a significant risk for developing AA, compared to that observed in controls (p= 0.0093, OR= 0.24, 95% CI= 0.06-0.84). Conclusions. Our data suggest that there is a plausible association between the presence of the TNFα -308G/A polymorphism and a higher susceptibility for developing AA. This risk might be due to overproduction of TNFα, which would facilitate an autoimmune response against the hair follicle.

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Promoter elements and protein function of TNIP1 indicate a regulatory feedback loop

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Nuclear receptor regulation of transcription is dependent on the receptor's ligand and auxiliary proteins known as coactivators and corepressors. We isolated TNFAIP3 interacting protein 1 (TNIP1) from a human keratinocyte cDNA library and identified it as a corepressor of RARs and PPARs, two receptors with demonstrated and emerging relevance to control of epidermal gene expression. Independent of our studies, TNIP1 protein has been reported to suppress NF-κB. Most recently, the TNIP1 gene locus was reported as strongly associated with psoriasis through a genome-wide scan. These results may place TNIP1 at the crossroads of keratinocyte normo- and pathophysiology and prompted us to investigate genomic elements controlling its expression. To this end, we isolated ~6 kilobases of the human TNIP1 promoter and subjected it to in silico, physical, and functional analysis. The TNIP1 promoter lacks a TATA box but is increasingly GC-rich towards the transcription start site and has several candidate constitutive and inducible regulatory sequences. Intriguingly, we found potential nuclear receptor half-sites of differing repeats throughout the 6 kilobase sequence. These were validated as functional PPAR and RAR binding sites through liganded receptor up-regulating the promoter in reporter constructs, receptor binding in mobility shift assays, and receptor occupying these sites in chromatin immuno-precipitation assays. Through similar approaches, we found two NF-κB sites, one each within proximal and distal promoter regions. Positive control of the TNIP1 promoter by PPAR, RAR, and NF-κB sets the stage for regulatory circuitry by the very proteins whose function is repressed by TNIP1. These results may place TNIP1, through its promoter and functions of its protein product, in a negative feedback loop limiting extremes in its expression. Such a system could contribute stability to nuclear receptor- and NF-κB-mediated gene expression networks in normal keratinocyte physiology or be subject to perturbation in instances of hyperproliferative or inflammatory disease such as psoriasis.

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High efficiency exogenous gene expression in primary human skin cells using Bacmam technology

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Primary cells provide a physiologically relevant model system for study of complex biological processes. Despite this evident advantage over immortalized cells, difficulty in efficient delivery and expression of exogenous genes has limited widespread use. Here, we demonstrate the use of BacMam technology for successful delivery of exogenous genes to primary skin cells, including keratinocytes, fibroblasts, and melanocytes. BacMam technology refers to a recombinant, non replicative baculovirus system that allows for highly efficient, transient gene delivery, and is capable of carrying large inserts (up to ~28Kb). We show efficient gene delivery in primary skin cells indicated by expression of multiple proteins including- cytoplasmic localized emGFP, Organelle LightsTM (Baculoviruses carrying fluorescent protein expression constructs targeted to specific subcellular structures), and the transcription factor, hOct4. All resulted in >70% expression. Comparable results were obtained using emGFP on cells that had undergone up to six passages, with detectable expression lasting multiple days. Similar results were obtained with BacMam encoded hOct4, a protein which is known to have a shorter half life than emGFP. Repeated dosage of the cells with viral particles enabled extension of detectable protein expression. In addition, simultaneous high efficiency delivery of multiple genes can be accomplished while having minimal effect on cell viability. Taken together, these results underscore that BacMam technology provides an effective platform for delivering exogenous genes to primary skin cells, and suggests it may have broad applicability as a research tool.

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A nonsense mutation in the SCN9A gene in congenital insensitivity to pain

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Pain is a highly conserved sensory response among all vertebrate species which confers protection against noxious and dangerous stimuli. Loss of pain sensation is associated with multiple injuries that compromise survival. On the other extreme, hypersensitivity to pain also adversely affects affected individuals, as they perceive several non-harmful stimuli as being noxious. Pain is categorized into several subtypes including: mechanical, visceral, inflammatory, thermal, chemical and neuropathic. Congenital insensitivity to pain (CIP) (OMIM 243000) is a rare autosomal recessive disorder, characterized by insensitivity to all modalities of pain except neuropathic pain, and recurrent injuries frequently go unnoticed. CIP is caused by deactivating mutations in the SCN9A gene encoding for the Na1.7 channel. At the opposite end of the spectrum, primary erythromelalgia (PE) (OMIM 133020) is an autosomal dominant disorder characterized by enhanced pain perception and hyperactivating mutations in the SCN9A gene. The pathway of pain mediated through the Na1.7 channel is most likely to be acting at the level of peripheral nociceptive transmission since these channels are mainly expressed in the peripheral nervous system. We analyzed the DNA from members of a consanguineous Pakistani family for mutations in the SCN9A gene through direct sequencing after performing linkage studies and haplotype analysis mapping to chromosome 2q24. We identified a novel nonsense mutation designated R523X in all affected individuals, further extending the spectrum of mutations in this gene. Our finding underscore the importance of the Na1.7 channel in altering the complex neural circuitry of pain which extends from sensory fibers in the skin to the thalamus.

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Evaluation of functional siRNA delivery in a human epidermal skin equivalent model

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Although RNA interference offers high potential as a novel therapeutic approach for treating skin disorders, delivery hurdles (e.g., stratum corneum penetration and siRNA uptake by keratinocytes) have hampered progression to the clinic. We have recently demonstrated that high pressure, resulting from intradermal injection of large volumes, facilitates nucleic acid uptake by keratinocytes in mouse skin. Furthermore, intradermal injection of large volumes of our lead clinical siRNA inhibitor TD101 to pachyonychia congenita foot lesions (Phase 1b clinical trial) likely facilitated uptake, leading to marked improvement in symptoms. However, the intense pain associated with hypodermic needle administration during the trial appears to limit future use. A number of patient-friendly technologies that allow siRNA to be delivered across the stratum corneum barrier are being developed. At TransDerm, we are developing a topical formulation (GeneCream) as well as a protrusion array device (PAD) that penetrates into the epidermis to release siRNA cargo. Unfortunately, without the pressure associated with injection of high volumes, the efficiency of functional siRNA delivery is diminished. In order to evaluate siRNA modifications or other delivery systems that facilitate keratinocyte uptake, human epidermal equivalents, in which pre-existing gene expression can be readily monitored, have been prepared and used to evaluate functional siRNA delivery. Using this model system, treatment with modified siRNAs (e.g., Dharmacon Accell technology) was shown to reduce pre-existing gene expression at the mRNA and protein levels, suggesting that human epidermal skin equivalents will be useful to investigate and optimize technologies that facilitate siRNA uptake in skin keratinocytes.

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Silencing of reporter gene expression in skin using siRNAs delivered by a soluble protrusion array device (PAD)

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Despite rapid progress in the development of potent and selective siRNA agents for skin disorders, transfer to the clinic is hampered by the lack of effective, patient-friendly delivery technologies. The stratum corneum poses a formidable barrier to efficient delivery of large and/or charged macromolecules including siRNAs. Delivery of siRNA by intradermal injection results in reproducible and effective knockdown of targeted gene expression but is painful and the effects are localized to the injection site. The use of microneedle arrays represents a relatively painless method to deliver therapeutic agents to larger areas of the skin. To test the effectiveness of this approach to deliver nucleic acids, including siRNAs, a dissolvable protrusion array device (PAD) was developed. Needle-like tips penetrate the skin barrier and rapidly hydrate upon insertion, forming a gel-like depot that releases functional cargo such as plasmid DNA or siRNA. PAD-delivered Accell siRNA distributed throughout the treated skin and silenced reporter gene expression in a transgenic mouse skin model. Furthermore, PAD-delivered reporter plasmid resulted in firefly luciferase expression in mouse ear, back and footpad skin as assayed by intravital bioluminescence imaging. These results indicate that PAD technology delivers functional nucleic acids to skin.

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Increased interstitial pressure improves nucleic acid delivery to skin enabling a comparative analysis of constitutive promoters

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Nucleic acid-based therapies hold great promise for treatment of skin disorders if delivery challenges can be overcome. To investigate one mechanism of nucleic acid delivery to keratinocytes, a fixed mass of expression plasmid was intradermally injected into mouse footpads in different volumes, and reporter expression was monitored by intravital imaging or skin sectioning. Reporter gene expression increased with higher delivery volumes, suggesting that pressure drives nucleic acid uptake into cells after intradermal injections, similar to previously published studies for muscle and liver. For spatiotemporal analysis of reporter gene expression, a dual-axis confocal (DAC) fluorescence microscope was used for intravital imaging following intradermal injections. Individual keratinocytes expressing hMGFP were readily visualized *in vivo* and appeared to preferentially express initially in the stratum granulosum and subsequently migrate to the stratum corneum over time. Fluorescence microscopy of frozen skin sections confirmed the patterns observed by intravital imaging. A comparative promoter study investigating CMV, ubiquitin C (UBC) eIF4A1 and EF1A promoters suggested that the UBC promoter is preferred in terms of promoter strength and uniform distribution through the epidermis. In summary, intravital imaging with the DAC microscope is a noninvasive method for probing spatiotemporal control of gene expression and should facilitate development and testing of nucleic acid delivery technologies.

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Differentiation of induced pluripotent stem cells into a multipotent keratinocyte lineage

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Recent breakthroughs in the generation of induced pluripotent stem cells (iPSCs) have provided a novel renewable source of cells with embryonic stem cell-like properties, which may potentially be used for gene therapy and tissue engineering. Although many obstacles remain to be solved before these cells can be applied clinically, the development of efficient protocols for differentiation of iPSCs into particular cell types and tissues is a prerequisite for their future application. Despite the significant progress in differentiation of iPSCs into a variety of cell types, iPSC-derived keratinocytes have not yet been obtained. We report the *in vitro* differentiation of murine iPSCs into a keratinocyte lineage through subsequent applications of retinoic acid and bone-morphogenetic protein-4. We show that mouse iPSCs generated using retroviruses expressing *Oct4*, *Sox2*, *Klf4*, and *c-Myc* can be differentiated into functional keratinocytes capable of regenerating a fully differentiated epidermis, hair follicles and sebaceous glands in an *in vivo* environment. Keratinocytes derived from iPSCs were analyzed with respect to gene and protein expression, as well as their ability to differentiate *in vitro* and to reconstitute normal skin and its appendages in an *in vivo* graft assay. Currently, no effective therapeutic treatments are available for the majority of genetic skin diseases. The development of a method for the efficient differentiation of iPSCs into a keratinocyte lineage will now enable us to determine whether genetically corrected iPSCs can be used to generate a permanent corrective therapy for inherited skin diseases using genetically engineered mouse models for epidermolysis bullosa simplex and epidermolytic hyperkeratosis.

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A wide spectrum of filaggrin mutations contribute to Ichthyosis Vulgaris and Atopic Dermatitis in the Singaporean Chinese population

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Filaggrin gene (FLG) mutations cause ichthyosis vulgaris (IV) and predispose individuals to atopic dermatitis (AD). Comprehensive AD cohort studies in Europe and Japan have reported a FLG null mutation carrier frequency between 18 – 48%, but prevalent FLG mutations found in the European population are rare or absent in Chinese patients with IV and AD. Japanese and Chinese populations share some mutations but previous reports have also highlighted the presence of differential population-specific mutations. Due to the large size (10 – 12kb) and repetitive nature of the gene, detection of new Chinese-specific FLG mutations remains challenging. We wished to clarify the profile of FLG mutations and compare the prevalence of variants between Singaporean Chinese and other populations. Singapore has a heterogeneous Chinese population, and is therefore a good choice for a comprehensive study of FLG mutations present in Asian populations. We completely sequenced FLG in 94 IV patients and identified 26 mutations, of which 14 are novel; these mutations were then screened in 449 Singaporean Chinese AD patients. Around 20% of the AD patients in our cohort carry one or more FLG null mutations and 5 of the 26 mutations make up about 75% of the FLG mutation spectrum in these. The contribution of FLG variants in Asian AD cases is not as well established as in Europe and therefore future replication studies will help to confirm these mutations as prevalent pan-Asian FLG mutations contributing to IV and AD. The wider genetic landscape of FLG mutations in Asia is slowly emerging. Rare FLG mutations will probably continue to surface in Asian populations and their collective role in predisposing to AD needs to be well-characterised to inform targeted therapy.

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Copy number variations upstream of SOX9 gene are associated with hypertrichosis

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Hypertrichosis refers to hair growth that is excessive for the age and body site of an individual. Inherited hypertrichoses are very rare human disorders whose incidence as a group has been estimated as low as 1 in 1 billion. The genetic basis of hypertrichosis is largely unknown, and no gene mutations have been directly associated with hypertrichosis. Recently, there have been several reports of hypertrichosis in association with chromosomal rearrangements as well as copy number variations (CNVs). Gene expression can be impacted by a variety of mechanisms, for example, disruption of transcriptional regulation; modification of the chromatin structure; or separation of the gene from a regulatory element. We previously reported several patients with Ambras syndrome in association with chromosomal rearrangement involving chromosome 8 that affected the expression of TRPS1 gene through a position effect. In this study, we analyzed Affymetrix Cytogenetics Whole Genome 2.7M array data from 12 individuals with hypertrichosis. An average of 724 gains and 267 losses per individual were detected, and for large CNVs over 1000 kbp, an average of 12 gains and 1 loss was detected. Interestingly, we observed a large 1.7Mb duplication located ~1Mb upstream of the SOX9 gene in a family with hypertrichosis, distinct from previously reported CNVs, which is potentially involved in disruption of gene expression. The upstream region of SOX9 contains ~3Mb of regulatory sequences which are prone to rearrangement and lead to syndromes with a wide variety of clinical features. Given the role of SOX9 in determination of hair follicle stem cells, we postulate that this duplication results in misexpression at a critical point in development, leading to the hypertrichosis phenotype.

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The first Asian case of Dowling Degos Disease described with a recurrent keratin 5 mutation

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Dowling-Degos disease (DDD, OMIM no. 179850) is an autosomal dominant genodermatosis that is characterized by reticulate pigmentation of the flexures. A *KRT5* gene association has been documented in several European families. We have identified a 2 base-pair deletion mutation (c.442delAG) in exon 1 resulting in a premature termination codon in the V1 (head) domain of keratin 5 (p.S148fsX30) in a Singaporean Chinese family with DDD. This mutation was previously reported in a Spanish family, confirming this mutation as the cause of the genodermatosis, and suggesting that this disease is not confined to Europeans. Biopsies were collected from the patient and used for histology and electron microscopy studies, and to isolate cells for use in three-dimensional organotypic cultures. Immunohistochemistry showed a normal expression pattern of keratin markers indicating normal epidermal differentiation, though histology of the skin showed characteristic features of the disease: elongated and branched rete pegs or ridges and dermal pigmentary incontinence. The keratin intermediate filament network appeared to be intact by electron microscopy, and no obvious differences in the melanocytes or keratinocytes were observed. However there was an increase in the number of melanosome clusters in lesional (hyperpigmented) skin compared to non-lesional skin from the same patient, and more melanosomes were observed in suprabasal cells in lesional skin, possibly indicating a failure in melanosome turnover. Though the mechanism of disease is unclear, these results confirm a role for keratins in pigment biology, possibly via impacts on melanosome trafficking. In addition, a polymorphism in the keratin 5 head domain (p.G138E) has recently been identified as a susceptibility variant for basal cell carcinoma. This finding together with the spectrum of DDD mutations now identified, suggests changes in the head domain of keratin 5 can severely impact the structural integrity of the epidermis.

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MicroRNAs in the epidermis and dermis are associated with skin and hair follicle morphogenesis

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MicroRNAs (miRNA) are small 21-23 nucleotide non-coding RNA molecules that regulate expression of target genes through repression of mRNA translation and mRNA degradation. Previous studies have shown a number of miRNAs expressed in developing mouse skin tissue. To assess whether miRNAs may be involved in regulating skin morphogenesis and hair follicle development in a lineage-restricted manner, we performed global profiling of miRNA expression in mouse backskin epidermis and dermis that was microdissected at 24 hour intervals, corresponding to days E12.5 to E16.5. Using miRNA microarrays and computational algorithms to detect regulatory RNA molecules, we detected multiple miRNAs expressed in the skin during this time period. The miRNAs fell broadly into 4 categories based on expression patterns. The first group showed high levels of expression in the epidermis but no expression in the dermis, suggesting that these miRNAs are crucial for epidermal fate specification, such as miR-203. The second group of miRNAs showed restricted dermis-only specific expression patterns. Interestingly, we found several miRNAs that were co-expressed in both the dermis and epidermis. One group of these miRNAs had peak expression at E15.5 in both dermis and epidermis, the time when hair follicle development is underway, indicating that their targets of must be down-regulated in both skin compartments, whereas the final group of miRNAs showed low levels of expression at E15.5 in both skin layers, indicating that increased expression of their target genes play a role in hair follicle development. We postulate that miRNAs play distinct roles in epidermal, as well as dermal fate specification and hair follicle development and induction.

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Gene-breaking mutagenesis in zebrafish identifies the novel epidermal mutant wicked witch of the Midwest

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The zebrafish (Danio rerio) is a powerful vertebrate model system increasingly appreciated for understanding epidermal development and disease. We have developed protein trap gene-break transposons (PT-GBTs) as a novel insertional mutagenesis technology, and have applied PT-GBTs as a new tool for studying epidermal biology in the zebrafish model. Combining a 5′ mutagenicity cassette and a 3′ gene-finding cassette within a single transposon vector, PT-GBTs function by transcriptional disruption. The 5′ mutagenicity cassette terminates the endogenous transcript, fusing it with an mRFP tag to permit tissue-specific prioritization of PT-GBT events. The 3′ gene-finding transcript, derived from an internal promoter, includes a fluorescent reporter plus the endogenous downstream exons. Using these new PT-GBT vectors, we have identified novel transgenic zebrafish lines labeling skin, neurons, neural crest, bone, muscle, and other tissue types. From morphologically-based recessive forward genetic screening we identified the novel epidermal mutant we have named wicked witch of the Midwest (wwm). wwm mutants display defective epidermal development, followed by extensive cell death and embryonic lethality. wwm mutants develop ΔNp63-positive epidermal aggregates by 48hpf. Using conditional reversion, we have confirmed that the wwm mutant allele is a PT-GBT insertional allele. Antisense morpholino-mediated restoration of wild-type splicing reduces the severity of the wwm phenotype. Cre recombinase efficiently rescues the wwm phenotype by excising the PT-GBT 5′ and 3′ cassettes to restore a wild-type chromosome. The novel epidermal mutant wwm and the novel PT-GBT technology serve to expand the growing role for zebrafish in dissecting the molecular foundations of skin development and disease.

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Pitavastatin suppresses lipid peroxidation-induced VEGF expression in the skin of KKAy metabolic syndrome mice

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Recently, multiple observational studies have demonstrated an association between psoriasis and metabolic syndrome. A potential common link to explain the relationships between each component of psoriasis and metabolic syndrome appears to be lipid- oxidation- induced expression of vascular endothelial growth factor (VEGF). We investigated the levels of lipid peroxidation products and VEGF expression in the skin of KKAy metabolic syndrome mice, and also the effects of lipid peroxidation derivatives, namely oxidized low density lipoprotein (oxLDL) and lysophosphatidylcholine (LPC), on VEGF production by a human epidermal keratinocyte cell line (HaCaT cells). We observed increased lipid peroxidation and VEGF expression in the skin of KKAy metabolic syndrome mice, and pitavastatin suppressed the increase in lipid peroxidation and VEGF expression. oxLDL and LPC increased the VEGF production by HaCaT cells. p44/p42 mitogen-activated protein kinase (MAPK) and CD36 were involved in the VEGF production *in vivo* and *in vitro*. Our results suggest that metabolic syndrome enhances the risk of development of psoriasis by, at least in part, increasing lipid peroxidation-induced VEGF expression via p44/p42 MAPK and CD36 activation, and suggest the possible clinical usefulness of pitavastatin in the treatment of psoriasis.

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Calcium-induced epidermal differentiation is a reversible process regulated by PKD

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Although commitment to epidermal differentiation is generally considered to be irreversible, differentiated keratinocytes have been shown to maintain a regenerative potential and to reform multilineage skin epithelia when placed in a suitable environment. To obtain insights into the mechanism of re-initiation of this proliferative response in differentiated keratinocytes, we examined the reversibility of commitment to calcium (Ca2+)-induced differentiation in primary cultures of mouse keratinocytes. Lowering Ca2+ concentration in these cultures to micromolar levels, triggered culture-wide morphological and biochemical changes as indicated by de-repression of cyclin D1, re-initiation of DNA synthesis and acquisition of basal cell-like characteristics. Pretreatment of differentiated keratinocytes with Goedecke 6979, an inhibitor of protein kinase D (PKD) and protein kinase C (PKC)-alpha, but not with GF109203X, a general inhibitor of PKCs, specifically blocked re-initiation of proliferation and morphological reversion of differentiated cultures suggesting PKD activation by a PKC-independent mechanism. PKD activation followed biphasic kinetics with an early transient phosphorylation within the first 6 hrs followed by sustained and progressive phosphorylation beginning at 24 hrs. In turn, PKD activation induced prolonged extracellular signal regulated kinase 1/2 (ERK1/2) signaling and progression to DNA synthesis in response to the low Ca2+ switch. Specific knockdown of PKD-1 by RNA interference or expression of dominant negative form of PKD-1 did not have a significant effect on normal keratinocyte proliferation and differentiation but did inhibit Ca2+-mediated re-initiation of proliferation and morphological reversion in differentiated cultures. The present study identifies PKD as a major regulator of a proliferative response in differentiated keratinocytes through sustained activation of the ERK-mitogen activated protein kinase pathway and provides new insights into the process of epidermal regeneration and wound healing.

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Upregulation of nuclear factor-κB (NF-κB) expression by SLURP-1 via Keratinocyte α7 Nicotinic Acetylcholine Receptor (nAChR) involves both ionic events and activation of protein kinases

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The nAChRs are classic representatives of the superfamily of ligand-gated ion channel proteins, or ionotropic receptors, mediating the influx of Na+ and Ca2+ and efflux of K+. SLURP-1 (secreted mammalian Ly-6/urokinase plasminogen activator receptor [uPAR]-related protein-1) is a novel auto/paracrine cholinergic peptide that binds to and activates α7 nAChR, a high Ca2+-permeable ion channel coupled to upregulation of NF-κB expression. SLURP-1 exhibits anti-tumor activity by inducing apoptosis of transformed cells. In this study, we elucidated relative contribution of the ionic events elicited by influxes of Na+ and Ca2+ to SLURP-1 signaling and the roles of signaling kinases using the immortalized line of human keratinocytes Het-1A. A multifold upregulation of the NF-κB expression at the mRNA and protein levels by recombinant (r)SLURP-1 was only slightly diminished due to elimination of Na+, whereas in Ca2+-free medium the effect of rSLURP-1 was inhibited by >50%. Both in the absence of extracellular Ca2+ and in the presence of Cd2+ or Zn2+, the SLURP-1-dependent elevation of NF-kB was almost completely blocked by inhibiting MEK1 activity. Downstream of α7 nAChR, the SLURP-1 signaling coupled to upregulation of NF-κB also involved Jak2 as well as the Ca2+/calmodulin-dependent kinase II (CaMKII) and protein kinase C (PKC) steps whose inhibition significantly ($p<0.05$) reduced the rSLURP-1-induced upregulation of NF-κB. These results demonstrated the existence of the non-ionic signaling mechanism downstream of keratinocyte α7 nAChR. Activation of α7 nAChR by auto/paracrine SLURP-1 leads to upregulation of the NF-κB gene expression due to activation of the Raf-1/MEK1/ERK1/2 cascade that proceeds via two complementary signaling pathways. One is mediated by the Ca2+-entry dependent CaMKII/PKC activation and another one by Ca2+-independent involvement of Jak2.

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Role of Smad-interacting protein 1 (SIP1) in collagen synthesis of dermal fibroblasts

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TGF-β/Smad signaling is implicated in the pathogenesis of scleroderma through the synthesis of extracellular matrix proteins including collagens. Smad-interacting protein 1 (SIP1) was originally identified as a protein binding to the Smads and belongs to family of transcriptional repressor. SIP1 contributes to epithelial mesenchymal transition (EMT) process through transcriptional downregulation of E-cadherin. However, its role in collagenogenesis remains unknown. To study the role of SIP1 in collagenogenesis, the expression of SIP1 was silenced in murine dermal fibroblasts by siRNA. Transcript levels of Col1a2 and Col3a1 were decreased in SIP1-knockdown fibroblasts. To examine the role of SIP1 *in vivo*, SIP1 conditional KO mice were generated by crossing $SIP1^{flox/flox}$ mice with Prx-1-Cre mice in which Cre was expressed in mesodermal cells under the regulation of prx-1 promoter. The SIP1 KO mice showed deformity of teeth, poor development of skull and atrichia in the area of head, limbs and abdomen. Furthermore, flaccidity in the skin, particularly of limbs, was observed. Quantitative RT-PCR using whole skin samples revealed that the gene expressions of Col1a2 and Col3a1 were decreased in the SIP1 KO mice compared with those in wild-type mice. We conducted the skin fibrosis experiment by subcutaneous injections of bleomycin in the duration of four weeks. While wild-type mice developed distinct skin fibrosis, SIP1 KO mice showed attenuated lesions, in which Col1a2 and Col3a1 mRNAs were significantly down-regulated compared with wild-type mice. These results suggest that SIP1 in dermal fibroblasts contributes to collagenogenesis and could be a novel therapeutic target for treatment of scleroderma.

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ErbB2 interacting protein (Erbin) binds unique domains of the desmoglein 1 C-terminus to form a complex that cooperatively promotes epidermal differentiation

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Desmogleins exhibit differentiation-dependent expression in the epidermis and differ significantly in their intracellular domains, which extend well beyond those of classical cadherins. This suggests unique binding partners may confer distinct functions on desmogleins during epidermal morphogenesis. We previously showed that desmoglein 1 (Dsg1), which is first expressed between proliferative basal and differentiating suprabasal cells, is crucial for suppressing EGFR/ErbB2 to allow epidermal differentiation. A yeast two-hybrid screen revealed Dsg1 interacts with Erbin, an ErbB2 binding protein that is recruited to the cell surface in the suprabasal epidermis, coincident with Dsg1 expression. Interestingly, Erbin staining was perturbed in Dsg1-deficient organotypic cultures, suggesting a role for Dsg1 in localizing this signaling modulator. Co-immunoprecipitation from keratinocytes detected a Dsg1:Erbin complex; moreover, we showed the two purified proteins interact directly *in vitro*. In addition, ectopic Dsg1 shifted Erbin density in lysates separated by sucrose gradient fractionation. However, a truncated Dsg1 lacking the desmoglein unique region downstream of the catenin-binding segment (Dsg1-ICS) did not bind Erbin in yeast and failed to shift Erbin density in keratinocytes. Dsg1-ICS was also impaired in inducing differentiation compared to full-length Dsg1, defining the first potential function for the desmoglein-specific domains of Dsg1's C-terminus. As well, Dsg1-mediated differentiation was inhibited in Erbin-deficient cells, indicating Erbin is specifically required for Dsg1 functionality. Finally, we showed that Erbin depletion in organotypic cultures suppressed differentiation, similar to Dsg1 knock-down, and in both cases the phenotype was rescued by inhibiting Erk/MAP kinase signaling. Thus, we propose that Dsg1 and Erbin form a complex that is crucial for proper down-regulation of EGFR/ErbB2 signaling to promote epidermal differentiation.

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Neurotrophins stimulate human fibroblast differentiation, migration and contractile strength

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Neurotrophins (NT), NGF, BDNF, NT3 and NT4, belong to a family of growth factors involved in the control of skin homeostasis. These proteins act through two kinds of receptors: the low-affinity receptor p75 (p75NTR) and the high-affinity receptors TrkA, TrkB and TrkC. Previous studies have shown that skin cells, such as keratinocytes and melanocytes express NTs and their receptors. Because fibroblasts are critical in many physiopathologic processes at the skin level, such as wound healing and fibrosis, we wanted to evaluate the expression and function of NTs and their receptors in these cells. Here we show that human dermal fibroblasts and myofibroblasts express Trks and p75NTR at both mRNA and protein level, as shown by RT-PCR and Western Blot, respectively. Moreover, fibroblasts and myofibroblasts secrete all NTs, as shown by ELISA. In particular, myofibroblasts express higher levels of p75NTR and TrkB than fibroblasts. On the contrary, myofibroblasts express TrkA at lower levels and both cellular types don't express TrkC. Fibroblasts and myofibroblasts also secrete NGF and NT3 at higher levels, as compared with NT4 and BDNF. NGF, BDNF, NT3 or NT4 promote fibroblasts differentiation into myofibroblasts, as shown by the induction of α-sma expression at 24 and 48 hours, by Immunofluorescence and Western Blot, with an effect similar to the one produced by TGFβ Scratching assay at 24 hours demonstrates that NGF, BDNF or NT3 (100ng/ml) also induce fibroblast migration in statistically significant manner, the effect being more evident at 48 hrs. Moreover, NGF or BDNF statistically increase the tensile strength of fibroblasts in a dose dependent manner, as measured through the Glasbox® device. By contrast, NT3 or NT4 do not exert any effect. These results suggest that paracrine or autocrine NTs play an important role in tissue remodeling and wound healing, by inducing fibroblast differentiation and migration.

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Targeted inducible expression of wnt5a in adult mouse epidermis

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Wnt5a is the prototypical "non-canonical" type of Wnt proteins, mediating signal transduction not through β-catenin activation. Previously, we have shown that Wnt5a and its various receptors are expressed in adult epidermis in a highly layer-specific pattern and that induction of Wnt5a causes type 1 interferon hypersensitivity *in vitro* (Romanowska et al, PloS One, 2009), suggesting that it contributes to epidermal differentiation and that its massive upregulation contributes to the upregulation of interferon responsive signalling in psoriasis. The function of Wnt5a in adult skin has not been studied *in vivo* since overexpression of Wnt5a causes severe developmental abnormalities in several organ systems. Here, we describe the generation and initial characterization of mice with inducible Wnt5a expression in the epidermis. To circumvent prenatal lethality we used a bi-cistronic system where full-length Wnt5a, including a C-terminally located haemagluttinin-tag, is followed by an internal-ribosome entry site upstream of a second open reading frame encoding a KRAB repressor fused to a tet-transactivator protein. The entire cassette is placed under a modified mini-keratin14 promoter with several Tet-responsive elements. In the absence of doxycycline, any background transcription in K14-active epithelial cells is feedback-inhibited by the KRAB repressor, leading to tight expression control. Upon doxycycline administration, Wnt5a is expressed in the basal layer of the epidermis. Wnt5a overexpression as such causes no major epidermal changes in adult mice. Strikingly, however, enforced overexpression of Wnt5a causes massive hypersensitivity to injection of poly-inosine/cytosine (pIC), thereby confirming our previous in vitro observations. These mice will be useful for the detailed analysis of the regulation of interferon responses and toll-like receptor signalling by Wnt5a *in vivo*.

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Opposing actions of insulin and arsenite converge on PKCδ to alter keratinocyte proliferative potential and differentiation

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When cultured human keratinocytes reach confluence, they undergo a program of changes replicating features of differentiation *in vivo*, including exit from the proliferative pool, increased cell size and expression of specialized differentiation marker proteins. Previously, we showed that insulin is required for some of these steps and that arsenite, a human carcinogen in skin and other epithelia, opposes the differentiation process. In present work, we show that insulin signaling, probably through the IGF-I receptor, is required for the increase in cell size accompanying differentiation and that this is opposed by arsenite. We further examine the impact of insulin and arsenite on PKCδ, a known key regulator of keratinocyte differentiation, and show that insulin increases the amount, tyrosine phosphorylation and membrane localization of PKCδ. All these effects are prevented by exposure of cells to arsenite or to inhibitors of downstream effectors of insulin (phosphotidylinositol 3-kinase and mammalian target of rapamycin). Retrovirally-mediated expression of activated PKCδ resulted in increased loss of proliferative potential after confluence and greatly increased formation of cross-linked envelopes, a marker of keratinocyte terminal differentiation. These effects were prevented by removal of insulin, but not by arsenite addition. We further demonstrate a role for src family kinases in regulation of PKCδ. Finally, inhibiting epidermal growth factor receptor kinase activity diminished the ability of arsenite to prevent cell enlargement and to suppress insulin-dependent PKCδ amount and tyrosine 311 phosphorylation. Thus suppression of PKCδ signaling is a critical feature of arsenite action in preventing keratinocyte differentiation and maintaining proliferative capability.

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Endogenous galectin-3 positively regulates keratinocyte migration by controlling EGFR intracellular trafficking through Alix

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One crucial step in skin wound re-epithelialization is keratinocyte migration. By studying keratinocytes from transgenic mice deficient in the carbohydrate-binding protein galectin-3 (gal3-/- mice), we found that endogenous galectin-3 promotes keratinocyte migration *in vitro* and *in vivo*. Importantly, we found a significant decrease in cell surface epithelial growth factor receptor (EGFR) expression in gal3-/- keratinocytes compared to wild-type cells as determined by flowcytometry. By immunofluororescence analysis, EGFR was found to accumulate in the cytoplasm of gal3-/- keratinocytes *in vitro* as well as in the peri-wound area. Gal3-/- keratinocytes exhibited reduced rates of endocytosis and recycling of EGFR, as well as aberrant activation of a proteasome degradation pathway after epithelial growth factor (EGF) stimulation. Galectin-3 was translocated to multivesicular bodies (MVBs) 30 minutes after EGF stimulation, and was colocalized with the MVB component protein Alix. Alix could be co-precipitated with galectin-3 from keratinocytes stimulated with EGF. In addition, Alix was associated with EGFR in keratinocytes treated with EGF and this association was diminished in gal3-/- keratinocytes. Our results suggest that galectin-3 controls keratinocyte migration and reveal a critical role of galectin-3 in transport and expression of a key cell surface receptor.

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STA-21, a stat3 inhibitor, induces differentiation of normal human epidermal keratinocytes and human keratinocyte derived cell lines

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Signal transducer and activator of transcription 3 (Stat3) plays critical roles in biological activities including cell proliferation, migration, survival, and carcinogenesis. We have previously reported that Stat3 deficient (K5-Cre.Stat3flox/flox) mice did not develop skin tumors in DMBA/TPA induced carcinogenesis and were resistant to ultraviolet B (UVB) irradiation induced carcinogenesis. On the other hand, K5.Stat3C transgenic mice, whose epidermal keratinocytes expressed constitutively activated Stat3, showed more susceptible to the DMBA/TPA induced and UVB irradiation- induced skin carcinogenesis as compared with non-transgenic littermates. We recently showed that topical application of STA-21, which is a Stat3 inhibitor of small molecular weight compound, significantly suppressed the development and progression of tumors induced by UVB irradiation in K5.Stat3C mice. From this finding, we evaluated the effect of STA-21 on keratinocytes to understand the roles of Stat3 signaling in the cells. Cell proliferation of normal human epidermal keratinocytes was suppressed with STA-21 treatment in dose dependent manner. Reduced expression of cyclinD1 and c-myc, which were the down stream molecules of Stat3 signaling, was observed with the treatment. In the meantime, we noted that normal human epidermal keratinocytes came into differentiated morphology and the expression of involucrin, tranglutaminese 1 and cytokeratin 10 was up-regulated with STA-21 treatment. We then examined whether STA-21 could induce the differentiation marker molecules even in immortalized keratinocytes or SCC cell lines (HaCaT, A431, HSC-2 and HSC-4). The gene expression of involucrin, transglutaminse 1 and cytokeratin 10 was up-regulated in the cell lines with STA-21 treatment for 48 hours. The tumor suppression by STA-21 treatment might be explained as the result of induction of differentiation as well as inhibition of cell growth.

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Spermidine stimulates hair follicle elongation and anagen prolongation, accompanied by increased keratin K15 expression

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Polyamines are crucial for multiple different cell functions, including proliferation, growth, differentiation, migration and angiogenesis. As one of the most highly proliferative organs in the mammalian system, the hair follicle (HF) has long been thought to be particularly susceptible to regulation by polyamines, and eflornithine, which inhibits ornithine decarboyxlase, the rate-limiting enzyme of polyamine synthesis, inhibits human hair growth. However, the direct effects of polyamines on human HFs remain to be investigated. Therefore, in order to study whether and how the key polyamine spermidine impacts human hair growth, normal human anagen VI scalp HFs were microdissected and organ-cultured with spermidine (0.1-1 μM). Spermidine significantly promoted hair shaft elongation, and prolonged the growth phase of the hair cycle (anagen). This was accompanied by a tendency towards increased proliferation of hair matrix keratinocytes. Quantitative immunohistomorphometry also revealed that spermidine upregulated protein expression of K15, the epithelial stem cell-associated keratin, and K15-promoter activity *in situ*. Spermidine may also upregulate expression of K19, another epithelial stem cell-associated keratin. These experiments provide the first evidence that spermidine is a potent stimulator of human hair growth under organ culture conditions and suggest that oral spermidine may be a useful adjunct therapy for treating hair loss associated with telogen effluvium. In addition, our data indicate a novel role for spermidine in human epithelial stem cell biology. These results highlight the importance of polyamines in human HF biology and encourage one to systemically dissect the underlying mechanisms of action.

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EphA2 negatively regulates epidermal Erk1/2-MAPK signaling and growth

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The Raf-Mek-Erk1/2 pathway promotes keratinocyte proliferation and is dampened to allow for cell cycle exit and differentiation. In addition, normal human epidermal keratinocytes (NHEKs) switched from low (< 0.1 mM) to high (>1.2 mM) Ca2+ dynamically regulate Erk1/2-MAPK activity during the stabilization of cadherin-based cell-cell contacts and subsequent differentiation. EphA2 is a receptor tyrosine kinase that becomes activated by GPI-linked ephrin ligands on adjacent cells. Since NHEKs express both receptor and ligand, we hypothesized that adhesion-dependent EphA2/ephrin-A1 complex formation would suppress epidermal Erk1/2 signaling. In support of this possibility, pharmacological targeting of EphA2 using a recombinant ephrin-A1-Fc (efnA1-Fc) peptide mimetic was capable of rapidly inhibiting Raf-Mek-Erk1/2. Chronic peptide treatment led to EphA2 loss and restricted the colony forming capacity of NHEKs in a Ca2+-dependent manner. To determine whether native EphA2/ephrin-A1 complexes were capable of dampening basal Erk1/2-MAPK signaling in elevated Ca2+, we switched NHEKs from low to high Ca2+. As expected, EphA2 was rapidly recruited to cell-cell contacts and phosphorylated in a manner that was inversely related to Erk1/2. EphA2 activation during a Ca2+ switch was inhibited by: a) preventing E-cadherin-mediated adhesion with a function-blocking antibody, b) cleaving GPI-anchored proteins with PI-PLC, c) occupying ephrin-A1 receptor binding sites with an EphA2-Fc ectodomain peptide mimetic, or d) directly interfering with the EphA2-ephrin-A1 binding site using a pyrrole-based compound. Collectively, these observations indicate that downstream Erk1/2 signaling through EphA2 is normally activated by ephrins on adjacent keratinocytes. Importantly, oscillatory Erk1/2 signaling, cell cycle exit and terminal differentiation were all disrupted in NHEKs lacking EphA2, highlighting its potential importance in epidermal homeostasis. EphA2 may further serve as a therapeutic target in hyperproliferative skin disorders and cancer where it can be harnessed to blunt mitogenic signaling pathways.

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Th2 cytokines-induced Dual oxidase 1 in human epidermal keratinocytes makes a positive feedback loop for IL-4/IL-13 signaling by augmenting STAT6 phosphorylation via oxidative inactivation of protein tyrosine phosphatase 1B.

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Hydrogen peroxide plays a crucial role in eukaryotic signal transduction besides its well known cytotoxic effects. In air way epithelial cells, dual function NADPH oxidase/heme peroxidase (DUOX) 1 and 2 have been identified as the cellular source of H_2O_2. In keratinocytes, however, the expression of DUOX1 or 2 has not been examined yet. In this study, we first demonstrated by DNA microarray that IL-4/IL-13 stimulation augments only DUOX1 mRNA expression among 7 members of NOX/DUOX family in normal human epidermal keratinocytes (NHEK). Next, we confirmed the induction of DUOX1 by IL-4/IL-13-stimulated NHEK at the mRNA and protein level by quantitative real-time PCR and Western blotting, respectively. Moreover, we demonstrated that the augmented DUOX1 expression was accompanied by increased H_2O_2 production, which was significantly suppressed by diphenylene iodonium, an inhibitor of NADPH oxidase or small interfering RNA against DUOX1 (DUOX1-siRNA). Next, using DNA microarray, we showed that more than 60% of 220 genes upregulated in IL-4/IL-13-stimulated NHEK were significantly suppressed by DUOX1-siRNA, suggesting the positive role of DUOX1 in the IL-4/IL-13 downstream signaling. Finally, we demonstrated that the increased expression of DUOX1 in IL-4/IL-13-stimulated NHEK augments STAT6 phosphorylation by the Western blotting. In addition, based on the recent findings that protein tyrosine phosphatase 1B (PTP1B) functions as a nonredundant negative regulator of IL-4 and IL-13 signaling in hematopoietic and nonhematopoietic cells, we demonstrated that DUOX1 induces oxidation of the catalytic cysteine of PTP1B by the Western blotting using Ab against oxidized PTP-active site. These results revealed a novel role of epidermal DUOX1 expression in making a positive feedback loop for IL-4/IL-13 signaling in NHEK.

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Vegetable peptide SP005 promotes activation of mTOR pathway through ERK/MAPK signal in stem cell proliferation

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Various substitutions may be insufficient to fully replace animal serum. Therefore, alternatives to such chemically defined medium such as protein-free medium supplemented with protein digests and plant hydrolysates have also been evaluated. This study was conducted to investigate the proliferative effect of vegetable peptide SP005 on adult stem cells (ASCs) in the absence of serum and its possible mechanisms of action. Cell viability and proliferation were determined by MTT assay and a Click-iTTM EdU flow cytometry assay, respectively. In addition, changes in the expression of cytokine genes were analyzed using a MILLIPLEXTM human cytokine ELISA kit. The viability of human adipose-derived mesenchymal stem cells (ADSCs) and cord blood-derived mesenchymal stem cells (CB-MSCs) treated with SP005 increased significantly. Also, the median value of the group treated with SP005 shifted to the right when compared with the untreated control group in cell proliferation assay. SP005 phosphorylated stepwisely the ERK, mTOR, p70 S6 Kinase (p70S6K), S6 ribosomal protein (S6) and eIF4E in ADSCs. Furthermore, quantitative analysis of the cytokines revealed that the production of vascular endothelial growth factor (VEGF), transforming growth factor-beta1 (TGF-β1), and interleukin-6 (IL-6) increased significantly in response to treatment with SP005 in both ADSCs and CB-MSCs. In the same breath, SP005 inducing-phosphorylation of ERK/mTOR/S6/eIF4E pathway was blocked in a row when pretreated with the PD98059, a specific ERK inhibitor. Also, inhibition of TGF-β1 through PD98059 pretreatment and consecutive decrease of ADSC proliferation revealed that TGF-β1 induces the phosphorylation of mTOR/S6/eIF4E. Collectively, the findings of this study indicate that TGF-β1 through ERK phosphorylation plays a crucial role in the SP005-induced proliferation of ADSCs in serum-free condition.

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Eotaxin-CCR3 interaction promotes survival of anaplastic large cell lymphoma cells via ERK1/2 activation

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The CC chemokine receptor (CCR)3 is expressed as a specific marker of CD30+ anaplastic large cell lymphoma (ALCL). ALCL cells also express eotaxin, a ligand for CCR3, leading to the hypothesis that eotaxin may play an autocrine role in ALCL. In this study, we demonstrate the cell survival effect of eotaxin–CCR3 interaction on ALCL. Human CD30+ Ki-JK cells, established from an ALCL, and murine EL4 lymphoma cells expressed CCR3 on the cell surface. Eotaxin increased cell survival rates of Ki-JK cells in a dose-dependent manner under serum-free conditions (survival rate of Ki-JK cells in a control medium alone was 40%, while that with 1.0 μg/ml eotaxin was 65%). Authentic examination by cell counting and BrdU-uptaking showed that eotaxin also promoted proliferation of EL4 cells (100% increase from the baseline with 1.0 μg/ml eotaxin). Western blotting and intracellular flow cytometry demonstrated that eotaxin induced phosphorylation of ERK1/2 in Ki-JK cells. ERK1/2 was constitutively phosphorylated in EL4 cells with medium alone, which was modestly upregulated by addition of eotaxin. The cell survival effect of eotaxin was completely blocked by addition of MEK inhibitors (PD98059 and U0126) in Ki-JK and EL4 cells. Interestingly, intracellular flow cytometry revealed that phospho-ERK1/2-positive Ki-JK cells cultured with eotaxin were all alive (nearly 100%) but that survival rate of phospho-ERK1/2-negative cells exposed by MEK inhibitors was only 30%. Eotaxin also enhanced tumor formation by injection of EL4 cells into the subcutaneous space of C57BL/6 mice. Moreover, tumor cells of cutaneous ALCL expressed CCR3 and increased levels of phospho-ERK1/2 by immunohistochemistry. Thus, these data demonstrated that signaling through CCR3 on ALCL cells was involved in tumor survival via ERK1/2 activation.

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Adipose-derived stem cell-cultured media improves oxazolone induced atopic dermatitis skin lesions

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Stem cells are undifferentiated cells which have the potentials of self-renewal and differentiation. Adipose-derived stem cell (ADSC) has relative advantages in the accessibility and abundance compared to other kinds of stem cells and produces many kinds of growth factors and hormones not determined yet. Therefore, we investigated whether the cultured media of ADSC could be used as a novel therapeutic material for atopic dermatitis (AD), a representative inflammatory skin disease. ADSC cultured media was applied topically, twice daily for 5days on oxazolone induced AD hairless mice model. Topical administration of ADSC cultured media improved the visible AD lesions through an improving mechanism of epidermal permeability barrier and differentiation compared to control of fibroblast cultured media. ADSC cultured media increased the LB number and the expression of anti-microbial peptides (CRAMP, mBD3). Our result implicated that topical ADSC cultured media would be useful in atopic dermatitis.

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Molecular regulation of neuropeptide receptors by peptidase endothelin-converting enzyme-1: Impact on inflammation and pruritus

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Neuropeptide (NP) receptors play an important role in inflammation, immuno-modulation and pruritus. Substance P, for example, plays an important role in inflammation and pruritus. NP receptors are characterized by prolonged desensitization and fine-tuned cell signaling after agonist stimulation. Thus, the molecular mechanisms which regulate NP receptor resensitization and signaling are of importance for understanding NP-mediated cell regulation and disease. We report that endosomal endothelin converting enzyme-1 (ECE-1) is critically involved in NP receptor regulation and inflammation. We found ECE-1 to be colocalized with the receptors for SP, CGRP and SST in early endosomes. NP binding to their cell surface receptors induced membrane translocation of β-arrestins followed by internalization of the NP-receptor-β-arrestin complex into early endosomes. During pH decrease in endosomes, ECE-1 became activated and induced NP degradation. This step liberated the internalized NP-receptor which subsequently recycled much faster back to the cell surface as controls in which ECE-1 was inactivated. Thus, restimulation of cells for NP receptors is dramatically enhanced in the presence of ECE-1. This is supported by *in vivo* findings in mice where ECE-1 inhibition by SM-19712 abolished rapid NP receptor resensitisation. In neurons, the ECE-1/receptor/β-arrestin complex also regulated downstream signaling of MAPkinases: ECE-1 inhibition caused endosomal retention of the NP receptors and Src, resulting in markedly sustained ERK2 activation, whereas ECE-1 overexpression attenuated ERK2 activation. ECE-1 inhibition also enhanced SP-induced expression and phosphorylation of the nuclear death receptor Nur77, resulting in cell death. The discovery that ECE-1 regulates recycling, resensitization, signaling of NP receptors and neuronal cell death establishes a novel function of intracellular endopeptidases in the regulation of inflammation and pruritus.

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Genetic deletion of *Egfr* results in delayed catagen associated with defective cell cycle progression

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Treatment of cancer patients with epidermal growth factor receptor (EGFR) inhibitors causes perifollicular inflammation in the skin. Mouse models with deficient EGFR signaling predicted this folliculitis and revealed that abrogation of EGFR disrupts hair follicle cycling. The mechanisms through which EGFR regulates the hair cycle were investigated using a skin-targeted *Egfr* mutant model generated by crossing floxed *Egfr* mice with *Keratin 14* promoter-driven *Cre recombinase* mice. Histological examination revealed synchronous catagen onset at 17d in the control mice. In contrast, *Egfr* mutant follicles asynchronously entered catagen between 18 and 20d. Follicular proliferation, as measured by bromodeoxyuridine incorporation, ceased by 18d in control skin, but was maintained in mutant skin. Phospho-Ser10 histone H3 immunofluorescence indicated follicular bulb cells accumulated in late G_2 and M phases prior to the cessation of DNA synthesis in control but not mutant mice. To identify EGFR-dependent signaling regulating cell division in catagen, transcriptional profiling was performed in *Egfr* mutant and control hair follicles at 17d, the time of normal catagen onset, using follicular RNA obtained from laser capture microdissection. *In silico* analysis of these data identified two mitotic regulatory genes, *Rcc2*, and *Sfi1*, that were upregulated by 1.7 and 2.0 fold, respectively, in *Egfr* mutant follicles and validated using real time RT-PCR. Tubulin immunofluorescence and confocal microscopy revealed an accumulation of metaphase cells in control but not mutant hair follicle bulbs prior to catagen induction at 17d. Thus, EGFR-mediated signaling triggers catagen entry and regulates the cell cycle in the hair follicle.

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Unravelling RIP4 signalling in the skin

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The Receptor Interacting Protein (RIP) kinases constitute a family of S/T kinases, generally involved in activation of the transcription factors NF-kappaB and AP-1. In contrast to other RIP kinases the RIP4 kinase activity is required for activation of NF-kappaB and AP-1. Inactive RIP can homodimerize and becomes autophosphorylated and ubiquitinated upon activation. Our data suggest that RIP4 phosphorylation is a prerequisite for its poly-ubiquitination. By mutation analysis we could correlate RIP4 modifications to its capacity to activate NF-kappaB and AP-1. Current data indicate a possible function of RIP4 in PKC-dependent NF-kappaB activation pathways. In addition, RIP4 was shown to be required for epidermal differentiation; however the molecular pathways are still elusive. RIP4-deficient mice are perinatally lethal and show skin hyperplasia, absence of a functional stratum corneum, parakeratosis and abnormal development of hair follicles and sebaceous glands (Holland et al., 2002). We found that, in analogy with IKKalpha-deficient mice (Hu et al., 1999; Takeda et al., 1999), RIP4-deficient mice display a severe skin barrier defect. Our current data point to a defect in periderm development affecting the skin, rather than a mere defect in keratinocyte differentiation. The limb defects and other skeletal defects found in IKKalpha knockouts are not occurring in RIP4-deficient mice. However, similar to IKKalpha knockouts RIP4-/- mice display a cleft palate phenotype.

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The TβRII Expression Levels determine whether TGFβ activates or inhibits ERK without participation of TβRI

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The transforming growth factor-beta (TGFβ) family cytokines play critical roles in skin homeostasis and skin diseases, most noticeably cell growth control, cell differentiation, tissue inflammation, fibrosis and skin cancer progression. TGFβ signals are transmitted from the extracellular environment to intracellular signaling networks via its cell surface receptor complex, the TβRII/TβRI heteodimer. TGFβ binds to TβRII, which in turn recruits, transphosphorylates and activates TβRI, whereby achieving an across the cell membrane signaling. TGFβ stimulation is known to activate R-Smad-dependent and R-Smad-independent post-receptor signaling pathways, such as the extracellular signal-regulated kinase (ERK1/2) pathway. However, two long-standing questions remained: 1) why TGFβ-stimulated activation of ERK occurs only in certain, but not all TGFβ-responding, cell types and 2) whether TβRII alone is able to mediate TGFβ signaling without the participation of TβRI (also called Alk5). We chose human dermal fibroblasts (DFs) and human keratinocytes (HKs) as the cell models to study these questions. We found that TGFβ activates ERK in DFs and inhibits ERK in HKs. In contrast, TGFβ equally stimulates R-Smad activation in both cell types. While the TβRI expression remains similar in both cell types, the expression level of TβRII in DFs is more than seven fold higher than that in HKs. Down-regulation of TβRII in DFs blocked TGFβ-stimulated ERK, just like that in HKs. In reverse, up-regulation of TβRII in HKs to the level in DFs changed their responses to TGFβ from TGFβ stimulated ERK inactivation to TGFβ-stimulated ERK activation. Most intriguingly, the TβRII-mediated TGFβ-stimulated ERK activation or inactivation did not require any participation of TβRI. Thus, this study illustrates that the expression levels of TβRII determine how TGFβ regulates ERK in various cell types and provides direct evidence for a TβRI-free signaling by TβRII for the first time.

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Receptor type protein tyrosine phosphatase beta directly regulates hepatocyte growth factor receptor function in human keratinocytes.

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Reversible protein tyrosine phosphorylation is a fundamental biochemical mechanism that regulates essential eukaryotic cellular functions. The level of tyrosine phosphorylation of specific proteins is intricately balanced by the dynamic action of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Hepatocyte growth factor receptor (HGFR, also known as Met), a receptor PTK, is a major regulator of proliferation, migration, and survival for many epithelial cell types. We report here that receptor type protein tyrosine phosphatase-beta (RPTP-β) specifically dephosphorylates Met, and thereby regulates its function in human keratinocytes. Over-expression of RPTP-β, but not other RPTP family members or catalytically inactive forms of RPTP-β, reduces HGF stimulated endogenous Met tyrosine phosphorylation in HEK293 cells (reduced 60%, n=2, $p<0.05$). Over-expression of RPTP-β in primary human keratinocytes reduces both basal and HGF-induced Met tyrosine phosphorylation at tyrosine 1356 by 30% and 60% respectively (n=3, $p<0.05$), and inhibits downstream MEK1/2 and ERK activation by 70% (n=3, $p<0.05$) and 80% (n=5, $p<0.05$), respectively. Furthermore, shRNA-mediated knockdown of endogenous RPTP-β increases basal and HGF-stimulated Met tyrosine phosphorylation at tyrosine 1356 2-fold (n=5, $p<0.05$), in primary human keratinocytes. Purified recombinant RPTP-β preferentially dephosphorylates purified Met at tyrosine 1356 directly. In addition, substrate-trapping mutant of RPTP-β specifically interacts with Met in intact cells. Over-expression of RPTP-β in human primary keratinocytes reduces HGF induction of VEGF expression by 75% (n=3, $p<0.05$), inhibits keratinocyte proliferation by 60% (n=6, $p<0.05$), and nearly abolishes migration in scratch assay. Taken together, the above data indicate that RPTP-β is a novel regulator of Met function in human keratinocytes.

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UV irradiation reduces Type II TGF-β receptor expression by transcriptional repression through a 38 base pair promoter sequence 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 through its cell surface type I (TβRI) and type II (TβRII) receptors. Ultraviolet (UV) irradiation impairs TGF-β signaling via reduced TβRII gene expression, thereby decreasing type I procollagen synthesis, in human skin fibroblasts. UV irradiation does not alter either TβRII mRNA or protein stability, indicating that reduction of TβRII expression results from transcriptional or translational repression. To understand how UV irradiation regulates TβRII transcription, we used a series of TβRII promoter-luciferase 5′-deletion constructs (covering 2kb of the TβRII proximal promoter) to determine transcriptional rate in response to UV irradiation. We identified a 137 bp region upstream of the transcriptional start site that exhibited high promoter activity, and was repressed 70% (n=5, $p<0.05$) by UV irradiation. Whereas, all other TβRII promoter reporter constructs exhibited either low promoter activities or no regulation by UV irradiation. Mutation of potential transcription factor binding sites within the promoter region revealed that an inverted CCAAT box (-83 bp from transcription start site), is required for promoter activity. Mutation of the CCAAT box abolished UV irradiation regulation of the TβRII promoter. Protein binding to the inverted CCAAT box probe (-100/-62 bp), determined by EMSA, was significantly enhanced in response to UV irradiation. Super shift experiments indicated that nuclear factor Y (NFY) is able to bind to this sequence, but NFY-binding was not altered in response to UV irradiation, indicating additional protein(s) are capable of binding this sequence in response to UV irradiation. Taken together, these data indicate that UV irradiation reduces TβRII expression through transcriptional repression. This repression is mediated by a 38bp sequence in TβRII promoter, in human skin fibroblasts.

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Deacetylated ganglioside GM3 activates uPAR/p38 MAP kinase signaling in caveolar domains

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GM3, the simplest ganglioside, regulates cell proliferation, migration and invasion by influencing cell signaling (e.g. EGFR, integrin and uPAR) at the membrane level. Although N-acetylated GM3 is commonly expressed, deacetylated GM3 (d-GM3) is mainly found in metastatic tumors. We have discovered that 82-95% of GM3 deacetylates in metastatic melanoma cells, and d-GM3 is a novel metastatic marker. d-GM3 increases urokinase plasminogen activator receptor (uPAR)-dependent cell migration and invasion by elevating uPA and activating uPAR/p38 MAP kinase signaling. Lacking both transmembrane and intracytoplasmic domains, uPAR requires other transmembrane molecules to initiate signaling. We hypothesize that d-GM3 expression activates uPAR signaling by promoting its association with integrins in caveolar domains. We modified d-GM3 expression genetically and biochemically in metastatic melanoma C8161 cells, and examined the effect of d-GM3 expression on the association of uPAR with integrins and caveolin-1. The impact of integrins and caveolin-1 on d-GM3 regulated uPAR signaling was also studied. Using immunoprecipitation and immunoblotting techniques, we found that disrupting d-GM3 expression prevents the formation of a complex containing uPAR, caveolin-1, and integrin α5β1 or α3β1, which is critical for suppressing p38 MAP kinase activity. Knocking down caveolin-1, but not integrin subunits (α5, α3 or β1), abolishes uPAR co-localization with d-GM3 at the plasma membrane. In addition, decreases in the expression of d-GM3 or caveolin-1 reduce the phosphorylation and activation of uPAR-dependent focal adhesion kinase (FAK), phosphotidylinositol 3-kinase (PI3K) and Src kinase. As a result, d-GM3 induced uPAR-dependent cell migration and invasion are inhibited. We conclude that d-GM3 enhances the metastatic phenotype by activating uPAR/integrin/p38 MAP kinase signaling through FAK, PI3K and Src kinase in specific membrane rafts. Elucidation of the mechanisms by which d-GM3 modulates uPAR signaling will help us to understand uPAR function in metastatic melanomas and lead to novel GM3-based cancer therapy.

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p75NTR mediates apoptosis in transit amplifying (TA) cells and its overexpression restores cell death in psoriatic keratinocytes

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p75 neurotrophin receptor (p75NTR) belongs to the TNF-receptor superfamily and signals apoptosis in many cell settings. The aim of the present study was to evaluate the proapoptotic role of p75NTR in human keratinocytes. p75NTR mRNA and protein are highly expressed in transit amplifying (TA) cells that are more susceptible to apoptosis than keratinocyte stem cells (KSC). On the other hand, p75NTR expression is low in KSC and absent in PM cells. Brain derived neurotrophic factor (BDNF) or beta-amyloid that specifically bind p75NTR, induce apoptosis in TA, but not in KSC cells, not in KSC. BDNF or beta-amyloid fail to induce cell death in TA cells transfected with p75NTRsiRNA. Furthermore, BDNF induce JNK1 phosphorylation, and NFkappaB DNA binding activity was significantly lower in keratinocytes infected with a p75NTR retroviral vector, which are both critical steps in p75NTR signal transduction. In psoriasis, that is characterized by a low rate of apoptosis, nerve growth factor (NGF) and its high affinity receptor TrkA, that signal survival in keratinocytes, are increased, whereas p75NTR is absent in lesional psoriatic skin and in cultured keratinocytes. The rate of apoptosis in psoriatic TA cells is significantly lower, as compared to normal TA cells. p75NTR is significantly lower in TA cells from psoriatic skin than in TA from normal skin. Moreover, beta-amyloid or BDNF fail to induce apoptosis in TA from psoriatic keratinocytes. Infection of psoriatic keratinocytes with p75NTR retroviral vector restores beta amyloid or BDNF-induced apoptosis. ($p<0.05$ by MTT and subG1 peak). These results indicate that p75NTR acts as a proapoptotic receptor in human keratinocytes. Absence of p75NTR in TA cells could account for the resistance of psoriatic keratinocytes to apoptosis.

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Adipocytes and adiponectin secretion plays a major role in skin physiology and in diabetes wound healing pathology

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A growing body of evidence suggests subcutaneous adipocytes as effectors, regulating skin maintenance and response to injury. So far, direct functional association between subcutaneous fat and skin cells has not been recognized. Utilizing several wound healing animal models, we show that subcutaneous adipocytes are recruited early in the wound healing process and secrete specific factors to promote healing. Impaired wound healing is the most serious diabetes-related skin disorder. In diabetes mice models subcutaneous adipocytes display abnormal morphology which correlates with wound healing impairment. Next, we show that when screening the ability of various adipokines to induce wound healing *in vitro*, adiponectin exhibits a prominent migratory effect on keratinocytes (70%) and fibroblasts (100%). Moreover, adiponectin and its receptors AdipoR1/R2 are expressed in murine and human epidermis where adiponectin inhibits keratinocytes terminal differentiation. Adiponectin expression is induced during wound healing at the wound site distributed specifically around adipocytes. In diabetic wounds, adiponectin expression is diminished and adipocytes display an abnormal morphology. Finally, treatment of animal wounds with adiponectin versus vehicle significantly improves all wound healing parameters *in vivo* including: epidermal closure (67% vs. 33%), dermal closure (83% vs. 50%) and reduced inflammation (83% vs. 17%). In keratinocytes, adiponectin activates selectively the insulin signalling cascade without affecting AMPK activation, including activation of the insulin receptor, activation of IRS1, Ras and Erk1/2 concomitantly with the activation of p70S6 Akt and mTOR pathway. In conclusion, adiponectin is secreted upon skin injury from adipocytes recruited at the wound site to induce wound healing parameters. In diabetic wounds, adipocytes are deformed and fail to secrete adiponectin, suggesting adiponectin as a causative factor in diabetes wound healing impairment and as a candidate for development of novel therapeutics in healing of diabetic ulcers.

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The role of the PLD2/AQP3/PG signaling module in keratinocyte proliferation and differentiation

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The balance between keratinocyte proliferation and differentiation is key for normal skin function. Our laboratory has shown previously that phospholipase D2 (PLD2) and aquaporin 3 (AQP3) colocalize in caveolin-1-rich microdomains of primary mouse epidermal keratinocytes. Indeed, PLD2 and AQP3 were found to interact in a direct protein-protein-mediated manner in Sf9 cells, which lack caveolin-1, and the specific domain necessary for this interaction is under investigation. Based on the idea that AQP3 can transport glycerol, which is a physiological primary alcohol substrate of PLD2, we hypothesized that AQP3 and PLD2 function together to form phosphatidylglycerol (PG), which serves as a lipid second messenger to inhibit keratinocyte proliferation and promote differentiation. First, using adenovirus-mediated infection, we determined that overexpressed PLD2 enhanced keratinocyte proliferation under control conditions and inhibited differentiation induced by a moderately elevated calcium level. However, PG synthesis was inhibited with PLD2 overexpression; this decrease may result from disruption of the interaction between endogenous PLD2 and AQP3 in membrane microdomains, and/or reduced AQP3 activity following overexpression of PLD2 and increased PLD2-mediated lipid signal generation. Next, to determine the functional interaction between AQP3 and PLD2, primary mouse keratinocytes were co-infected with adenoviruses expressing PLD2 and AQP3. Overexpression of either PLD2 or AQP3 inhibited the calcium-induced transglutaminase activity, a marker of keratinocyte differentiation. However, co-overexpression of AQP3 and PLD2 together returned transglutaminase activity to levels observed in empty adenovirus-infected cells, under both control and calcium-stimulated conditions. Similarly, PG synthesis was inhibited by either PLD2 or AQP3 over-expression, but PG levels were returned to control values with co-overexpression. These results are consistent with our hypothesis that PG is a differentiation signal; that is, less PG leads to proliferation and inhibition of differentiation.

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Tissue inhibitor of metalloproteinase 1 is induced in th1 and th17 t-helper cell subsets in a stat-dependent manner

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Naïve CD4+ T helper (Th) cells differentiate into effector T-cell subsets including: Th1, Th2, and Th17. These specialized subsets are important for host defense but are also major contributors to the pathogenesis of immune-mediated diseases. To better define the unique and common features of these subsets, we employed transcriptional profiling. In the current study we investigated T cell production of Tissue Inhibitor of Metalloproteinase 1 (TIMP1), a secreted protein that has pleotropic effects on cellular growth and survival and has been implicated in both cancer prognosis and the regulation of autoimmune diseases. We found that TIMP-1 was secreted in large amounts *in vitro* by T cells stimulated specifically under Th1 and Th17 conditions. We also showed that Th1 and Th17 cell production of TIMP-1 is regulated by separate mechanisms with Stat3 required for Th17 cell TIMP-1 expression and Stat4 required for Th1 mediated TIMP-1 expression. Using Chromatin Immunoprecipitation with massive parallel sequencing, we demonstrated that both Stat3 and Stat4 can bind to the promoter regions of the TIMP-1 gene. The highly regulated secretion of TIMP-1 by Th cells would suggest a novel mechanism whereby Th cell subsets influence the course of inflammatory disease and possibly oncogensis and metastasis.

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WITHDRAWN

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Irritant induced purine release mediates EGFR activation *in vitro*

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In addition to its role in energy transfer, ATP is recognized as an extracellular signaling molecule in the nervous system. More recently ATP and other purines have also been recognized for serving potential signaling roles in epithelial wound repair. Previously, we reported the model irritant sodium lauryl sulfate (SLS) activates the extracellular signal-related kinase (ERK) via epidermal growth factor receptor (EGFR), and that the ATP degradign enzyme apyrase inhibits this SLS mediated activation. These data suggested that ATP might serve as a signaling molecule in irritant mediated skin injury. In order to more fully define the role for ATP, we examined SLS-mediated ATP release *in vitro*. We determined that treatment of HaCaT cells with 0.004% SLS could induce ATP release at points as early as 2 min with levels 5 fold over untreated control (193 + 65 vs 38 + 10 nM). As previously described, SLS treatment induced the *de novo* egr-1 synthesis of the transcription factor egr-1, as did a spectrum of purines including ATP, ADP, UTP, and UDP, all of which were also inhibited by apyrase. Similarly, purine stimulation also resulted in EGFR dependent, and protease sensitive Erk activation, compatible with previously described SLS-induced activation events. Addition of exogenous ATP at concentrations as low as 62.5 nM, well within the range of release noted after SLS treatment, induced de novo egr-1 expression and pretreatment of HaCaT cells with apyrase prior to SLS significantly reduced the induction of *de novo* egr-1 protein synthesis. Finally, the purinergic receptor inhibitor suramin prevented the activation of ERK by SLS at early time points and also inhibited downstream genes activated by SLS after longer treatments. These data support the role of released purines as mediators of irritant skin injury and support the potential use of inhibitors of purinergic receptors as targets for the treatment or prevention of irritant induced skin injury.

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Cathepsin S is a cysteine protease, elicits itch and signals via protease-activated receptors

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Cathepsin S is a cysteine protease linked to inflammatory processes including atherosclerosis and asthma. The possibility that this or other cysteine proteases might evoke itch or be part of a classical ligand-receptor signaling cascade has not been considered previously. The sensation of itch is mediated by two distinct non-overlapping populations of cutaneous nerve fibers that evoke comparable degrees of itch. One set of fibers, the mechano-insensitive population, is more responsive to histamine than to cowhage. The other set is mechanosensitive and is more responsive to cowhage than to histamine. Histamine itch is associated with a wheal and flare. Since most clinical itches do not have a wheal or flare and do not respond to antihistamines, histamine is not thought to contribute to most itches. Cowhage refers to a tropical legume or, in this case, the loose hairs that cover the pods of Mucuna pruriens. It is the prototypic test substance that evokes non-histamine itch. We recently demonstrated that the active component of cowhage, is mucunain, a cysteine protease that serves as a ligand for protease-activated receptors 2 and 4. The identification of an endogenous mediator with properties similar to cowhage could lead to insights into non-histamine mediated itch. We focused on human cathepsin S because it shares active site sequence homology with mucunain and is selectively up-regulated in human keratinocytes upon stimulation with IFN-γ, consistent with a possible pruritic role in inflammatory skin disease. We show here that TNF-α also induces cathepsin S, that cathepsin S evokes itch, activates protease-activated receptors 2 and 4, and is present in itchy, but not normal, skin lesions. Cathepsin S or its receptors may thus be therapeutic targets for the treatment of pruritus.

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Galectin-12 deficiency enhances lipolysis and reduces adiposity in mice

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The breakdown of triglycerides, or lipolysis, is a tightly controlled process that regulates fat mobilization in accord with an animal's energy needs. It is well established that lipolysis is stimulated by hormones that signal energy demand, and suppressed by the anti-lipolytic hormone insulin. Yet much still remains to be learned about the control of lipolysis by intracellular signaling pathways in adipocytes. Here we show that galectin-12, a member of a β-galactoside-binding lectin family specifically expressed by adipocytes, functions as an intrinsic negative regulator of lipolysis. It is specifically localized on lipid droplets and regulates lipolytic protein kinase A (PKA) signalling by modulating phosphodiesterase (PDE) activity to control cyclic adenosine monophosphate (cAMP) levels. Galectin-12-deficient mice on a regular diet have reduced adipose tissue mass as a result of decreased adipocyte triglyceride content, and exhibit improved insulin sensitivity and glucose tolerance, compared to their wildtype counterparts. Galectin-12 ablation also ameliorates obesity in adult leptin-deficient (ob/ob) mice, and accelerates fasting-induced fat reduction in mice with diet-induced obesity. This study identifies a potential target for treatment of human metabolic disorders, a unique intracellular galectin that specifically localizes to an organelle to perform a critical function in lipid metabolism and glucose homeostasis.

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TGF-β suppresses β-catenin-dependent response to mechanical stimulation in dendritic cells

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In this study we set out to better define the molecular details that uniquely regulate dendritic cell (DC) maturation in response to tolerogenic mechanical stimulation versus inflammatory stimuli. Using the well-established murine bone marrow-derived DC (BMDC) system, we find that TFG-β dose-dependently suppresses mechanically-stimulated maturation while having no effect on LPS-induced maturation. Poorly defined mechanical stimuli used in previous studies have made it difficult to define the underlying molecular signaling mechanisms. Therefore, we used antibodies directed against an integrin to mimic mechanical signals. Under these conditions integrins are capable of initiating signaling consistent with mechanical signals. Using a lentiviral knock down strategy, we demonstrated that β-catenin is necessary for the transmission of mechanical signals. We furthermore determined, using a TCF/LEF reporter assay, that TGF-β inhibits the functional activity of β-catenin in DCs. Collectively, our data demonstrate a novel mechanism by which the immunosuppressive cytokine TGF-β can regulate tolerogenic mechanically-stimulated DC maturation independently from inflammatory maturation. By antagonizing β-catenin function in DCs, TGF-β selectively suppresses mechanically-stimulated β-catenin-dependent signaling, while leaving β-catenin-independent inflammatory signaling intact. This data also establish a model that may potentially explain several properties of tolerogenic steady state versus immunogenic inflammatory DC maturation observed *in vivo*.

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A study of NGF levels in atopic dermatitis. NGF does not seem to be a reliable marker for severity of atopic dermatitis.

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It has been reported that levels of NGF are markedly increased in atopic dermatitis (AD) patients and NGF was proposed as a marker of disease severity. Serum NGF is increased at evening & night time in healthy humans, while it is also known that itch of atopic eczema is much more severe at night. Subsequently, we aimed to assess NGF levels in the morning and evening hours and to examine whether there is a correlation between itch intensity and NGF levels using an experimental model of itch. 13 atopic patients and 17 healthy volunteers participated. We measured NGF levels in the skin and serum at two intervals, between 8-10 AM and 5-7 PM. Dermal levels of NGF were investigated by skin microdialysis in eczematous and non-eczematous skin in atopic patients, as well as in healthy subjects; blood samples were taken at 8 AM and 5 PM. Microdialysis was conducted for two hours. Itch was induced experimentally by histamine iontophoresis in the second part of the study. In the first hour, samples were collected in the absence of itch stimuli, while in the second hour microdialysis was carried out after the induction of experimental itch. Dermal levels of NGF were consistently found lower in the eczematous areas compared to non-eczematous areas in atopic dermatitis subjects ($p = 0.06$), and significantly lower compared to healthy skin of healthy volunteers ($p = 0.03$). Lower serum of NGF concentrations were found in the atopic group at both time points in comparison with healthy controls. Dermal NGF levels did not change significantly upon induction of exogenous itch via histamine iontophoresis. No significant differences were noted between morning and evening levels of dermal and serum NGF in either group. Our results suggest that NGF is not a suitable marker for severity of atopic eczema and does not appear to be related to the increased itch at night time.

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The phospholipase D2-aquaporin-3-phosphatidylglycerol lipid signaling pathway in corneal epithelial proliferation and migration

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In previous studies, our laboratory discovered that a novel cell signaling pathway composed of phospholipase D2 (PLD2), aquaporin-3 (AQP3), and phosphatidylglycerol (PG) plays an important role in the regulation of epidermal keratinocyte proliferation and differentiation. Because corneal epithelium and the epidermis are of common embryologic origin, we hypothesized that this cell signaling pathway is present and functional in corneal epithelial cells. SV40-immortalized human corneal epithelial cells were cultured in defined keratinocyte serum-free (dKSF) medium (Gibco) until approximately 70-100% confluent. The existence of PLD2 and AQP3 in the above cells was determined by sucrose gradient ultracentrifugation and western blotting with specific antibodies against PLD2 and AQP3. The effects of glycerol, glycerol with calcium, egg PG and dioleoyl PG (DOPG) on cell proliferation were measured through DNA synthesis tests with [3H]Thymidine. Also, in a wound closure assay the effects of glycerol, glycerol with calcium, egg PG and DOPG on cell migration were measured as the change in width of wound scratch made in fully confluent cells. We found that both PLD2 and AQP3 were localized in fractions 8 to 12, the heavy membrane fractions. 0.5% Glycerol significantly inhibited DNA synthesis under a control calcium concentration of 90μm. While egg PG liposomes inhibited DNA synthesis in a dose-dependent manner, with a maximal about 50% inhibition at a concentration of 100μg/mL, DOPG liposomes had little or no effect on DNA synthesis. More interestingly, in wound closure assay, 0.5% glycerol increased migration rate under both control and elevated extracellular calcium concentration (90μm versus 1mM). Also, egg PG decreased, while DOPG increased, the migration rate. These results showed the likely involvement of the novel cell signaling module in the regulation of proliferation and migration of corneal epithelial cells, processes important in corneal wound healing. In addition, different PG species may have different roles in this regulation.

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UVR induction of snail and slug is independent of lipid raft composition

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Ultraviolet radiation (UVR) is known to activate multiple receptor tyrosine kinases, including the epidermal growth factor receptor (EGFR), in a ligand independent manner, resulting in activation of downstream signaling cascades. We have previously shown that UVR upregulates expression of Snail and Slug, zinc finger transcription factors that modulate epithelial mesenchymal transformation, in human and mouse keratinocytes, at least in part through ERK and p38 MAPK signaling cascades. Moreover, Slug and Snail are downstream mediators of EGFR signaling. UVR is also known to cause lipid raft reorganization and activated EGFR localizes to non-lipid raft membrane fractions. We investigated the effect of lipid raft composition on UVR activation of EGFR and downstream induction of Slug and Snail. Both total and tyrosine phosphorylated (activated) EGFR protein was reduced in lipid raft fractions isolated from UVR treated HaCaT keratinocytes. Modulation of lipid raft composition by addition of cholesterol to the culture medium resulted in enhanced phosphorylation of EGFR after UVR exposure but did not alter Snail or Slug induction. Cholesterol sequestration by Nystatin treatment altered neither UVR-mediated activation of EGFR nor induction of Snail and Slug. These results indicate that EGFR activation by UVR results in its movement out of lipid raft microdomains and activation of downstream signaling, presumably via the MAPK pathway, resulting in enhanced Slug and Snail expression. However, UVR induction of Snail and Slug is not affected by the cholesterol composition of lipid raft microdomains. In addition, pretreatment of cells with the antioxidant N-acetylcysteine partially inhibited phosphorylation of EGFR but did not alter Slug induction, suggesting that, although oxidative stress contributes to EGFR activation, it does not mediate Slug induction.

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Slug modulates ultraviolet radiation induction of calprotectin in keratinocytes

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In keratinocytes, the calcium binding proteins S100A8 and S100A9 serve both as homodimeric intracellular calcium-binding proteins and as the secreted proinflammatory heterodimer calprotectin. Calprotectin is a RAGE ligand that stimulates phagocyte chemotaxis, keratinocyte proliferation, and cytokine production by keratinocytes. S100A8 and S100A9 genes are encoded in the Epidermal Differentiation Complex, a coordinately regulated set of genes located in 1q21, a chromosomal region frequently amplified in cutaneous squamous cell carcinoma (SCC). We have demonstrated markedly elevated expression of S100A8 and S100A9 in human cutaneous SCC by microarray. In addition, our studies of the role of the zinc finger transcription factor Slug in ultraviolet radiation (UVR)-induced SCC showed that Slug null mice were resistant to both UVR-induced inflammation and SCC. We examined a possible link between calprotectin expression and UVR-induction of inflammation, as well as a potential role for Slug in this process. UVR induced Slug and S100A8 mRNA with similar kinetics in HaCaT cells, but S100A9 mRNA was not UVR-inducible. UVR also stimulated release of calprotectin into the culture medium. UVR induction of S100A8 was evident immunohistochemically in the epidermis of wild type mice, but was much attenuated in the epidermis of Slug null mice. In contrast, immunohistochemical staining for S100A9 revealed little difference in the response of wild type and Slug null mice. These findings suggest that Slug has a role in regulation of S100A8 expression in response to UVR and that decreased calprotectin induction may account, at least in part, for the sunburn resistance of Slug null mice.

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Modulation of signal transduction by (-)-epigallocatechin-3-gallate (EGCG) in sebocyte

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EGCG, a major biologically active constituent of green tea, regulates proliferation and lipid accumulation in many cell types. It has been shown in other cell type that EGCG regulates the various types of receptor tyrosine kinases (RTK), which is associated with multiple downstream signaling pathways. However, the action mechanism of EGCG in sebaceous gland has been incompletely characterized. In this study, we examined the molecular mechanisms underlying the effects of EGCG on proliferation and lipogenesis of immortalized sebocyte cell line (SEB-1). Sebocytes were assayed for activation of epidermal growth factor receptor (EGFR), insulin-like growth factor-1 receptor (IGF-1R), mitogen-acivated protein kinase (MAPK) pathway (MAPK/extracellular signal-regulated kinase (ERK), p38 MAPK, and stress-activated protein kinase/c-Jun-N terminal kinase), and Akt. EGCG caused the inhibition of activation of EGFR, IGF-1R, ERK, JNK, and Akt, while it did not change p38 kinase activity. EGCG also caused a decrease in the level of both sterol response element-binding protein-1 (SREBP-1) protein and mRNA and an increase in the levels of mRNA that encode transforming growth factor-beta 2 (TGF-beta2). The present data show that the level of EGFR phosphorylation is decreased more rapidly than IGF-1R phosphorylation at the time points and the inhibition is selective for this MAPK pathway. These findings expand the roles of EGCG as an inhibitor of critical RTKs involved in sebocyte proliferation and interruption of this pathway in the sebaceous gland could be a desirable approach for reducing sebum and improving acne.

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Amphiregulin carboxy-terminal domain regulates autocrine keratinocyte growth and differentiation

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Autocrine KC proliferation can be selectively blocked by antibody (Ab)-mediated neutralization of secreted amphiregulin (AREG) and by small hairpin RNA (shRNA)-mediated AREG knockdown (JID 129:573A). Intriguingly, addition of exogenous EGF receptor ligands to KC cultures reversed the growth inhibition in response to AREG blocking Abs but not to shRNA-mediated AREG knockdown. To explore the mechanisms of differential inhibition of KC growth by AREG knockdown vs AREG neutralizing Abs, we investigated the importance of the cell-associated domains of AREG in regulating KC growth and differentiation. Immortalized but non-transformed N-TERT KCs (MCB 20:1436) previously engineered to conditionally express AREG-specific shRNA were infected with lentiviral constructs for the AREG extracellular domain (ECD) or the full-length AREG transmembrane (TM) precursor. As assessed by QRT-PCR, these constructs, which are not targeted by the AREG-specific shRNA, were effectively transcribed in the newly generated cell lines. In the absence of AREG shRNA, both cell lines grew equally well under autocrine conditions. However, after AREG knockdown, only the AREG-TM precursor (but not the AREG ECD) restored KC growth, rescued normal KC morphology and prevented the formation of tightly-packed colonies of differentiated cells. Consistent with its effects on KC growth and morphology, QRT-PCR revealed that the AREG-TM precursor significantly ($p<0.005$) ameliorated the AREG shRNA-induced expression of several KC differentiation markers, relative to the AREG ECD (K1: 8.3 vs 72% of shAREG for TM vs ECD; K10: 14.6 vs 72.1%; loricrin: 0.5 vs 26.1%, n=4-7). Similar trends were observed for involucrin and Tgase1 (31.1 vs. 68.8% and 33.1 vs 67.7%, respectively). Taken together, our data demonstrate that autocrine human KC growth and differentiation are highly dependent on the AREG transmembrane precursor protein and suggest a novel function of the carboxy-terminal domain of AREG.

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Rutin efficacy in hair loss

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The assay performed in this study has been designed to evaluate the effect of rutin on 5α-reductase activity in fibroblast cell line. This enzyme is expressed in skin melanocytes, fibroblasts and keratinocytes and transforms testosterone into dihydrotestosterone, a key metabolite implicated in various skin disorders such as acne vulgaris, hirsutism, seborrhea and androgenic alopecia. In this test, fibroblasts were pre-treated with testosterone in order to induce the production of 5α-reductase and were exposed subsequently to the test substances for 24 and 48 hours. At the end of the treatment period, the rates of conversion of testosterone into DHT is measured by a double ELISA assay in the treated and untreated cell cultures. These data values allow for estimating the rate of inhibition or stimulation of 5α-reductase activity by rutin. a titred extract of Serenoa repens (Saw palmetto), a well known pharmacologically active compound, and Bohemeria nipononivea extract were used as positive controls. The second part of the study was undertaken to investigate rutin effect on human dermal papilla cells (DPCs) *in vitro*. The results show that rutin inhibits significantly the enzyme 5α-reductase. particularly, rutin causes, on average, a higher inhibition than the positive controls, at doses 20-fold lower than Serenoa repens and Bohemeria nipononivea extracts. Moreover, data obtained on DPCs show rutin antiapoptotic effects *in vitro*. Thus, we suggest that rutin is useful in prevention and treatment of hair loss (telogen effluvium and androgenetic alopecia) through its dual effects.

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Probiotic Lactobacillus rhamnosus LR-04 oral supplementation improves dandruff condition

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Dandruff, a very common condition affecting adult population, is a chronic condition of the scalp characterized by flakes, erythema and itch. Moreover, recently, was reported that dandruff not only creates a discomfort in subjects affected but adversely affects also hair quality. The oral intake of Lactobacillus rhamnosus LR 04 for improving dandruff condition was investigated in a randomized, double-blind, placebo controlled clinical trial. 40 healthy volunteers (male, female, mean age 40 yrs) with moderate dandruff took part in the study. Subjects received LR 04 (109 CFU/day) or placebo for 1 month. Clinical valuation of dandruff was assayed instrumentally (corneocyte counting, measurement of the amount, size and thickness of scales). Dermatologist evaluation was performed at 7th, 15th, 30th days of treatment, with a follow-up at 2 and 4 weeks after treatment and subject self-assessment evaluation at the end of supplementation and on 30th days of follow-up. Results obtained indicated that daily consumption of L. rhamnosus LR 04 resulted in a significant decrease of dandruff associated scales versus placebo, as assessed both by dermatologist and subject self-assessment. Moreover the clinical improvement of dandruff status persisted also during 30th days of follow-up. In conclusion, this study demonstrated that the oral supplementation of Lactobacillus rhamnosus LR 04 is efficient, safe and well tolerated treatment of dandruff.

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A novel supplement for hair loss

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The hair follicle is a dynamic structure that generates a hair shaft through a tightly controlled cycle of growth, remodeling, and loss.The cycle of hair growth includes three stages: anagen (follicle generation and hair production), catagen (follicle regression), and telogen (resting phase). The hair follicle needs a lot of energy (oxygen, amino acids, glycogen, substances delivered by blood) to live and to cycle, and it involves complex mechanisms of protection, cell homeostasis and epithelial interactions. Many recent scientific studies proved that, in most frequent hair pathologies (Androgenetic Alopecia, Telogen Effluvium, Alopecia Areata), different cell components of hair follicle undergo a premature apoptosis process induced by the alteration of mechanisms of cell control, such as cell to cell communication, leading to the activation of caspase cascade causing premature entry of hair in the catagen phase. Oxidative stress combined with many specific factors particularly in male and female androgenetic alopecia, with the alteration of Androgen Receptors in the hair bulb, contributes to the premature senescence of the dermal papilla. Starting from this studies basis, we tested a specific food supplement performing a clinical trial in order to verify if its active ingredients, Spermidine,Zeaxantine and Rutin are able to control the hair bulb pathology (therefore the process of cell senescence and of cell apoptosis) that triggers hair loss. Experimental design: an open clinical trial was conducted on 50 subjects (26 women and 24 men) suffering from male and female AGA and Telogen Effluvium. At the same time we evaluated the efficacy of its active ingredients conducting an *ex-vivo* study on the same subjects: thanks to this study we could measure the reduction of caspase in bulbs after the systemic therapy with the food supplement. The data resulting from the clinical trial prove that the administration of the food supplement produces a clear and radical change in the clinical parameters that are significant to evaluate the severity level of hair pathology (AGA and TE)

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Defective metabolic degradation of retinoic acid in mice lacking Cyp26b1 alters skin development

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Retinoic acid (RA), a metabolite of vitamin A, plays a key role in a variety of biological processes and is essential for normal embryonic development. Recently, it has proven to be effective in the treatment of photodamaged skin especially in women. Although the specific role of RA signaling during skin development remains unresolved, excess RA intake during pregnancy results in skin anomalies in both human and rodent fetuses. The intracellular level of active RA is determined by the balance between its synthesis through the activity of retinaldehyde dehydrogenases (Raldhs) and its degradation by Cyp26 enzymes. Mouse embryos lacking Cyp26b1, one of the Cyp26 gene family members, display hyperproliferative and metaplastic epidermis beginning at E16.5 and impairment of the epidermal skin barrier formation in Cyp26b1 knockout fetuses is evident at E16.5 prior to birth. Moreover, Cyp26b1 knockout fetuses at E18.5 exhibit a significant increase in the rate of transepidermal water loss across their skin surface, compared with control wild-type fetuses. Late stage differentiation of keratinocytes is defective in Cyp26b1 knockout fetuses with respect to nuclear degradation and cornified envelope (CE) maturation as well as a reduced yield of CE from 18.5 embryonic skin. Microarray analysis and immunohistochemistry comparison between wild type and Cyp26b1-/- skin reveal that a number of genes associated with barrier formation are perturbed in Cyp26b1-/- mice. Our results establish that RA degradation mediated by Cyp26b1 plays an essential role during skin development and differentiation.

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A two-step mechanism for stem cell activation during hair regeneration

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The bulge stem cells of the adult hair follicle undergo cycles of activation and rest to sustain regeneration throughout life. Several lines of evidences revealed a crucial role for the mesenchymal dermal papilla (DP) cells in triggering a new hair follicle growth at each cycle. However, how these transitions between rest (telogen) and growth (anagen) phases are orchestrated remains unknown. In particular how the stem cell niche avoids stem cell depletion and which pathways are required for this process are still not well understood. To understand this process, we have examined the transition between telogen to anagen, focusing on the spatial relationship between the epithelial stem cells and the mesenchymal dermal papilla. During telogen, the hair germ separates the DP from the bulge stem cells. Hair germ cells become responsive sooner than the bulge, proliferating and contributing to the early stages of hair follicle regeneration. Moreover when purified and cultured, hair germ cells proliferate more rapidly than bulge cells but fail in long-term transfer. Molecular analyses show that the hair germ cells down-regulate bulge SC markers, but they more closely resemble the activated bulge than the transit-amplifying (matrix) cells. Transcriptional profiling reveals precocious activity of both hair germ and DP at the end of the resting phase, accompanied by Wnt MAPKinase signaling in the hair germ and elevated FGFs and BMP inhibitors in DP. We show that FGFs participate with BMP inhibitors in exerting selective and potent stimuli to the HG *in vivo* and *in vitro*. Our findings show how an efficient growth can be achieved by organizing the stem cell niche in two groups. One group, the hair germ, with the ability to respond quickly to growth promoting signals, which provides the first cellular body giving rise to the new organ. The other composed by the majority of stem cells which is the real engine contributing later to the organ growth.

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Dkk4 and Eda regulate distinctive developmental mechanisms for subtypes of mouse hair

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The mouse hair coat comprises protective "primary" and thermo-regulatory "secondary" hair. Primary hair formation is ectodysplasin (Eda) dependent, but it has been puzzling that Tabby (Eda-/y) mice still make secondary hair. We report that Dickkopf 4 (Dkk4), a Wnt antagonist, affects an auxiliary pathway for Eda-independent development of secondary hair. A Dkk4 transgene in wild-type mice had no effect on primary hair, but secondary hairs were severely malformed. Dkk4 action on secondary hair was further demonstrated when the transgene was introduced into Tabby mice: the usual secondary follicle induction was completely blocked. The Dkk4-regulated secondary hair pathway, like the Eda-dependent primary pathway, is further mediated by selective activation of Shh. The results thus reveal two complex molecular pathways that distinctly regulate subtype-based morphogenesis of hair follicles, and provide a resolution for the longstanding puzzle of how hair formation is maintained in the Tabby mice lacking Eda.

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Gene expression profiling reveals similarities and distinct differences between Lymphocytic and Neutrophilic Cicatricial Alopecias

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We recently reported that PPARγ-regulated pathways play an important role in the etiology of the lymphocytic cicatricial alopecia (CA) and lichen planopilaris (LPP). Treatment of LPP patients with PPARγ agonists showed a marked reduction in severity score and decrease in inflammatory infiltrate. Inflammatory cells detected during the active phase of disease, CA are classified into lymphocytic, neutrophilic and mixed. The clinical features include destruction of hair follicles, progressive hair loss, and permanent replacement of the follicle with fibrous tissue. Gene expression profiling and bioinformatics were utilized to dissect the molecular pathogenesis of disease and to discover novel biomarkers for differential diagnosis of clinical subtypes. Cholesterol biosynthesis was significantly decreased both in lymphocytic (LPP, FFA, CCCA) and neutrophilic (TF) CA. On the other hand, fatty acid metabolism was decreased in all three lymphocytic (LPP, FFA, CCCA) but not in neutrophilic (TF) CA. We are exploring the mechanisms by which these lipids affect stem cell function. Cytochrome P450 panel genes are upregulated in the unaffected tissue of all CA suggesting a role for xenobiotic metabolism in the pathogenesis. PXR is upregulated only frontal fibrosing alopecia while CAR/RXR activation occurs only in central centrifugal CA. Thus, PXR and CAR may be used as biomarkers to distinguish between these two CA sub-types. Our data shows that the LPS/IL1 pathway activation occurs in the lymphocytic but not neutrophilic CA. The activation of this pathway is thought to inhibit RXR, the heterodimerization partner of PPARγ. In addition, we identified distinct patterns of interferon and cytokine genes in neutrophilic and lymphocytic CA. These studies allow the molecular classification of CA subtypes and provide new therapeutic strategies for these challenging hair diseases.

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Expression of MPZL3 during mouse skin differentiation

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MPZL3 (myelin protein zero-like 3) is a newly described transmembrane protein that belongs to the immunoglobulin super family (IgSF). MPZL3 shares the highest levels of sequence homology with two IgSF cell adhesion proteins myelin protein zero (MPZ) and myelin protein zero-like 2 (MPZL2). A recessive missense mutation in *Mpzl3* was identified in the rough coat (*rc*) mice, which develop severe skin abnormalities, including cyclic and progressive hair loss, sebaceous gland hypertrophy, and spontaneous skin ulcerations. To better understand MPZL3 function, we analyzed MPZL3 expression using affinity-purified polyclonal antibodies. We detected abundant MPZL3 expression in the epithelial cells of various mouse organs, including the skin, the oral epithelia, and the intestine. We also examined MPZL3 expression during epidermal development and at different stages of hair follicle differentiation. MPZL3 was localized to the plasma membrane, and seemed to co-localize with E-Cadherin. Our results provide new insight on the functions of MPZL3 and a better understanding of the *rc* phenotype development.

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The role of Prostaglandin D2 and its receptor DP-2 in promotion of Androgenetic Alopecia

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To better understand the pathogenesis of androgenetic alopecia (AGA), we have performed global gene expression analysis on paired haired and bald scalp samples from 5 individuals undergoing hair transplantation for male pattern baldness. The Lipocalin type of Prostaglandin D2 Synthase (L-PGDS) was upregulated ($p<0.01$) in bald compared to haired scalp. RT-PCR ($n=4$, $p<0.04$) and western blotting ($n=4$, $p<0.01$) confirmed increases in L-PGDS mRNA and protein expression respectively. By ELISA ($n=3$, $p<0.01$) and mass spectrometry ($n=17$, $p<0.01$), we found increased PGD2, the enzymatic product of L-PGDS, in bald versus haired scalp. PGE2, which often antagonizes PGD2 actions, was decreased in bald scalp ($n=17$, $p<0.01$). As a mouse model of increased levels of PGD2, the K14-COX2 mouse develops alopecia and sebaceous hyperplasia reminiscent of that seen in AGA. In an *in vitro* explant model ($n=16$, $p<0.01$), PGD2 and 15d-PGJ2 inhibit human hair growth. PGD2 and 15d-PGJ2 also inhibit mouse hair growth when applied topically ($n=3$, $p<0.05$) to wild type mice. To investigate the mechanism, we sought to determine which receptors were required for PGD2 inhibition of hair growth. PGD2 was applied to mice null for the DP-1 and DP-2 canonical PGD2 receptors, as well as L-PGDS null mice as a control. PGD2 inhibited hair growth in DP-1 and L-PGDS but not DP-2 null mice ($n=3$, $p<0.05$). Together these data support a role for PGD2 and the DP-2 receptor in the development of AGA thus provide new targets for possible hair loss treatments.

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Common mechanism of hair follicle formation from dissociated trichogenic epidermal and dermal cells.

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In previous work we and others have shown that under the proper conditions dissociated trichogenic epidermal and dermal cells reform mature, cycling hair follicles. The morphogenetic steps dissociated cells take to produce a hair follicle in the mouse have been defined. The question addressed in this study is if the mechanism defined for the mouse is representative of all mammals. Using trichogenic cells generated from newborn or fetal animals we demonstrate here that cells from rat, dog, opossum and human all undergo the same formative steps: epidermal cells aggregate, dermal cells cluster in foci on the epidermal platform, the epidermal nests undergo apoptosis with cyst formation, peg-like structures grow centrifugally from the cyst to form hair follicles, and mature shafts enter the cyst lumen. From this comparative study we conclude that hair follicles form from dissociated cells in all mammals by one common, or universal, morphogenetic pathway.

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***Fuz* is a PCP effector gene that controls the morphogenesis and differentiation of hair follicles**

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Planar cell polarity (PCP) signaling is essential in determining the polarity of cells within the plane of an epithelial sheet. Core PCP genes were recently shown to control the global polarization of hair follicles in mice. *Fuz*, a homologue of the Drosophila PCP effector gene, *fuzzy*, plays a critical role in ciliogenesis in vertebrates, and is required for the development of a wide range of organs in mice. Here, we report that disruption of the *Fuz* gene in mice severely blocked the development of hair follicles in dorsal embryonic skin. In contrast to the loss of hair follicle polarization in mice deficient in core PCP genes, hair follicles in mice lacking the *Fuz* gene retained their typical anterior-posterior orientation. We show that disruption of *Fuz* impaired the formation of primary cilia in epithelial cells in developing hair follicles and that the hedgehog (Hh) signaling pathway was disrupted in mutant skin. Our data suggest that unlike other members of the PCP family, Fuz plays a unique role in the formation of primary cilia on epidermal keratinocytes, which are required for processing Hh signals during hair follicle morphogenesis.

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Dermal Sonic Hedgehog signaling is required for hair follicle morphogenesis and regeneration

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Hair follicle (HF) morphogenesis requires reciprocal inductive signalings between HF stem cells and dermal papilla (DP) cells. Sonic hedgehog (Shh) is one of the critical inductive signals produced from the HF epithelium. While both the HF epithelial and DP cells show Shh target gene induction, and autocrine epithelial Shh signaling facilitates epithelial progenitor expansion, the role for Shh signaling to the underlying dermis remains poorly understood. Here we investigate the dermal role of Shh signaling in HF growth and further dissect Shh signaling cross-talk using both mouse mutants and a genetically tractable hair regeneration assay that we have developed. We found that *Prx1-cre* dermal specific genetic knockout or dermal shRNA knockdown of Shh signal transducer Smoothened (Smo), reduced HF numbers and significantly retarded HF development. Although the dermal Smo deficient HFs were growth delayed, they displayed normal differentiation markers. Dermal Smo knockout HFs have reduced numbers of dermal condensate cells *in vivo*, with no detectable differences in proliferation or apoptosis, while Smo knockdown dermal cells expressed reduced levels of a subset of DP markers *in vitro*. These findings suggest Smo is required to maintain normal dermal condensate signaling. Using the hair regeneration assay, we tested if a DP signal Noggin could rescue dermal Smo knockdown hair regeneration defects. We found that Noggin overexpression alleviated the loss of dermal Smo by increasing epidermal Shh expression. We conclude that dermal Shh signaling is required to maintain DP, thereby promoting DP morphogenic signals to the HF epithelial progenitors. Since hedgehog signaling to the stroma is critical in a number of hedgehog-dependent epithelial tumors in humans, these results may facilitate new cancer therapeutics.

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Deletion of dermal integrin beta-1 leads to adhesion, but not hair follicle morphogenesis, defects

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Initiation of the hair follicle placode, its subsequent growth and maturation and its eventual cycling in post-natal skin requires signaling interactions between epithelial cells and adjacent dermal cells that form the specialized mesenchyme called the dermal papilla (DP). In addition, the primary cilium, a microtubule based organelle crucial for various signal transduction pathways, is required in DP for hair follicle development and Sonic Hedgehog (Shh) signal reception in DP cells. Previous work indicated a requirement for laminin-511 in hair follicle development and suggested that laminin-511 interaction with integrin beta-1 is required for primary cilia formation and thus Shh signal reception in DP. Reexamination of the skin of laminin alpha-5 knockout mice using adenylyl cyclase III antibodies for primary cilia demonstrate the presence of cilia in E14.5 dermal cells and E16.5 dermal condensates. E16.5 dermal condensates also show normal maturation markers such as CD133, P75 and syndecan-1. Furthermore, conditional knockout of integrin beta-1 in dermal cells using Prx1-Cre results in mild adhesion defects of the DP. However, hair follicle morphogenesis is preserved, as mature hair follicles develop in P1 skin lacking dermal beta-1 integrin. In conjunction with previous data on beta-1 integrin, our results indicate that epithelial-derived laminin-511 may control hair follicle morphogenesis by acting on the hair epithelium rather than the dermal compartment.

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Patterning dissociated heterotypic adult cells into functional folliculoid organ germs by substratum-faciliated self-assembly

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The aim of this study is to develop a method for efficient production of folliculoid keratinocyte-dermal papilla (DP) microtissues to facilitate epithelial-mesenchymal interaction. The behavior of DP cells and adult keratinocytes from hairless skin on poly(ethylene-co-vinyl alcohol) (EVAL) surface is investigated. Keratinocytes, poorly adherent both to substrate and between homotypic cells, become suspended disperse cells after homotypic cell seeding. Seeded simultaneously, keratinocytes and DP cells are able to aggregate into more than 2000 spheroidal microtissues after one single seeding. Dynamical analysis shows that DP cells act as a carrier in the process due to the heterotypic intercellular adhesion. DP cells attach faster to EVAL and start to aggregate. Keratinocytes adhere to DP cells and are then carried by DP cells to form initial hybrid aggregates. Due to the high motility of DP cells, these hybrid aggregates move collectively as clusters and merge into larger spheroids which subsequently detach from the substratum and can be easily collected. Compared with random cell distribution in spheroids generated in hanging drops, these hybrid spheroids have a preferential compartmented core-shell structure: an aggregated DP cell core surrounded by a keratinocyte shell. In addition to ameliorated DP signature gene expression, keratinocytes show down-regulated epidermal terminal differentiation and enhanced follicular differentiation. Functionally, these microtissues are able to grow hairs *in vivo*. This work sheds light on the complex effects and dynamics of cell-cell and cell-substratum interaction in the patterning of heterotypic cells into tissue forms and is of potential to be applied to mass generation of other epithelial organ germs *in vitro*.

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VEGF stimulates proliferation of human outer root sheath cells through VEGF receptor-2 mediated ERK signal pathway

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Vascular endothelial growth factor (VEGF) is a key regulator of physiological and pathological angiogenesis. The biological effects of VEGF are mediated by receptor tyrosine kinases. VEGF receptor-2, the primary receptor for VEGF, is thought to mediate most functional effects. Recently, VEGFR-2 was found to be expressed in normal human epidermis and associated with proliferation of epidermal keratinocytes. We investigated the expression and roles of VEGF receptor-2 on human outer root sheath cells (ORS), which localize the outmost part of epidermal layers of hair follicle. The expression of VEGFR-2 on ORS cells at mRNA and protein levels was determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. Localization of VEGFR-2 in ORS cells was detected by immunofluorescent method. The effect of VEGF on ORS cell proliferation was determined by MTT assays. The expression of VEGFR-2 on ORS cells was demonstrated both at mRNA and protein levels. Immunostaining for VEGFR-2 showed strong signal mainly on the membrane of ORS cells. Exogenous VEGF165 stimulated proliferation of ORS cells and up-regulated expression of VEGFR-2 in a dose-dependent manner. Moreover, Exogenous VEGF165 induced phosphorylation of VEGFR-2, PLC-γ1, PKC-α, MEK1/2, and p44/42 MAPK (ERK1/2) in a time-dependent manner. Take together, these results show that human ORS cells express functional VEGF receptor-2 and exogenous VEGF up-regulates expression of VEGFR-2 and stimulates proliferation of ORS cells via VEGFR-2 mediated ERK signaling pathway. Our findings suggest that molecular targeting of VEGF/VEGFR-2 may be a useful and novel strategy for treatment of hair disorders such as androgenetic alopecia.

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VEGF receptor-2 mediates proliferation of human dermal papilla cells through ERK pathway, but not through p38 MAPK, JNK, AKT pathways

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VEGF receptor-2 is the primary receptor for VEGF and is thought to mediate most functional effects. Recently, VEGFR-2 was found to be expressed in normal human epidermis and mediates proliferation of keratinocyte. We previously demonstrated that both VEGF and VEGFR-2 were expressed in human hair follicles including outer root sheath and dermal papillae by immunofluorescence. Therefore we further investigated the expression and roles of VEGF receptor-2 on human hair follicles at cell level. The dermal component of hair follicle dermal papilla (DP) is believed to play very important roles during the development, reformation and maintenance of hair follicle and control of hair cycle. The expression of VEGFR-2 on dermal papilla cells (DPCs) at mRNA and protein levels was determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. Localization of VEGFR-2 in DPCs was detected by indirect immunofluorescent method. The effect of VEGF on DPCs proliferation was determined by MTT assays. The expression of VEGFR-2 on DPCs was demonstrated both at mRNA and protein levels. Immunostaining for VEGFR-2 showed strong signal mainly on the membrane of DPCs. Exogenous VEGF165 stimulated proliferation of DPCs in a dose-dependant manner. And this was blocked by VEGFR-2 neutralizing antibody (MAB3571). In addition, ERK1/2 phosphorylation induced by 10ng/ml of VEGF165 was detected in a time-dependent manner and abolished by ERK inhibitor PD98059. But the phosphorylation of p38 MAPK, JNK, AKT were not detected with the treatment of 10ng/ml of VEGF165. In conclusion, these results show that functional VEGF receptor-2 was expressed by human DPCs and mediates the proliferation of DPCs through ERK pathway, but not p38 MAPK, JNK, AKT pathways. Our findings indicate that molecular targeting of VEGF/VEGFR-2 may be a useful strategy for treatment of hair disorders.

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Cholesterol enhances the dry skin relief benefits of some but not all lamellar bilayer-forming lipids

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The barrier properties of the stratum corneum (SC) controlled its total architecture but the highly ordered lipid bilayers play a vital role. The aim of this study was to determine the effects of bilayer-forming mixtures of phospholipids and pseudoceramides together with cholesterol and fatty acid for their effectiveness in treating dry skin. Double-blind, fully randomized, placebo controlled clinical trials in which dryness on the outer lateral lower legs was visually assessed by expert grading using a six point scoring scale (0-5) were conducted. Thirty five female subjects, with severe dry skin (dryness grade 3 to 4) underwent an initial fourteen day mild syndet bar soap washing pretreatment phase (Dove bar), followed by a four week treatment phase, during which test formulations (5% of bilayer-forming lipids and 1% of stearic acid or 4% of bilayer-forming lipids, 1% of cholesterol and 1% of stearic acid) were applied twice a day (2 mg/cm2). A significant reduction in skin dryness was observed for all lipid mixtures. However, only mixtures containing either phospholipids alone (AUC difference = 5.16, $p<0.01$), or phospholipid with an optimised combination of fatty acids and fatty alcohols (AUC difference = 3.44, $p<0.01$) and an omegafatty acid-containing pseudoceramide (AUC difference = 4.83, $p<0.01$), showed a better improvement in the presence of cholesterol. A synthetic pseudoceramide did not show a significant difference in the presence of cholesterol. These findings show that bilayer-forming mixtures of specialised lipids, cholesterol and fatty acid are efficient treatment for dry skin. However, only specific lipids in a specific ratio with cholesterol and fatty acid such as phospholipids and an omegafatty acid-containing pseudoceramide show greater improvement. This is highly likely to be related to the stereochemistry of the particular lipids tested as the interaction of cholesterol with the lipids is dependent on this.

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The effect of environmental stresses on hair follicles in human scalp skin grafts

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Hair scalp is constantly exposed to different environmental stresses including UV radiation, oxidative stresses, and chemical stresses that can influence hair growth and also affect hair color. Evidence suggests that even if hair follicles (HF) are not directly exposed to environmental stress at the same level as the skin, they can nevertheless exhibit signs of alteration similar to those observed in the skin. In this study, we investigated the effects of UV, oxidative stress induced by H2O2, and chemical stress induced by SDS on human scalp skin grafts maintained in culture. For UV irradiation, the biopsies were submitted to successive exposure to 5 and 0.2 J/cm2 of UVA and UVB, respectively. For oxidative and chemical stress, H2O2 at 5mM and SDS at 1% were topically applied. Outcomes on hair follicle structure were studied by hematoxylin-eosine (HE) staining 24 hours after stress exposure. Damage was mainly observed in the outer root sheath, and included apoptotic cells, vacuoles, oedemas, and disorganization of the basal layer of the ORS. Using immunofluorescence staining, we observed an increase in catalase expression for the three stress conditions studied. We were also interested in following p63 expression, as it is one of the regeneration capacity markers of HF. Interestingly, our studies showed that, after stress, UV irradiation was accompanied by a decrease in p63 expression. Moreover, studies on the application of IV09.003 (a specific antioxidative stress) on human scalp skin grafts 24 hours before UV stress showed that HF in these grafts exhibited less damage, as seen by HE staining. Moreover, the protective effect of the active ingredient prevented UV-induced increase in catalase expression, and maintained p63 expression at a level close to that of non-irradiated skin. This study demonstrates the importance of hair follicle protection against environmental stress that can damage hair structure, growth, and color.

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Modulation of hair follicle stem cell markers and hair growth

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Hair follicles (HF) are stem cell-rich structures that undergo recurrent phases of growth (anagen), regression (catagen), and relative quiescence (telogen) followed by hair shedding. At the end of telogen, stem cells become activated in order to start the next growth phase and regenerate a new hair shaft. Here we were interested in evaluating the expression of different stem cell markers in HF and in studying a possible modulation of hair growth. Human scalp skin grafts were cultured at the air-liquid interface and fed with a previously defined serum-free medium. A selected active ingredient (IV09.004) was applied topically for 48h. Scalp grafts were then embedded in paraffin or frozen in OCT and processed for immunofluorescence evaluation. Hair follicle structure was controlled by hematoxylin-eosin staining. Among the different markers described as stem cell markers, we studied keratin-15, alpha-6 integrin, beta-catenin, and p63. These markers showed positive staining in the outer root sheath (ORS) of HF. Keratin-15 and alpha-6 integrin were more specifically located in the outermost layer of ORS and showed more intense staining following treatment with IV09.004. The staining of beta-catenin, which plays a key role in hair follicle growth and differentiation, also became more intense in the presence of the active ingredient. P63 has been previously shown to decrease in areas affected by alopecia, indicating the role played by p63 positive cells in hair growth and regeneration. This staining was more intense in treated human scalp skin grafts. Hair growth was measured on human scalp skin grafts maintained in culture for up to 14 days. We observed a positive effect on hair growth from 10 days of treatment onward. In conclusion, the control of hair growth by IV09.004 may be related to its activity in modulating stem cell markers, and this effect may have implications for hair regeneration and vitality.

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p63 and IRF6 are components of a common molecular pathway disrupted in ectodermal dysplasias

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Mutations of IRF6 and p63 cause human congenital syndromes characterized by defects in development of ectoderm-derived structures. p63 and Irf6 null mice display consistent phenotypes affecting the same tissues. Thus, p63 and IRF6 control tissue-specific transcription programs essential for the regulation of ectodermal cell proliferation and differentiation. We report that in keratinocytes IRF6 and p63 constitute a feed-back regulatory cascade: ΔNp63 activates transcription of IRF6 and this, in turn, induces proteasome-mediated ΔNp63 degradation. This loop allows keratinocytes to exit the cell cycle, thereby limiting their proliferation. p63 mutations causing ectodermal dysplasias are unable to activate IRF6 transcription, and mice with mutated or null p63 show reduced Irf6 expression in palate and ectoderm. These results identify a novel mechanism regulating the proliferation-differentiation balance of keratinocytes essential for palate fusion and skin differentiation and links the pathogenesis of two genetically different groups of ectodermal dysplasia syndromes into a common molecular pathway.

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Effects of extracellular calcium on cultured sebocytes

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Calcium plays a vital role in regulating physiologic events in many tissues. Especially in the epidermal keratinocytes, calcium shares in the control of major functions including proliferation, differentiation and cell-cell adhesion. It is known that characteristics of cultured human sebocytes are significantly different from those of cultured human epidermal keratinocytes. Human sebaceous glands, isolated from adult human occipital scalp, were cultured on Dulbecco's modified Eagle's medium for first three days and after then on keratinocyte growth medium (KGM) to examine the possible involvement of calcium in cultured sebocytes. Extracellular calcium concentrations of the KGM were ranged from 0.09 to 100 mM (0.09, 0.2, 1.1, 10 and 100 mM). We used immunocytofluorescence and RNA amplification to investigate the expression of cytokeratin, peroxisome proliferator-activated receptor-γ (PPAR-γ), melanocortin 5 receptor (MC-5R), mel5 and involucrin on the cultured sebocytes. Human follicular cDNA chip made by Kyungpook National University was used for microarray. Calcium-induced morphological changes of cultured sebocytes were induced. High calcium levels in culture media resulted in death of cultured sebocytes. Immunocytofluorescence and RT-PCR revealed changes in expression of MC-5R and involucrin, and no changes in expression of cytokeratin and PPAR-γ. This result leads us to further evaluation about an effect of extracellular calcium concentration on cultured sebocytes.

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Modulation of basement membrane components in the hair follicle of human scalp skin grafts

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Hair follicles result from the concentric organization of different compartments, including epithelial and mesenchymal components separated by a basement membrane (BM). In the skin, the BM is the seat of numerous molecular signals, which regulate cell adhesion, migration, proliferation, and differentiation. Though the role of the BM in hair follicles (HF) is not well characterized, recent studies have underlined the particular importance of molecular communication in hair growth and cycling. Specifically, interactions between α3β1integrin and laminin 511 have been shown to be implicated in hair follicle morphogenesis. Moreover, α6 and β1 integrin subunits play a role in the cohesion of the basal layer of the outer root sheath (ORS). Using an immunohistochemical approach, in the present study we characterized different components of the BM in hair follicles. Human scalp skin grafts were maintained in culture at the air-liquid interface and treated topically with an active ingredient for 48h. Biopsies were then processed for immunofluorescence staining, using antibodies against γ2 and α5 chains of laminin 332 and 511, respectively. We were also interested in evaluating collagen IV, as well as the α6, α3, and β1 integrin subunits. Our results showed that these markers were positively regulated by IV09.005, a previously described hair energizer. In order to study the consequences of the upregulation of BM components on hair growth, human scalp skin grafts were maintained in culture for up to 17 days, with a daily application of the active ingredient. Hair shaft elongation was measured and evaluated. Our results showed that hair growth was higher in treated biopsies, compared to placebo. Using this model of human scalp skin grafts, our studies demonstrated that reinforcing the expression of different BM components may enhance hair growth.

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WITHDRAWN

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New pointers towards a role of perifollicular mast cells in Alopecia areata

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Alopecia areata (AA) is an organ-restricted, T-cell-dependent autoimmune disease. In AA, the typical inflammatory cell infiltrate around the anagen hair bulb is connected with a collapse of the constitutive hair follicle immunoprivilege (IP). Although an increase of mast cell (MC) numbers has been described in AA, the role of MCs in AA pathogenesis remains to be dissected. As a basis for such studies, we have compared the number, degranulation and proliferation status of perifollicular MCs in AA and normal human scalp skin. Since MCs have recently been found to regulate CD8 T-cells in mice, MC numbers and activation status were correlated with CD8 T cell numbers and with the expression of transforming growth factors β-1 (TGFβ-1), a potent immunosuppressant that maintains HF IP and is also expressed by MCs. Paraffin sections of AA and healthy human scalp skin were stained for MCs by using c-kit or TGFβ-1 immunohistochemistry, Ki-67/Tryptase and CD8/Tryptase double-staining and Toluidine blue. This was followed by quantitative statistical analysis of MCs density, degranulation and proliferation, CD8 T-cells number and of TGFβ1-immunoreactivity in designated reference areas. Our results demonstrate a significant increase in perifollicular MC numbers, degranulation status and proliferation in AA patients compared to controls, especially in the HF connective tissue sheath in early stage AA. In addition, many of these MCs appear to engage in enhanced contact with numerous CD8+ T-cells. In late-stage AA, we found a decrease of follicular TGFβ-1 expression, in particular, in those patients that display low MC numbers. These findings encourage one to systematically dissect the as yet under-investigated and likely complex contribution of MCs to AA pathogenesis.

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A safe and effective new topical treatment for nail fungus (Onychomycosis)

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Oral drug therapies for treatment of Onychomycosis are problematic because of systemic toxicity, depletion of drug due to metabolism, long treatment regimen, adverse side effects and high expense for the patient. The only US FDA approved topical treatment (Penlac® with ciclopirox) has very low cure rates (10 to 13%). What is needed for an effective topical therapy is a drug active that has the ability to penetrate the highly impervious keratinaceous nail plate, with strong anti-fungal properties against the causative organisms (Trichophyton mentagrophytes, and Trichophyton rubrum). Furthermore it should be cost-effective, and have no adverse toxicity or side effects during the treatment regimen. In this presentation, we describe a pyrithione salt that is a very strong antifungal (MIC against T. mentagrophytes is <0.25 ppm) and is able to permeate through the nail bed via a molecular cascade mechanism by sequentially forming disulfide bonds with the sulfhydryl groups of the nail keratin. We also describe experimental data demonstrating its high degree of efficacy and permeability when applied as a topical nail lacquer. The *in vitro* and *in vivo* efficacy data show the high potency of the drug when compared against other known anti-fungals recommended for this condition. One of the pyrithione variants (pyrithione zinc) is a Category I (GRAS, GRAE) drug that has been widely and successfully used to treat dandruff for over 50 years. Pyrithione has many advantages over other drug candidates. Its low molecular weight (127 daltons), lipophilic character, and the ability to easily form disulfide linkages (pKa = 4.3) enable permeation through the nail bed and its fungicidal effect by catalytically disrupting proton gradients does not induce resistance. Its well documented human safety data, lack of systemic bioaccumulation and relative low cost for treatment regimen, all make this drug a very attractive therapy for the topical treatment of Onychomycosis.

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Inhibition of sebaceous gland differentiation and production of sebum-specific lipids by a melanocortin receptor 5 antagonist

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The melanocortin receptor 5 (MC5R) is present in human sebaceous glands where it is expressed only in differentiated sebocytes. The targeted disruption of MC5R in mice resulted in water repulsion and thermoregulation defects due to reduced production of sebaceous lipids. Studies presented here document the suppression of sebum production by the inhibition of the sebaceous MC5R. JNJ-10229570 blocked the binding of ligands to MC5R-transfected cells in a dose-dependent manner and inhibited the production of sebum-specific lipids by cultured primary human sebocytes. Most importantly, topical treatment of human skin transplanted on the backs of severe combined immunodeficient (SCID) mice with JNJ-10229570 led to distinct reductions in sebaceous gland size, sebaceous cell size, and differentiation level within the gland, and markedly reduced sebum secretion. We suggest that MC5R antagonists such as JNJ-10229570 could be used as topical sebum suppressive agents for the treatment of acne and other sebaceous gland disorders.

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Characterization of A20 epidermal-specific knockout mice

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Numerous stimuli lead to modifications and proteosomal degradation of IκB, resulting in nuclear localization and activation of NF-KB. Genetic deletion or overexpression in the epidermis of different key proteins in NF-KB signaling shows that this pathway has both an anti-apoptotic role and regulatory function in skin inflammation. The NF-KB pathway is tightly controlled and one critical brake is A20, known as a dual inhibitor of both NF-KB and apoptosis. Polymorphisms in or near the human A20 gene are associated with psoriasis. We genetically deleted A20 in the mouse epidermis. The A20EKO mice display an hyperproliferating epidermis with keratin 14 expression in the suprabasal layers. Although differentiation markers have a normal expression pattern and skin barrier is not affected in adult mice, EM analysis shows incomplete cornification. The $A20^{EKO}$ mice also have thinner and damaged hair, which resembles the phenotype of K14-EDA-A1 transgenes. Mutations in mouse and men in the ligand EDA-A1, its receptor EDAR or downstream molecules EDARADD or TRAF6 all acquire ectodermal dysplasia. Also nails are longer in the $A20^{EKO}$ mice, the tail is more scaly and the skin displays sebocyte hyperplasia. In HEK293T cells A20 can act as an inhibitor of EDAR-induced NF-KB and currently we are investigating whether the EDAR pathway is hyperactive in $A20^{EKO}$ mice. In addition, $A20^{EKO}$ mice show increased expression of several cytokines that may be responsible for the aquired skin hyperproliferation.

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Genome organiser and special AT-rich binding protein Satb1 controls the establishing tissue-specific chromatin organization during development of the epidermis

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During development, multipotent progenitor cells establish tissue-specific programmes of gene expression that underlie a process of differentiation into specialized cell types. Genome organiser and special AT-rich binding protein Satb1 plays an important role in the control of higher order chromatin remodelling and establishing distinct three-dimensional conformations in a number of tissue-specific gene loci required for maintenance of their transcriptionally active status. Here we show that during epidermal morphogenesis in mice, epidermal differentiation complex (EDC) located on mouse chromosome 3 and containing large number of genes activated during keratinocyte terminal differentiation becomes incorporated into the Satb1+ nuclear network. Genetic ablation of the Satb1 results in alterations of three-dimensional EDC structure and marked decrease in expression of the loricrin, involucrin, fillagrin and other genes involved in the control of epidermal barrier formation. These alterations are accompanied by significant decrease of the epidermal thickness and abnormal granular layer formation in Satb1 -/- mice compared to wild-type controls. Furthermore, ChIP analysis revealed that Satb1 expression in keratinocytes is directly controlled by p63 transcription factor. Thus, Satb1-dependent step in the p63-mediated program of higher order chromatin remodeling in the EDC locus is very important for proper execution of the differentiation programs in epidermal progenitor cells. These data demonstrate a fundamental significance of the Satb1 in establishing three-dimensional chromatin organization in epidermal keratinocytes and raise a possibility for further analyses of its role in alterations of gene expression programs seen in patients with the disorders of epidermal differentiation.

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Autoimmunity, skin morphology and hair cycling in a mouse model of Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE), a chronic autoimmune disorder, arises when the body's own immune system produces antibodies against healthy tissue including the kidneys, brain, skin and hair follicle. Skin abnormalities and alopecia are often accompanied by emotional disturbances including depressive mood. Better understanding skin and hair morphology in an SLE like model is a critical step towards improved patient outcomes like quality of life. This study examines hair growth in a well-established SLE model, by comparing MRL-lpr mice (disease develops at 7-8 weeks) and congenic MRL+/+ mice (disease develops around 4-5moths) on four main determinants: hair cycle score (HCS), epidermal and dermal thicknesses and gender at 12 weeks of age. Skin specimens from the neck and back regions were harvested and cryosections were processed for immunohistochemistry and AP staining. To calculate the HCS, every stage of anagen and telogen was assigned an arbitrary number in ascending order (telogen=0, anagen I = 1, anagen II=2). The total number of HFs in each stage was multiplied by its corresponding unit number. The results of each sum were totaled and divided by the total number of HFs counted, thus defining the average HCS of all HFs within the entire examined group. No significant differences between lpr and +/+ mice were detected. However, females in both groups exhibited higher HCS (>0.05), suggesting that they enter the hair growth phase at a different time point then males. Furthermore, when comparing +/+ females to males, females exhibited a thicker epidermal layer (>0.01) and males a thicker dermal layer (>0.05). Current results support the notion that estrogens and androgens play an important role in skin morphology. These findings suggest that hormonal interactions account for sex-dependent differences in hair cycling and skin morphology. Further hormonal analysis is required to elucidate how higher HCS relates to alopecia and increased prevalence of SLE in females.

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Dicer is required in adult skin for maintenance of rapidly proliferating hair follicle matrix cells, hair shaft formation, and catagen

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Multiple miRNAs are expressed in developing and adult skin, and constitutive deletion of the miRNA processing enzyme Dicer in embryonic mouse skin causes failure of hair follicle morphogenesis. To determine whether Dicer function is required in adult hair follicles and epidermis we generated Krt5-rtTA tetO-Cre Dicerfl/fl mice in which the Dicer gene can be inducibly deleted in the basal epidermis and hair follicle outer root sheath, including hair follicle stem cells, by oral doxycycline. Induction of Dicer deletion in the telogen resting phase reduced miRNA expression but did not immediately result in histological abnormalities or loss of the stem cell markers K15 and S100A4. Initiation of a new anagen growth phase by hair plucking stimulated stem cell proliferation, but Dicer mutant hair follicles were unable to develop a normal matrix or produce a hair shaft. Instead, compared with controls, plucked Dicer mutant hair follicles showed increased cell death and expression of phosphorylated Histone H2.AX, a DNA damage marker, in the matrix, and rapidly degraded. When Dicer depletion was initiated during mid-anagen, hair shaft formation failed and hair follicles did not enter the catagen regression phase, remaining in an abnormal anagen, before degrading. In either case, long term Dicer depletion resulted in loss of K15 and S100A4 expression. Interestingly, in contrast to hair follicle loss, the epidermis was maintained in induced mutant mice, and displayed hyperproliferation and increased expression of the stem cell-associated protein p63. These results demonstrate a specific requirement for Dicer in maintaining the ability of adult hair follicles to grow and regenerate, and indicate that Dicer functions to maintain the viability of the transient amplifying matrix cells, as well as in long term maintenance of hair follicle stem cells and structures.

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Clinical evaluation of embryonic-like fibroblast secreted proteins to induce hair growth in humans

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Research has shown the importance of Wnt 7a and wound healing growth factors on the stimulation of bulge cells and inter-follicular stem cells to induce hair growth. We have developed a bioengineered human cell-derived formulation, termed Hair Stimulating Complex (HSC), consisting of growth factors and morphogens recognized to be critical to the induction and maintenance of hair follicle growth. Following preclinical safety and efficacy studies suggesting the increased induction of anagen in the C57Bl model by HSC, a clinical pilot study was undertaken. The double-blind, placebo-controlled, randomized single site trial was primarily designed to evaluate safety of the HSC product, with efficacy as a secondary goal. Data analysis indicated that HSC is safe and showed effectiveness in stimulating hair growth in subjects with MPHL. All subjects tolerated the procedures well, no adverse reactions were reported. Histopathological evaluation of the treatment site biopsies taken at 22 & 52 wk post-treatment revealed no abnormal morphology, hamartomas or other pathological responses. Trichoscan image analysis of placebo sites at 12, 22 & 52 wk showed no significant improvements in any of the measured hair growth indicators over the initial 12wk evaluation period whereas the HSC treated sites demonstrated an increase in all hair growth indicators except vellus hair density. The improvements from HSC treatment were significantly greater than that observed in placebo treated sites: hair shaft thickness (6.3% + 2.5% vs. -0.63% + 2.1%; p = 0.046), thickness density (12.8% + 4.5% vs. -0.2% + 2.9%; p = 0.028), and terminal hair density (20.6 + 4.9% vs. 4.4 + 4.9%; p = 0.029). Similar trends were seen at 22 & 52 wk, with total number of hairs increasing on the HSC-treated site only over one year. These results clearly demonstrate that a single intradermal administration of HSC improved hair growth in subjects with androgenetic alopecia and is a clinical substantiation of previous preclinical research with Wnts and wound healing.

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Endo- and phytocannabinoids differentially regulate biology of human epithelial skin cells

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Recent findings unambiguously argue for the functional existence of the endocannabinoid system (ECS) in the human skin. In the current study, we investigated the effects of endocannabinoids, such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and a non-psychoactive phytocannabinoid from *Cannabis sativa*, Cannabidiol (CBD), on the biology of cultured human skin cells. All cannabinoids tested suppressed viability (MTT assay) and induced mostly apoptosis-driven cell death (series of fluorimetric assays) of human epidermal HaCaT keratinocytes and human immortalized SZ95 sebocytes. Using a combined pharmacological and molecular-cell biological approach, we have furthermore shown that both AEA and 2-AG stimulated lipid production of SZ95 sebocytes, a hallmark of sebocyte differentiation and hence a model of holocrine sebum production (fluorimetric Nile Red assay). Of further importance, these actions were mediated by the metabotropic CB2 cannabinoid receptor and intracellular signaling mechanisms involving e.g. the MAPK system and various transcription factors. In contrast, CBD did not induce lipid production in SZ95 sebocytes. However, at non-cytotoxic doses, it intriguingly inhibited lipid synthesis induced by AEA, 2-AG or other lipogenic factors. Finally, we have also found evidence that the sebostatic action of CBD is most likely mediated by the opening of one of the transient receptor potential vanilloid (TRPV) ion channels, i.e. TRPV4, expressed on these cells, and the concomitant Ca^{2+}-influx. Taken together, our findings strongly suggest that modification of ECS activity exerts profound yet differential effects in the regulation of growth, survival and differentiation (lipid synthesis) of various skin cells. Therefore, pharmacological targeting of certain ECS elements may have a therapeutic value in the management of such a common skin disorder as acne vulgaris.

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MicroRNA-31 regulates a complex program of gene expression during murine hair cycle and associated tissue remodeling

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The hair follicle is a cyclic biological system that progresses through stages of the growth, regression and quiescence, which involves dynamic changes in its gene expression programs. MicroRNAs are small non-coding RNAs, which are critically important for the control of gene expression and silencing. Here, we show that miR-31 expression in mouse skin is markedly increased during anagen and decreased during catagen and telogen stages of spontaneous and induced hair cycle. Administration of anti-sense miR-31 inhibitor into the skin during the early- and mid-anagen phase resulted in acceleration of anagen development accompanied by the alterations in keratinocyte differentiation and hair shaft formation. To determine the putative miR-31 targets, primary epidermal keratinocytes were transfected with either anti-mir-31 or pro-mir-31. miR-31 inhibition resulted in the increase of mRNA and protein levels of Fgf10, selected components of Wnt and BMP signalling pathways (Sclerostin, Bambi) and Dlx3 transcription factor. Alterations in the miR-31 activity also resulted in changes of the expression of Krt14, Krt16, and Krt17 transcripts and proteins *in vivo* and *in vitro*. To validate whether miR-31 directly regulate expression of these keratins, a luciferase reporter assay was conducted. In HaCaT cells, miR-31 strongly inhibited the luciferase activity from the reporter constructs containing the 3'UTR segment of Krt16 and Krt17, but not Krt14. Thus, miR-31 can negatively regulate the expression of Krt16 and Krt17 transcripts, which represent the genuine miR-31 targets. Collectively, these data suggest a role of miR-31 in the control of complex program of gene expression during hair cycle, which includes a direct regulation of the Krt16 and Krt17 expression, as well as indirect effects on the expression of the growth regulatory molecules that control anagen development and hair shaft formation.

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Role of Sostdc1 in hair follicle and mammary gland development

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Sostdc1 (Ectodin, Wise, Usag-1) is a secreted protein known to regulate both Wnt and Bmp signaling. Modulation of Wnt activity is thought to be mediated by the ability of Sostdc1 to interact with Lrp6, which may lead to either repression or stimulation of Wnt signaling. Inhibition of Bmp signaling by Sostdc1 is brought about by its ability to bind several Bmps with high affinity. Sostdc1 is expressed in a number of skin appendages including developing vibrissae and hair follicles, mammary glands, and teeth. In teeth, loss of Sostdc1 affects the morphology of molars and also leads to formation of supernumerary teeth. Little is known about the function of Sostdc1 in other ectodermal organs, but it has been proposed to be one of the key mediators of the nuclear receptor corepressor Hairless during hair follicle regeneration. Here we report the hair and mammary gland phenotype of Sostdc1 null mice. Absence of Sostdc1 led to enlarged and/or ectopic hair, vibrissa, and mammary placodes. Surprisingly, the adult hair phenotype was not grossly affected, and hair follicle cycling appeared normal. The enlarged mammary buds of Sostdc1 -/- embryos were associated with ectopic Wnt activity, and in the adult, hair follicles were found in the nipple area which is normally devoid of hair follicles.

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"Tonic inhibition" of human and mouse skin mast cell functions *in situ* by endocannabinoids and cannabinoid receptor 1 (CB1)-mediated signaling

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Neuroendocrine controls are increasingly appreciated as important modulators of skin MC functions. This includes exo- and endocannnabinoids. We have previously shown that locally produced prototypic endocannabinoids (e.g. anandamide, AEA) markedly inhibit the growth of organ cultured human hair follicles (HFs) via CB1 (Telek et al, FASEB J 2007). Since perifollicular MCs are important regulators of murine hair growth, we have now investigated the role of CB1 signaling in normal skin MCs, exploiting organ cultured human HF which is rich in c-Kit+ and CB1+ MCs within its connective tissue sheath (CTS). While AEA and the CB1-specific agonist ACEA did not change CTS-MC number or its degranulation pattern, the CB1-specific antagonist AM251, as well as CB1 knockdown in human HF organ culture significantly up-regulated the number of CTS-MCs and stimulated their degranulation. The same was seen in CB1 knockout mice. Finally, CB1 agonists counteracted the activating effects of MC secretagogues on CTS-MC in situ. These data suggest that normal skin MCs have an important, novel "inhibitory tones" by endocannabinoids and CB1 signaling is crucial not only for avoiding excessive MC stimulation, but also excessive MC maturation from resident precursors. Furthermore, we provide the first evidence that routine gene knockdown technology also works in an intact, human organ *ex vivo* and illustrate that the CTS of human HFs offers an excellent, physiologically and clinically relevant model system for investigating and manipulating the biology of human skin MCs within their natural tissue context.

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Identification of human ABCB5$^+$ dermal progenitor cells with multipotent differentiation plasticity

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Skin stem cells posses promising therapeutic potential. Here we report identification of a novel skin-associated cell population based on expression of the ATP-binding cassette transporter, ABCB5, which is found in and can be isolated from the dermis of healthy humans or human patients. ABCB5$^+$ skin cells reside in the reticular dermis, can co-express the stem cell marker CD133, and are distinct from CD31$^+$ stromal cells and CD34$^+$ dermal cells. Comparative analysis of early developmental and lineage-specific gene expression patterns demonstrated ABCB5$^+$ dermal cells to be distinct from mature human fibroblasts, and to exhibit the more primitive molecular phenotype of human fibroblast-derived induced pluripotent stem (iPS) cells, and of human embryonic stem (ES) cells, with respect to down-regulated expression of vascular endothelial differentiation markers. In differentiation assays, purified ABCB5$^+$ dermal cells were capable of giving rise to all three embryonic lineages (ectodermal, mesodermal and endodermal) *in vitro*. Moreover, in a human to mouse skeletal muscle injury xenotransplantation model, human ABCB5$^+$ dermal cells possessed the capacity to differentiate into human spectrin- and delta-sarcoglycan-positive skeletal myofibers and to contribute to skeletal muscle regeneration *in vivo*. Interestingly, while ABCB5$^+$ dermal cells could be consistently detected in the skin of healthy humans of all ages, a significant decline in ABCB5$^+$ cell frequency was observed in older individuals. Thus, ABCB5 expression defines a novel dermal progenitor cell population in human skin that posseses multipotent differentiation plasticity. These results suggest a physiological role of ABCB5$^+$ progenitor cells in tissue repair and regeneration. Moreover, they point to potential therapeutic utility of purified ABCB5$^+$ dermal cells in regenerative medicine.

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Regulation of Shh and Sox9 by Trps1 in the developing murine hair follicle

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Trichorhinophalangeal syndrome I (TRPS1) is a vertebrate transcription factor containing a GATA-type zinc finger and two Ikaros-like zinc fingers. Patients with TRPS I have sparse scalp hair in addition to craniofacial and skeletal abnormalities. Trps1 is expressed in the mesenchyme-derived dermal papilla and the highly proliferative epithelial cells of adult mouse hair follicles. Mice in which the GATA domain of Trps1 has been deleted (*Trps1*$^{\Delta gt/\Delta gt}$) have a reduced number of pelage follicles and lack vibrissae follicles postnatally. During embryogenesis, we found that *Trps1*$^{\Delta gt/\Delta gt}$ mice have a reduced number of vibrissae follicles that are irregularly-spaced and smaller than wild-type vibrissae, which degenerate by birth. Immunofluorescence analyses revealed that *Trps1*$^{\Delta gt/\Delta gt}$ vibrissae exhibit increased proliferation and decreased expression of Shh. Furthermore, we found that expression of *Sox9*, a transcription factor required for specification of hair follicle stem cells whose expression depends on Shh signaling, was upregulated in *Trps1*$^{\Delta gt/\Delta gt}$ whisker pads during early morphogenesis. Histologically, *Trps1*$^{\Delta gt/\Delta gt}$ follicles share striking similarities with *Shh*$^{-/-}$ and *Gli2*$^{-/-}$ follicles, most notably a reduction in follicle number and arrest shortly after induction. We demonstrate that Trps1 and Shh colocalize in the matrix and inner root sheath of vibrissae follicles in *Shh*$^{Ires\text{-}LacZ}$ reporter mice. Quantitative RT-PCR analyses revealed reduced expression of multiple Shh pathway transcripts in *Trps1*$^{\Delta gt/\Delta gt}$ whisker pads. We also provide evidence that Trps1 forms stable complexes with Gli2 in co-immunoprecipitation assays. Finally, we demonstrate that SOX9 binds to the *TRPS1* promoter and intron 1 in an endogenous chromatin immunoprecipitation experiment, suggesting that Sox9 may in turn regulate *Trps1* expression. Our findings uncover a transcriptional hierarchy in mouse vibrissae including Trps1, Shh and Sox9 that controls the specification of early follicle progenitors and their subsequent proliferation.

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Dlx3 expression in neural crest-derived cells is required for normal hair and tooth development

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The homeodomain transcription factor Dlx3 is expressed in hair, teeth and bone during embryonic development. Mutations in the DLX3 gene are etiologic for Tricho-Dento-Osseous (TDO) syndrome, an ectodermal dysplasia characterized by defects in hair, teeth and bone. Dlx3 is expressed in post-migratory neural crest, and all three organs affected in TDO syndrome receive contribution from neural crest cells for their development. Dental mesenchyme and craniofacial bone have long been known to be neural crest derived, and more recent studies have identified epidermal neural crest stem cells in the hair follicle. We deleted Dlx3 in the neural crest by mating Dlx3flox/flox mice with Wnt1-cre mice. The conditional knockout mice grow hair but exhibit a disheveled coat, kinky vibrissae and sparse hair on the head. Analysis of the composition if the coat reveals a reduction in the proportion of zigzag hairs which are derived from the third wave of hair follicles, suggesting that Dlx3 expression in the neural crest is required for their differentiation. Histological analysis shows that hair follicles are not affected in their morphogenesis, but fail to enter anagen after the first telogen. At the tooth level, the absence of Dlx3 in the dental mesenchyme results in impaired polarization and differentiation of odontoblasts that fail to express dentin sialophosphoprotein, an essential determinant of dentin mineralization. The dentine is hypoplastic and almost absent from the lingual side of the incisors and the root processes of the molars, resulting in brittle teeth. Collectively, these data demonstrate that Dlx3 expression in the neural crest is required for the normal development of hair and tooth. While several studies previously described tooth phenotypes in neural crest conditional knockout models, to our knowledge, this is the first example of a gene deletion in the neural crest that results in impaired hair development.

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The miRNA-processing enzyme *Drosha* is required for hair follicle regression, hair shaft differentiation, long-term maintenance of hair follicle stem cells, and epidermal homeostasis

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Deletion of the miRNA-processing enzyme Dicer in mouse skin causes failure of hair follicle morphogenesis and maintenance, suggesting key roles for miRNAs in these processes. Dicer cleaves dsRNA and may possess additional non-miRNA-related functions. To determine the extent to which *Dicer* mutant skin phenotypes result from defects in miRNA processing, we investigated the requirements in epidermis and hair follicles for the nuclear RNaseIII endonuclease Drosha, an independent enzyme in the miRNA biogenesis pathway. We utilized *K5-rtTA tetO-Cre Drosha*$^{fl/fl}$ mice in which *Drosha* deletion can be induced in basal epidermis and hair follicle outer root sheath, including stem cells, by dosage with doxycycline. Inducible deletion of *Drosha* in late embryogenesis or in postnatal life reduced the expression levels of miRNAs such as miR-24, miR-203, and miR-205 that are normally highly expressed in the hair follicles and epidermis. Induced *Drosha* deletion during the anagen hair follicle growth phase caused failure of programmed regression (catagen) and absent expression of FGF5, which is required for the anagen-catagen transition. Long-term *Drosha* depletion resulted in defective expression of hair follicle differentiation markers, aberrant hair shaft production, and gradual loss of the stem cell marker K15. By contrast, *Drosha* mutant epidermis displayed thickening, hyperproliferation, and expanded expression of K14, p63, K10, and involucrin. These data demonstrate multiple requirements for *Drosha* in the anagen-catagen transition, hair shaft differentiation, long-term maintenance of hair follicle stem cells, and epidermal homeostasis. Defects in *Drosha*-deficient skin parallel those observed in constitutive and inducible *Dicer* mutants, suggesting that they result predominantly from miRNA depletion.

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Effect of the Lexington LaserComb on hair regrowth in the C3H/HeJ Model of Alopecia areata

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Alopecia Areata (AA) is one of the most common forms of alopecia, with a prevalence of nearly 2 percent in the population. The pathophysiology of AA is characterized by the infiltration of CD4+ and CD8+ T lymphocytes at the site of injury. The C3H/HeJ mice have been used as a model of AA. We have previously demonstrated that AA can be induced prematurely and locally by topical heat application in C3H/HeJ mice. Laser therapy administered at dermatology clinics, such as the 308nm Excimer Laser, has already been used in the treatment of AA with some success. In this study, we tested whether the HairMax LaserComb (Lexington International, LLC), a device used to treat androgenetic alopecia at home, may provide an alternative treatment for AA. To test this hypothesis, C3H/HeJ mice with heat-induced localized AA were treated with the HairMax LaserComb at the site of hair loss. Fourteen C3H/HeJ mice with heat-induced AA were used in this study. Two mice were used for histopathology to confirm the AA phenotype. The remaining 12 mice were randomized into two groups: group I was treated with the HairMax LaserComb for 20 seconds daily, three times per week for a total of six weeks; group II was treated similarly with the laser turned off. Group I showed hair regrowth two weeks after initiation of laser treatment, and complete hair regrowth in the alopecic lesion at six weeks. In contrast, mice in group II exhibited no regrowth of hair. Histopathological analysis confirmed these findings. Our results indicate that HairMax LaserComb was an effective treatment for heat-induced AA in C3H/HeJ mice. Our findings should be extrapolated to the treatment of AA in patients because the HairMax LaserComb is user friendly and non-invasive, which could translate to increased patient compliance and improved efficacy.

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Inductive signaling from the dermal papilla of the hair follicle

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The activity of keratinocytes in the hair follicle is regulated in part by signals from the dermal papilla. Mice expressing cre-recombinase in the dermal papilla were developed to probe the interaction between follicular keratinocyte populations and the dermal papilla in vivo. By ablating genes required for transduction of signals impinging on the DP and characterizing the resultant changes in DP gene expression and keratinocyte behavior, we have begun to unravel the complex feedback between these two compartments that controls follicular regeneration and cycling, and the size, shape and growth rate of the hair generated during the anagen phase. For example, inactivation of the β-catenin gene within the dermal papilla of fully developed hair follicles results in changes in gene expression in the DP that dramatically reduce proliferation of the progenitors that generate the hair shaft and IRS and lead to premature induction of the destructive phase of the hair cycle (catagen). Although β-catenin activity is not required in the dermal papilla during the catagen and telogen phases, regeneration of the cycling follicle is blocked in the absence of β-catenin in the DP. DP-specific blockade of other signaling pathways and transcriptional regulators result in equally dramatic effects. Progress in deciphering the feedback loops that govern hair morphogenesis and cycling will be discussed.

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Adult human epithelial stem cells are a novel target for thyroid hormone regulation *in situ* and *in vitro*

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The hormonal controls of human epithelial hair follicle stem cells (HFSCs) are still largely unknown. Since the HFSCs overexpress deiodinase-2, which activates thyroid hormone (TH) by converting T4 to bioactive T3, we assessed the effects of T3 and T4 on HFSCs *in vitro* and *in situ*. Here we demonstrated that physiological concentrations of T3 and T4 enhanced, first, the expression of cytokeratin 15, and secondary of CD200 (both key HFSC markers) in adult organ-cultured human skin. Accompanied, we found an up-regulation of K14, leading to proliferation of basal and suprabasal keratinocytes. We were able to show, that via transfected Cytokeratin 15 (K15)-promoter-driven GFP *in situ* demarcated or isolated human HFSCs underlie profound regulation by TH *in situ* and *in vitro*. In isolated and organ.cultured HF T3/T4 strongly enhanced the K15-promotor controlled GFP and CD200 expression in the stem cell rich HF bulge region. In the presence of T3/T4 isolated K15-GFP+ cells demonstrated reduced colony forming efficiency, proliferation, cell number, and viability but an significantly enhanced apoptosis. In addition, K15-GFP+ cells strongly up regulated the epithelial-mesenchymal-transition marker vimentin, assume a fibroblastoid morphology and up regulated POMC expression under T3/T4 stimulation *in vitro*. These data provide novel insights into (neuro-)endocrine controls of normal human epithelial progenitor/stem cells in general, and illustrate how primary human K15-GFP+ cells can be used to further characterize these controls *in situ* and *in vitro*.

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The nude mutant gene *Foxn1* is a HOXC13 regulatory target during hair follicle and nail differentiation

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Because of the remarkable overt phenotypic parallels between *Hoxc13* gene-targeted *(Hoxc13^{tm1Mrc})* and *Foxn1^{nu}* (nude) mutant mice, we sought to determine whether *Hoxc13* and *Foxn1* might act in common pathways of hair follicle (HF) differentiation. By performing comparative histopathological analyses, we show that the alopecia exhibited by both the *Hoxc13^{tm1Mrc}* and *Foxn1^{nu}* mice is due to strikingly similar defects in hair shaft differentiation and that both mutants suffer from a severe nail dystrophy. These phenotypic similarities are consistent with the extensive overlap between *Hoxc13* and *Foxn1* expression patterns in the HF and the nail matrix. Furthermore, DNA microarray analysis of skin from *Hoxc13^{tm1Mrc}* mice identified *Foxn1* as significantly down-regulated along with numerous hair keratin genes. This down-regulation apparently reflects the loss of direct transcriptional control by HOXC13 as indicated by our results obtained through co-tranfection and chromatin immunoprecipitation (ChIP) assays utilizing C2C12 cells. Combined, these data support a regulatory model of keratinocyte differentiation in which HOXC13-dependent activation of *Foxn1* is part of a regulatory cascade controlling the expression of terminal differentiation markers.

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The ΔNp63 isoform is an essential regulator of epithelial development and stem cell renewal

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The epidermis is a stratified epithelium, whose development and maintenance is dependent on the intricate balance between the proliferation and differentiation of resident stem/progenitor cells. The transcription factor p63 is important in the development of the skin as p63 null mice exhibit striking defects in embryonic epidermal morphogenesis. However, understanding the mechanisms that underlie this phenotype has been complicated by the lethality of p63 null mice and the existence of multiple p63 isoforms including TAp63 and ΔNp63. The fact that all isoforms of p63 are absent in the p63 knockout mouse models has precluded studies on the biological role of individual p63 proteins, in particular the ΔNp63 isoforms, which are predominantly and highly expressed in the stem cell compartments of the skin and hair follicles. To investigate the role of ΔNp63 in epidermal morphogenesis and homeostasis, we have generated ΔNp63 knock-in mice where the ΔNp63 specific exon has been replaced by the Green Fluorescence Protein. This strategy allows us to follow ΔNp63 positive cells that mark the epidermal and hair follicle stem cell niches. ΔNp63 knockout animals exhibit severe developmental anomalies including truncated forelimbs and the absence of hind limbs, phenocopying prior knockout animals in which all isoforms of p63 are deleted. Interestingly, the ΔNp63 null animals completely lack a stratified epidermis yet display isolated clusters of disorganized epithelial cells along portions of the exposed dermis. Despite a failure to develop a stratified epidermis, the patches of ΔNp63 null keratinocytes are able to undergo a program of terminal differentiation. Our results suggest that in the absence of ΔNp63, keratinocytes are capable of committing to an epidermal cell lineage but likely suffer from diminished renewal capacity of stem/progenitor cells. The generation of ΔNp63-knock-in mice reaffirms the indispensable role of this isoform of p63 in skin biology and serves as a valuable resource to isolate and probe the functional properties of ΔNp63 enriched keratinocytes.

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Important role of hair follicle stem cells for continuous sebaceous gland renewal

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Mammalian epidermis is a highly dynamic tissue which comprises a multi-layered epithelium consisting of the interfollicular epidermis, the hair follicles (HFs) and associated sebaceous glands (SG). Functional sebaceous glands release sebum into the hair shaft and are important for barrier function and protection against pathogens and/or environmental assaults. The high turn-over of the SG requires a constant renewal of cells, suggesting stem or progenitor cells to be involved in SG homeostasis. Although there are implications that unipotent progenitors participate in SG regeneration, the cellular process of SG renewal and maintenance still needs to be elucidated. We have generated an inducible Cre-mouse model which enables us to track the fate of single HF-derived stem cells and to investigate sebaceous commitment *in vivo*. From these lineage tracing experiments we have learned that HF-derived progenitors continuously reconstitute SGs. This process takes place independent from hair follicle regeneration by HF stem cells during the hair cycle. Directing progenitor cells into sebocyte lineage by manipulating Lef1, one of the crucial regulators of tissue maintenance and differentiation, we could identify HF-derived stem cells as cells of origin resulting in abnormal hair follicles, cysts and *de novo* formed SG.

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A novel, complex enzymatic control of mammalian pigmentation: UDP-N-GlcNAc 1-phosphotransferase regulates *POMC* gene expression, tyrosinase sorting and subsequent melanin synthesis *in situ* and *in vitro*

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The phosphorylation of mannose recognition residues on lysosomal enzymes, essential for endosomal/lysosomal targeting, is catalyzed by the UDP-GlcNAc 1- phosphotransferase. This is composed of three homodimers, α2β2γ2, encoded by two separate genes (*GNPTAB* and *GNPTG*). Defects of GNPTAB prevent or impair the formation of recognition residues, followed by missorting of lysosomal enzymes, and induce the lysosomal storage disease mucolipidosis (ML) II. Interestingly, besides excessive lysosomal storage, skeletal dysplasia and neurodegeneration, these patients show thickening of the skin and bright-blonde hair. In order to explore the largely unknown GNPTAB functions in human skin, we have silenced *GNPTAB* by siRNA transfection in organ-cultured adult human scalp hair follicles (HF) and isolated human HF melanocytes. This was complemented by examining the skin of knock-in mice engineered to overexpress ML II patient-specific mutated *gnptab*. These studies revealed that *GNPTAB* knock-down substantially reduced human HF melanin content and tyrosinase activity *in situ*. In isolated human HF melanocytes, *GNPTAB* knock-down reduced *POMC* gene and protein expression. The melanogenesis key-enzyme, tyrosinase, was missorted into the culture media and had reduced activity, whereas gp100 was unaffected. The knock-in mice showed a HF cycling blockade, along with impaired HF pigmentation and absent tyrosinase activity, a thickened dermis, and a dystrophic subcutis. Besides revealing a completely novel, complex enzymatic control of mammalian pigmentation by UDP-N-GlcNAc 1-phosphotransferase, the human and murine models employed here provide excellent tools to investigate the full range of functions of this phosphotransferase in mammalian biology under physiologically relevant conditions.

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Endocannabinoids regulate proliferation, differentiation, and survival of human sweat gland epithelial cells

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Recent findings suggested that certain members of the endocannabinoid system (ECS) are expressed *in situ* in the human sweat gland. In the current study, we investigated the functional properties of the ECS using the human eccrine sweat gland-derived cell line NCL-SG3. Expressions of both cannabinoid receptor subtypes (CB1, CB2) as well as of those enzymes which are involved in the synthesis and degradation of the endocannabinoids were unambiguously identified in NCL-SG3 cells (Western blot, Q-PCR). In addition, mass spectrometry analysis revealed that sweat gland cells produce the prototypic endocannabinoids anandamide (AEA) and 2-arachidonylglycerol (2-AG). Of further importance, these endocannabinoids dose-dependently suppressed the viable cell number and proliferation of NCL-SG3 cells (MTT assay), and induced mostly apoptosis-driven cell death (series of fluorimetric determinations). We also found that these actions were accompanied by the onset of the differentiation process of NCL-SG3 cells since both AEA and 2-AG significantly elevated expressions of late whereas suppressed levels of early differentiation markers (Q-PCR). Likewise, endocannabinoids markedly stimulated lipid synthesis of NCL-SG3 cells (Nile Red fluorimetry), another hallmark of differentiation. Intriguingly, these effects were not abrogated by either pharmacological or siRNA-mediated inhibition of CB1 and CB2 suggesting the lack of involvement of these receptor. However, since both AEA and 2-AG induced a marked phosphorylation of the MAPK Erk1/2; and, furthermore, since the MAPK inhibitor PD098059 prevented the above effects of the endocannabinoids, the MAPK pathway seems to be involved in mediating their actions. Taken together, our findings strongly suggest that a functional ECS is expressed in human sweat gland epithelial cells and that locally produced endocannabinoids exert profound effects in the regulation of growth, survival and differentiation of these cells.

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Inducible deletion of β–Catenin reveals essential roles in adult hair follicle proliferation and maintenance, and epidermal homeostasis

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β-catenin plays central controls in the development of skin appendages. However the precise functions of β-catenin in postnatal epidermal and appendage tissue homeostasis remain unclear. To determine whether β-catenin is required for maintenance of the epidermis and hair follicles in adult life, we generated Krt5-rtTA tetO-Cre Ctnnb1fl/fl mice in which deletion of β-catenin in basal epidermis and hair follicle outer root sheath, including stem cells, can be induced by treatment with oral doxycycline. To ask whether β-catenin is required for anagen onset, deletion was induced during telogen, and hair shafts were plucked to initiate a synchronized hair follicle growth cycle. Unlike in controls, hair follicle stem cells failed to proliferate in mutant skin and the follicles did not enter anagen. Deletion of β-catenin in early to mid anagen caused cessation of matrix cell proliferation, rapid regression of the matrix, and separation of epithelial cells from the dermal papilla. Expression of the bulge stem cell marker K15 was maintained during short periods of β-catenin deficiency, indicating that these phenotypes were not due to immediate loss of stem cells. However, following longer periods of β-catenin depletion, K15-positive, label retaining stem cells disappeared, follicular epithelium degraded, and cysts were formed in the dermis. By contrast, the epidermis was maintained, but displayed hyperproliferation, basal layer expansion, abnormal differentiation, and defects in cell-cell adhesion. These data reveal multiple distinct roles for β-catenin in regulating hair follicle stem and matrix cell proliferation and maintenance; follicular structural integrity; and epidermal homeostasis.

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Extrinsic regulation of hair follicle cycling

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The skin epithelium maintains tissue homeostasis and regeneration through dynamic interactions with multiple cell types in the underlying dermis, including fibroblasts, blood vessels, and adipocytes. However, despite the importance of these cellular interactions in the skin, the mechanisms that mediate cellular crosstalk during skin homeostasis and regeneration are not well understood. To define how cells within the skin epithelium interact with cells in the dermis, we have analyzed the biology and function of subdermal adipocytes during skin tissue homeostasis. Subdermal adipocytes compose a unique white adipose tissue depot that underlies the dermal fibroblasts and surrounds the hair follicle during hair growth. Recent data suggest that subdermal adipocytes express signaling molecules that regulate hair follicle cycling, but the role of these cells in the skin in not known. Our data show that a critical interplay exists between hair follicle stem cells and subdermal adipocytes. During regeneration and stem cell activation in the murine hair follicle, adipogenesis characterizes changes in the skin tissue microenvironment through *de novo* formation of subdermal adipocytes. We find that defects in subdermal adipocytes modulate the activity of murine adult follicular stem cells. Furthermore, epithelial stem cell activity controls subdermal adipogenesis. These data implicate adipocytes as niche cells for epithelial stem cells and define a vital crosstalk between adipocytes and adult epithelial stem cells in murine skin.

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Overexpression of microRNA miR-31 in mouse skin alters hair growth

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microRNAs are crucial regulators of gene expression networks. They have been implicated in many aspects of skin biology including the formation and maintenance of hair follicles. However, the role of the majority of the microRNAs expressed in the skin is unknown. Here, we show that microRNA miR-31 is associated with the wound healing process, photoaging, and skin carcinogenesis. To build a model for miR-31 overexpression and to explore the significance of miR-31 in skin biology, we have generated a doxycycline-inducible mouse model of miR-31 expression. miR-31 expression can be elevated up to 500-fold after maximal induction with doxycycline. These are levels of up-regulation that can be observed in human skin cancer, wound healing and photoaging. Postnatal induction of transgenic miR-31 expression causes a ragged fur phenotype compared to the smooth appearance of the fur of control littermates. However, the expression of the human target gene RhoA is unaltered indicating that RhoA may be a human specific target. Induction for more than a month of miR-31 in these transgenic mice does not cause aberrant growth in the epidermis. miR-31 expression is frequently up-regulated in human skin in response to stress or abnormal growth control. Our miR-31 transgenic mouse model will be a valuable tool to identify the significance of miR-31 during these processes and in the regulation of hair growth.

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Negative regulation of Shh expression in a model of the RAS/MAPK syndromes

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RAS/MAPK syndromes are a family of congenital syndromes, which are associated with germline activating mutations in RAS or their downstream effectors and characterized by multiple skin and hair abnormalities. Recently, we studied the effects of activating a single allele of RAS in the mouse skin to understand the pathogenesis of RAS/MAPK syndromes. These mice develop redundant skin, short hair, papillomas, and later hair loss, much like their human counterparts. Here we demonstrate that hair defects in this RAS gain-of-function mouse model are associated with a five-fold reduction in expression of a key developmental regulator, Sonic hedgehog (Shh), and downstream targets. To further understand the molecular basis for this inhibitory effect on Shh, we developed a skin explant system from normal mouse skin. Surprisingly, we found that Shh response to growth factor stimulation (i.e. FGF2, EGF, PDGF) in the skin was extremely rapid, 30 to 90 minutes and dose-dependent. Moreover, pharmacologic inhibition of RAS/MAPK signaling resulted in 2 to 3-fold increase in Shh. Results presented here suggest that RAS/MAPK signals affect Shh transcription through HDAC-independent mechanisms. The contributions of candidate transcription factors of the Ets-family, Etv4/5, are further explored in this novel regulatory pathway. In summary, these findings from rare diseases in humans and mouse models reveal an important role of RAS/FGF signals in modulating hair and skin phenotype. Our findings further suggest that RAS signal, particularly FGF, regulate morphogenesis via transcriptional modulation of Shh levels.

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Remodeling of hair-follicle associated lymphatic vessels is dependent on hair follicle development and hair cycle dependent changes in murine skin

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Hair follicle-associated vascular and neuronal networks undergo extensive remodeling throughout the hair cycle and in hair development. Lymphatic vasculature is a key component of the dermal architecture. The lymphatic system is an open-ended system that maintains fluid homeostasis and is essential for the immune response to infectious agents. Preliminary work revealed an intimate association of lymphatic vessels with the human and murine hair follicle. However it is unknown whether lymphatic architecture changes with skin remodeling during hair follicle development or the hair cycle. Therefore we quantified changes in hair follicle-associated-lymphatics in the C57BL/6J mouse. Dorsal skin samples were harvested from neonatal mice on days 1 (birth), 3, 5, 7, 8, 10 and 17 (initiation of first hair cycle). To quantify lymphatic changes during the hair cycle, synchronous anagen hair growth was initiated by wax depilation in day 42 mice. Dorsal skin samples were harvested on days 1, 3, 5, 7, 8, 12, 18, 19, 20, and 25 post-depilation. Skin samples were processed and immunostained with the lymphatic marker, LYVE 1 and PGP9.5, a pan-neuronal marker. All images were captured using the Cytoviva dual-mode fluorescence/dark field microscope system. (Cytoviva, Inc., Auburn, AL). ANOVA analysis of lymphatic vessel measurements (maximum length and width) revealed significant changes associated with hair follicle development as well as with the dynamic changes in the hair follicle throughout the hair cycle. The changes in hair follicle-associated lymphatic vessels reported here will improve our understanding of the inter-dependent triad of angiogenesis, lymphangiogenesis and neurogenesis and given the role of lymphatics in the immune response, could provide unique insights into our understanding of the immune privilege of the hair follicle and/or loss thereof in the disease state.

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Age, gender and ethnic variations in sebaceous lipids

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Different skin types have been reported to have different levels of skin hydration and barrier function, as documented with instrumental measurements of conductance and trans epidermal water loss (TEWL). The factors that account for these differences are unclear. Since sebaceous lipids are important to these functions, we compared the lipid components of sebum from three ethnic backgrounds: Caucasian, African-American, and Northern Asian individuals. Females, (18-25 and 35-45 years old) with no acne were evaluated for skin surface hydration and barrier function. Skin surface lipids were analyzed following sebum collection using sebutapes. Significant differences ($p<0.05$) were documented in skin hydration between African-American and Caucasian panelists in both age groups, with African-American > Northern Asian > Caucasian. African-American and Caucasian panelists significantly differ ($p<0.05$) in their TEWL, with the trend being the inverse of the hydration trend: Caucasian > Northern Asian > African-American. These data indicate a superior barrier function for African Americans. African Americans females produced more facial lipids than the other two ethnic group females, with African-American > Northern Asian > Caucasian. When analyzing the three lipid classes (re fatty acids, triglycerides, and wax esters), the trend became significant ($p<0.05$) in the wax ester fraction when comparing African-Americans to Caucasians directly. Additionally, seven lipids were identified in the wax ester fraction that were significantly different in quantity ($p<0.05$) between African-Americans and Caucasians. These data suggest the differential lipid composition in ethnic groups might contribute to the observed differences in barrier function and hydration

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Suppression of DNA degradation by inactivation of DNase1L2 leads to increased fragility of hair

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Nuclear DNA is degraded during terminal differentiation of keratinocytes in the interfollicular epidermis and in the hair follicle. Yet the physiological function of this breakdown process is elusive. To investigate the role of the keratinocyte-specific endonuclease deoxyribonuclease 1-like 2 (DNase1L2), we generated DNase1L2 knockout mice and determined the properties of epidermis and hair. Degradation of nuclear DNA was normal in the interfollicular epidermis, but incomplete in the epithelium of the tongue and in hair and nail. Most strikingly, virtually all corneocytes of the hair fiber and the nail plate contained nuclear remnants. As determined by quantitative PCR, DNA was retained at more than hundred-fold increased amounts in hairs and nails of DNase1L2-deficient mice. Western blot analysis showed that incomplete degradation of DNA was associated with retention of histones in hair. Hairs of mutant and wildtype mice were exposed to mechanical stress in a bead mill, and the size distribution of the resulting fragments was determined. In two independent experiments involving hairs from 5 and 8 mice per group, DNase1L2-deficient hair was broken into a significantly higher number of fragments than wildtype hair. Correspondingly, the maximum length of fragments was significantly reduced by the presence of aberrantly high amounts of DNA. In conclusion, this study suggests that defective degradation of DNA in corneocytes decreases the mechanical resistance of hair. These results provide an unexpected link between chromatin, programmed cell death and mechano-protective functions of the epidermal appendages.

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Follicle stem cell compartments differ in responsiveness to oncogenic Hedgehog signaling

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Deregulated Hedgehog (Hh) pathway activation contributes to tumorigenesis in multiple tissues and organs. The exact cell populations in skin which are capable of responding to oncogenic Hh signals and give rise to tumors are not yet well defined. We have begun to address this question by driving expression of an activated form of GLI2 (GLI2*) in hair follicle stem cells of the bulge and secondary hair germ (SHG), a unique site of low-level, endogenous Hh signaling in resting (telogen) hair follicles. We generated mice combining Cre-inducible and doxycycline-regulated gene switch technologies to achieve exceptionally tight control of transgene expression in K15 positive hair follicle stem cells and their progeny. Activation of GLI2* expression for two weeks during the resting phase of the hair cycle yielded occasional microscopic tumors arising from hair follicles in dorsal skin. In striking contrast, induction of anagen by depilation yielded massive tumors affecting nearly all hair follicles during the same two-week time-frame, revealing the potent effect of stem cell activation on GLI2*-driven skin tumorigenesis. Examination of non-depilated mice four weeks after GLI2* induction revealed spontaneous macroscopic tumor development on paws, whisker pads, and tails. Histology of early lesions suggests that tumors are derived from the telogen SHG, even though GLI2* transgene was detected by immunostaining in cells in the bulge and SHG. Moreover, in situ hybridization revealed expression of Hh target genes Gli1 and Ptch1 in both stem cell compartments. These data reveal that hair follicle stem cells in distinct niches can respond differently to oncogenic Hh pathway activation. In contrast to 'primed' Hh-responsive stem cells located in the SHG, stem cells in the hair follicle bulge appear resistant to oncogenic Hh signaling during the resting phase of the hair cycle.

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The living wave: Self-organizing regenerative behavior of stem cells revealed in the cycling of large hair follicle populations

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Stem cells cycle through a number of activated states. Among large stem cell populations, stem cells may cycle randomly, synchronously or as a coordinated unit. Regenerative behavior of stem cells at this level has not been addressed. Using the experimentally tractable hair cycling paradigm, we develop the concept that stem cells are modulated by a combination of micro-environmental factors and macro-environmental factors to regulate regenerative behavior (Plikus MV, et al., 2008. Nature. 451:340-344). We now combine experimental and computational approaches to further study how these regenerative behaviors are coordinated. We found that activation of stem cells in individual follicles requires the integration of inputs from an intrinsic clock and extrinsic environmental signals. The wave patterns in the same animal of different physiological status, in transgenic mutants of the same species and in different species vary. They range from random (adult human scalp), slow waves (normal mouse), fast trasversing waves (K14 noggin mouse) to fractal-like waves (rabbit) and can be reset and restarted (pregnant mouse). We develop computer simulations using a Cellular Automata model in which each hair follicle is simulated as one automaton. We demonstrate that underlying self-organizing principles show wave patterns with stochastic, scale-invariant, and non-autonomous properties, conferring the hair regenerative system with robust adaptability. New experimental data show more cellular and molecular mechanisms involved in follicle coupling, and the experimental manipulation of parameters led to wave patterns predicted by model simulations. We developed a diagram which uses simple parameters to integrate the diverse wave patterns examined in mice, rabbits and humans.

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Generation of induced pluripotent stem cells by reprogramming human dermal papilla cells

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The dermal papilla (DP) is a unique adult stem cell population located at the base of the hair follicle, which we and others have shown to be capable of directed differentiation into other cell types such as muscle, bone and neurons. Due to its multipotency, we sought to analyze the efficiency of the DP, an easily accessible cell type, for reprogramming into induced pluripotent stem cells (iPS). We isolated and established cultures of DP and dermal skin fibroblasts (DF) from hair follicles taken from the occipital scalp region of human donors. At passage 3 in culture, both DP and DF express Klf4 and cMyc, two of the four iPS-inducing factors. However, unlike mouse dermal papilla, human cells do not express Sox2, *in vivo* or *in vitro*. We therefore introduced Oct4, cMyc, Klf4 and Sox2 into both DP and DF cultures using retroviral transduction. Colonies appeared at the same time, after 21 days in DP and DF cultures, and we were able to establish multiple iPS cell lines from the DP and DF cultures that contained all 4 transduced factors. We detected the expression of undifferentiated pluripotent markers in these cell lines, including SSEA3, Lin28 and nanog, using both RT-PCR and immunocytochemistry. Both human DP-iPS, and DF-iPS were able to differentiate into all three germ layers after embryoid body formation. We are currently analyzing the iPS forming efficiency of human DP and DF cultures using just two factors, Oct4 and Sox2 to complement the endogenous factors, Klf4 and cMyc, that are expressed in the DP. These studies provide insight into the accessibility and efficiency of DP cells as a source of iPS cells in humans, which could be useful in clinical applications due to their inherent plasticity and ease of access.

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Wnt/β-catenin- and depilation-induced activation of hair follicle stem cells requires chromatin regulatory protein Pygo2

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The Wnt/β-catenin signaling pathway is known to be important for hair follicle development and regeneration. Pygopus 2 (Pygo2), recently shown to associate with histone-binding and modifying activities, is a PHD finger-containing protein of the Pygopus family implicated as coactivators of β-catenin/TCF-dependent transcription. However, whether Pygo2 regulates Wnt/β-catenin signaling and hair follicle stem cell development and homeostasis remains to be elucidated. Here we show that Pygo2 is dynamically expressed in both the mesenchymal and epithelial components, including the stem cell compartment, of developing and adult hair follicles. To elucidate the *in vivo* function of Pygo2, we generated and analyzed skin epithelia-specific Pygo2 knockout mice (SSKO), as well as compound mutant mice that are deficient for Pygo2 and overexpressing an undegradable form of β-catenin (ΔN-β-catenin) mimicking activated Wnt signaling. Loss of epithelial Pygo2 does not affect normal hair follicle morphogenesis and cycling. In contrast, Pygo2 is required for ΔN-β-catenin overexpression-induced premature entry into the anagen phase of hair cycle by regulating hair germ cell activation. Furthermore, Pygo2 deficiency abolishes trichofolliculoma formation induced by ΔN-β-catenin overexpression. Our findings uncover an important role for Pygo2 in Wnt-signaling induced stem cell activation and tumorigenesis. Current experiments address the involvement of Pygo2 in depilation-induced hair follicle regeneration, and results of such study will be presented.

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Defining BMP functions in hair follicle stem cells homeostasis by conditional ablation or activation of BMP receptor 1A

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During the hair cycle, the behavior of hair follicle stem cells (hf SCs) is tightly governed by an intricate balance of signaling pathways which induce bouts of SC quiescence and activation, resulting in new hair. We have previously shown that bone morphogenetic protein (BMP) signaling is essential for hf SC homeostasis. However, precisely how BMP signaling functions in hf SCs at the molecular level still remains to be determined. Since the stem cell marker CD34, the only available marker for the isolation of hf SCs, is lost upon BMPR1A deletion we addressed this problem by employing, a Keratin 15 (K15)-driven system to simultaneously label and specifically target BMPR1A ablation within the hf SCs during the second postnatal telogen. We were able to isolate hf SCs marked by eYFP from control and BMPR1A knockout (KO) mice by fluorescence activated cell sorting (FACS) before morphological changes in hair cycle were observed. Additionally, we also utilize an inducible, gain of function system to investigate the consequences of active BMP signaling in hf SCs. Microarray analysis revealed that, following inhibition of BMP signaling in the bulge, BMPR1A KO hf SCs showed down-regulation of approximately 25% of common up-regulated hf SCs signature genes (Greco et al., 2009 and Blanpain et al., 2004). Furthermore, we also revealed that 30% of BMPR1A KO genes overlapped with the previously characterized hair germ signature (Greco et al., 2009). Here we employ both loss and gain of function approaches to address the role of BMP signaling in regulating hf SC homeostasis. Our findings suggest a model where balancing BMP signaling in hf SCs is essential in maintaining their homeostasis and its inhibition could be important to switch them from their quiescence to activation towards hair germ.

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Epigenetic organ regeneration: Development of a model using amputated mouse vibrissae follicles

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Mammalian organ regeneration is the ultimate goal of regenerative biology and medicine. The most dramatic example of organ replacement is known as 'epimorphic regeneration' and occurs mainly in amphibians, in which phenotypically committed cells are reprogrammed at the amputation plane toward a stem cell phenotype. The result is the complete regrowth of an amputated structure from an anatomically complex stump. Although mammals cannot regenerate amputated limbs, the regeneration of the hair follicle end bulb following amputation results in regeneration of the dermal papilla (DP) and the entire organ structure of the hair follicle, and can serve as a model to interrogate this process. To analyze the molecular events underlying this phenomenon, we adapted a mouse model, from previous work carried out in rat and human HFs. The mystacial pads of adult mice were incised to expose vibrissa follicles and end bulbs were excised. DP and end bulb regeneration was examined at several time points up to 21 days by histology and immunohistochemistry. We followed the formation of the new DP using molecular markers, which appears to be reprogrammed from the remaining dermal sheath after amputation. We observed pronounced cellular invasion of the mesenchyme around the amputated end bulb. This preceded the restoration of the DP which coincided with positive Prom-1 expression. Following expansion of epithelial cells down the hair shaft to below the level of the cut, regression and consolidation of a P-cadherin labeled basal epithelium above the newly formed basement membrane preceded differentiation of a new matrix. Similar to the molecular mechanisms involved in epimorphic regeneration in lower vertebrates, dedifferentiation of dermal sheath cells to progenitor/stem cells and their reprogramming to repopulate the DP may occur via the epigenetic reactivation of developmental regulatory genes that function during embryogenesis.

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c-myb ablation affects hair follicle homeostasis

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The transcription factor, c-myb, is required for hematopoiesis, and its knockout is lethal at e15 due to the lack of erythropoiesis. c-myb expression is essential for proliferation and prohibitive of differentiation in multiple blood lineages. We have previously determined that c-myb expression is induced *in vitro* in human keratinocytes released from an induced block in proliferation and that downregulation of c-myb expression is a late marker of calcium-induced differentiation. We also showed that c-myb is expressed in basal and suprabasal layers of the murine epidermis and that it is expressed in the follicle in a hair cycle-dependent manner. A c-myb hypomorphic mouse had been generated which resulted in a significant reduction of c-myb expression. These mice are often runted and have reduced viability, in addition to hematopoietic abnormalities. Targeted ablation of c-myb expression in the crypt cells of the colonic lumen, the niche of gut epithelium stem cells, results in retarded proliferation, disrupted differentiation and reduced crypt length. We examined the skin of c-myb knockdown mice and noted that they shed their hairs in an anterior to posterior wave between days 15 and 18. Hair loss occurred on both the dorsal and ventral surfaces between the neck and the tail and was cyclic. Knockdown mice undergo a new round of hair growth coincident with the next anagen at around day 40. Subsequent cycles are often characterized by transient patchy hair loss, which is sometimes permanent. Histologic analysis reveals that the number of follicles in knockdown mice is severely curtailed and the length of remaining follicles is diminished. K14 and K15 cre-mediated ablation of c-myb expression do not result in the visible wave of hair shedding characteristic of the c-myb hypomorph. However, the histology of the targeted mice and the hypomorph share some pathologic features as compared to wild-type controls, suggesting that the role of c-myb in maintaining hair follicle integrity is divided among distinct cell types.

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Keratin 74 is a novel determinant of human hair texture and is mutated in autosomal dominant woolly hair

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Woolly hair (WH) is an inherited hair shaft anomaly characterized by tightly curled hair that can display either autosomal dominant (ADWH) or recessive (ARWH) inheritance. We and others have recently shown that ARWH is caused by mutations in P2RY5 or LIPH genes. However, the molecular basis of ADWH has not previously been reported. In this study, we identified a Pakistani family with ADWH in which all affected individuals had short and tightly curled hair with normal hair density. The family showed linkage to chromosome 12q12-q14.1, containing the type II keratin gene cluster. We discovered a heterozygous mutation N148K within the helix initiation motif of the keratin 74 (KRT74) gene in all affected family members. KRT74 encodes the inner root sheath (IRS)-specific epithelial (soft) keratin 74. We demonstrate that the mutant K74 protein results in disruption of keratin intermediate filament formation in PtK2 cells, most likely in a dominant-negative manner. Furthermore, to test whether mutations in the corresponding murine Krt74 gene might underlie a new mouse phenotype, we sequenced the mouse Krt71-74 genes in the dominant Caracul-like 4 (Cal4) allele which is characterized by wavy coat phenotype and maps to the same region of mouse chromosome 15 as other Caracul (Ca) alleles. We identified a novel heterozygous mutation E440K not in Krt74, but in the neighboring gene, Krt71. Krt71 was previously reported to harbor other Ca mutations, as well as coding SNPs that are associated with curly-coated dogs. In this study, we provide the first genetic evidence for a phenotype resulting from a mutation in a hair follicle-specific epithelial keratin in humans. Our findings further underscore the crucial roles of the IRS-specific epithelial keratin genes Krt71-74 not only in hair disorders, but open the possibility that these genes may function as genetic determinants of normal hair texture variation across mammalian species.

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Differences in mean current intensity required to evoke sensation in c-fibers in the scalp of Alopecia areata subjects and normal controls

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Alopecia areata (AA) is an immune mediated hair disease. The role of the nervous system in AA has long intrigued researchers. Previously, we demonstrated that functional differences in C fiber perception exist when comparing the affected ophiasis and unaffected non-ophiasis scalp of subjects with patchy AA. In this study, we sought to investigate nerve function in the scalp of control and AA subjects to determine if these differences were specific for AA. A total of 51 subjects were enrolled, 20 AA subjects (8M, 12F) and 31 controls (11M, 20F). Transcutaneous electrical stimulation was applied to the ophiasis scalp of AA subjects and the frontotemporal and ophiasis scalp of controls. Stimuli were generated at three frequencies:2kHz, 250 Hz, and 5Hz, allowing the assessment of myelinated ABeta (touch) and ADelta fibers (fast pain) and unmyelinated C fibers (slow pain). Current perception thresholds (CPTs) represented the minimum current intensity needed for sensation perception. Two-sided paired t-tests compared CPTs collected from AA subjects and controls. In control subjects, results at 2000Hz, 250 Hz, and 5Hz were not statistically significant, suggesting that sensory perception of the scalp is not location dependent. However, paired t-test evaluation comparing CPTs from AA subject affected ophiasis scalp and control ophiasis scalp was statistically significant ($p=0.006$) at 5Hz (C fibers), 226 microAmps (SD±128) and 116 microAmps (SD±138microAmps), respectively, demonstrating that C fibers require more stimulation to evoke sensation in AA subjects when compared to normal control ophiasis scalp. These results demonstrate that C fiber dysfunction is part of the pathophysiology of AA and specific for the AA disease process.

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Reconstitution of human hair follicles using fetal epidermal and dermal cells

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Reconstitution of hair follicles has been established by using dissociated cells from murine skin, but a reconstitution assay for human hair follicles is still lacking. The aim of our study was to develop a reconstitution assay for human hair follicles. We used human fetal scalp, 16 to 18 weeks gestational age, to provide the starting cell populations for hair follicle reconstitution. Epidermis and dermis were separated and single cells isolated from scalp tissue. The cells were recombined and injected into the back skin of immunodeficient mice. 3 weeks after injection, hair germs were evident. By 5 weeks, fully formed hair follicles and pigmented hair shafts were visible. The regenerated hair follicles contained all follicular epithelial lineages. The human origin of the regenerated follicles was confirmed using in situ hybridization for Alu DNA repeats specific for human. To test whether Matrigel facilitates survival and growth of the cell grafts, we added Matrigel to the suspension of cells before injection. We found that Matrigel rescued trichogenicity and resulted in a higher number of regenerated hair follicles.

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Suppression of Wnt/ β-catenin pathway maintains melanocyte stem cells

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Adult melanocyte stem cells (McSCs) are maintained in the hair follicle bulge in an undifferentiated state. They regenerate mature, pigment-producing melanocytes during the hair follicle cycle. This regenerative capacity of McSCs holds potential for the treatment of skin pigmentation diseases including vitiligo, gray hair and melanoma. To investigate the mechanisms governing McSCs, we focused on Wnt signaling whose inhibitors are highly expressed in the bulge. We have found by immunohistochemistry that β-catenin, a down stream mediator of Wnt-signaling is suppressed in McSCs, but is transiently activated in differentiated melanocytes in the hair bulb at the onset of the hair growth phase (anagen). To decipher the functional significance of β-catenin expression, we either stabilized or depleted β-catenin in McSCs using inducible Cre-based transgenic mouse models. Forced activation of Wnt signaling by β-catenin stabilization led to increased expression of melanocyte differentiation markers and ectopic pigmentation within the bulge. These inappropriately differentiated melanocytes displayed a compromised ability to repopulate bulb melanocytes, leading to reduced melanin in the hair shaft. Conversely, depletion of β-catenin suppressed pigment production in bulb melanocytes and ultimately resulted in unpigmented hair. These results indicate that Wnt activation is involved in the generation of differentiated progeny and suppression of this pathway is necessary for maintenance of McSCs.

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Effects of Fermented Rhus verniciflua Stokes extract (FRVE) on hair regeneration in cyclophosphamide-induced Alopecia model C57BL/6 mouse

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A unique feature of hair growth is its distinct cell cycle with anagen, catagen, and telogen phases. Hair loss is the result of premature entry, induced by apoptosis signals, into the catagen phase. In this study, the effects of Fermented Rhus verniciflua Stokes extract (FRVE) on the proliferation of human hair dermal papilla cells (HHDPCs) and on the stimulation of hair growth in a cyclophosphamide-induced alopecia model C57BL/6 mouse were investigated. In a cell-based MTT assay, the proliferation of cyclophosphamide-treated HHDPCs increased with the addition of FRVE. This suggests that FRVE may play a role in protecting HHDPC proliferation from apoptosis signals induced by cyclophosphamide. Using gene expression-based RT-PCR from HHDPCs treated with the extract, the ratio of Bcl-2/Bax gene expression was noted to be increased, though there was no change in Bcl-2 gene amplification. The extract also significantly decreased Bax gene amplification. Hair growth was increased in the mouse groups treated with the extract and cyclophosphamide-injection, as compared with the cyclophosphamide-only group. Also, anti-apoptotic Bcl-2 proteins were increased, and pro-apoptotic Bax proteins were decreased in the tissues of the mouse groups treated with the extract. According to tissue histology, the number of hair follicles and the thickness of the epidermis increased in the FRVE-treated groups. Based on these results, we conclude that FRVE promotes hair growth and may retard the catagen phase. Hence, FRVE might be a potential new therapeutic source for the prevention of hair loss.

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Impact of copy number variations in the human genome on hair patterns

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Copy number variations (CNVs) are large blocks of DNA (>1kb to megabases) which vary in number between individuals and add to the repertoire of human genetic polymorphisms. The impact of CNVs on human skin disease and variation is largely unknown. Here we report that a significant portion of small regulatory microRNAs are highly enriched in CNVs of the human genome. We found that 215 microRNAs are contained in CNVs, representing an approximate two-fold enrichment of known microRNA (29.95% versus an expected 18.4% of microRNAs). Interestingly, we find that many of these microRNAs are expressed in the human hair follicle. Since microRNAs regulate RNA translation and RNA stability, changes in CNVs between individuals could alter microRNA levels and target genes. The potential impact of these CNVs in human hair and phenotypic variation will be presented.

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Different effects of active or IgE-dependent passive systemic anaphylaxis on endothelin-1

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Endothelin-1 (ET-1) is a highly potent vasoactive peptide which has been implicated in various animal models of inflammatory diseases including septic peritonitis, solar dermatitis, and arthritis. Recently, it has been reported that ET-1 levels can increase during certain allergic responses and that ET-1 can increase the magnitude of certain allergic reactions in rodents. We measured ET-1 levels during active systemic anaphylaxis elicited in the presence or absence of mast cells, as well as during IgE- and mast cell-dependent passive systemic anaphylaxis. Although we previously showed that mast cells can reduce ET-1 levels during a model of sepsis, we found that active systemic anaphylaxis resulted in increased intraperitoneal concentrations of ET-1 in both WBB6F1-$Kit^{W/W-v}$ and corresponding wild-type mice. Moreover, pharmacological blockade of ET receptors in C57BL/6 wild type mice did not significantly reduce hypothermia or improve survival during active anaphylaxis. In contrast, while induction of IgE- and mast cell-dependent passive anaphylaxis did not result in significant changes in intraperitoneal concentrations of ET-1 in C57BL/6 wild type mice, treatment of these mice with a pharmacological inhibitor of the ET_A receptor reduced mast cell degranulation and resulted in a significant, albeit small, reduction in the hypothermia response. Our findings show that ET-1 levels can be elevated during active anaphylaxis in mice, but suggest that ET-1 does not contribute substantially to this largely mast cell-independent anaphylactic response. However, our data suggest that low levels of ET-1 may act via ET_A to increase mast cell degranulation and exacerbate clinical features of IgE- and mast cell-dependent anaphylaxis.

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Galectin-1 is a potent inducer of a regulatory phenotype on skin-resident memory T cells.

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Galectin-1 (Gal-1) is a soluble (S)-type lectin that exists natively as a homodimer and has anti-inflammatory features by virtue of its pro-apoptotic activity on pro-inflammatory T helper subsets (Th_1/Th_{17}). Thus, Gal-1–Gal-1 ligand interactions are recognized as pivotal elements in controlling immunological processes, such as autoimmunity, cancer progression, and fetomaternal tolerance. However, information on whether and to what degree Gal-1 impacts T cell fate and differentiation in skin is unknown. To this end, we generated a Gal-1–human IgG Fc1(Gal-1hFc)chimeric molecule along with mutant non-binding controls to probe the functional activity of Gal-1 binding on skin-resident memory T cells. Unlike commercially-available recombinant Gal-1, Gal-1Fc did not need stabilization with reducing agents to exhibit hallmark binding activity to lactosamine moieties and induce apoptosis on polarized murine Th_1 and Th_{17} cells or to trigger IL-4, -10 and -13 expression in activated mouse T cells. To study Gal-1 effects on human skin-resident T cells, we isolated and expanded skin-resident memory T cells from human skin explants. These T cell cultures expanded with IL-2 and IL-15 characteristically segregated into prominent populations of $CD25^{hi}/FOXP3^+$ (34%) and $IL\text{-}17^+$ T cell (55%) subsets. Using non-growth-inhibitory concentrations of Gal-1hFc or non-binding controls, we found that, while $IL\text{-}17^+$ T cell levels were dramatically lowered, the percentage of IL-4, IL-10 and TGF-β-producing T cells were significantly elevated ($p<0.001$) in cultures treated with Gal-1hFc. Interestingly, $CD25^{hi}FOXP3^+$ T cell numbers remained unchanged following Gal-1hFc treatment. Collectively, these results indicate that Gal-1hFc creates a Th_2 skewing environment while also shifting the effector/regulatory T cell balance towards a more regulatory phenotype. These studies provide exciting new insights into the immunoregulatory effects of Gal-1 and support its potential use as an anti-inflammatory therapeutic in T cell-mediated autoimmune diseases.

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Cigarette smoking inhibits the acquisition of contact hypersensitivity (CHS) by immunization through oral mucosa

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TS is a risk factor for cancers of the oral cavity. We tested the hypothesis that TS may induce oral cancers, in part, by suppressing immunity. To examine whether TS exposure alters induction of immunity through oral mucosa, C3H/HeN mice breathed cigarette smoke from a smoke-delivering manifold for 1 hr daily for 21 d. To quantify smoke exposure, total suspended particulate matter (TSP) was determined by aspirating smoke through a glass microfiber filter attached to a manifold port. The weight of smoke product collected on the filter divided by the aspirated volume was calculated as TSP in mg/m^3. TSP averaged 444 mg/m^3 per smoking session. Pos control mice were treated identically except that no TS was delivered. One d after the last exposure to TS all mice were immunized by application of 10 µl of 1% oxazolone in acetone:olive oil (1:20) to each buccal mucosa. Neg control mice were mock-immunized by application of vehicle only. Mice were challenged 5 d later on each side of each ear with 5 µl of 1% oxazolone and 24 hr ear swelling assessed as a measure of CHS. Mice exposed to TS had a significant decrease in the CHS response (4.25 ± 1.67 mm x 10^{-2}) vs pos control mice (9.15 ± 4.23), $p=0.003$. The response of the non-immunized group was 3.22 ± 1.58. Upon subsequent reimmunization (via dorsal skin) and repeat challenge, immunologic tolerance was observed specifically in the TS-exposed mice. In a preliminary experiment, by real-time PCR a significantly higher level of langerin (CD207) mRNA was found in buccal mucosa from TS-exposed mice compared to controls, suggesting an increase in Langerhans cell (LC) number as has been reported in human buccal mucosal samples from smokers vs. nonsmokers. In this regard, recent data suggesting that LCs may serve to downregulate induction of immunity. Exposure to TS for 3 weeks inhibits the ability of buccal mucosa to present a hapten for induction of CHS. Abnormal presentation of putative tumor antigens by TS-perturbed mucosa may contribute to escape of incipient cancers from immunologic control.

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Exposure of Langerhans cells (LCs) to pituitary adenylate cyclase-activating peptide (PACAP) or vasoactive intestinal peptide (VIP) biases antigen presentation towards a $CD4^+$ T cell IL-17 response

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We previously reported that exposure of LCs to PACAP or VIP enhances IL-17 production during antigen presentation to T cells. We now provide evidence that LC exposure to PACAP or VIP results in enhanced presentation for induction of IL-17-producing $CD4^+$ T cells. BALB/c LCs enriched to ~95% homogeneity were incubated in PACAP, VIP, or medium alone for 2 hrs and then washed 4 times. LCs (10^4/well) were plated in 96 well round bottom plates with 2×10^5 $CD4^+$ spleen cells from DO11.10 transgenic mice (BALB/c background). These mice have T cell receptor transgenes that allow for recognition of a fragment of chicken ovalbumin ($OVA_{323-335}$). $CD4^+$ cells were obtained by removal of NK cells and NKT cells from DO11.10 splenocytes with the use of magnetic microspheres linked to anti CD49b. Remaining cells were negatively selected for CD4 expression using anti-CD45R, anti-CD11b, anti-Ter119, anti-CD16/32, and anti-CD8 antibodies linked to magnetic microspheres. Cells were cultured for 48 hrs in the presence of graded concentrations of $OVA_{323-335}$. Supernatants were collected for assessment of cytokine content by ELISA. Exposure of LCs to PACAP or VIP enhanced IL-17 production while decreasing IL-22 production. In separate experiments, cells were harvested at 24 hr (mRNA analysis) or 48 hrs (FACS analysis) with coculture in phorbol myristic acetate and ionomycin for the last 5 hrs followed by removal of LCs by magnetic capture. For mRNA analysis, RNA was extracted for real-time PCR; IL-17 mRNA was significantly increased by exposure to PACAP or VIP. FACS analysis of $CD4^+$ cells stained for various cytokines was performed. Cells expressing IL-17 (and IL-22) were increased in number while their expression of γIFN was decreased. Cells expressing IL-22 but not IL-17 were decreased. Exposure of LCs to PACAP or VIP appears to enhance differentiation of $CD4^+$ cells towards production of IL-17 while decreasing γ-interferon. PACAP and VIP may be important endogenous immune modulators.

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Muramyl dipeptide induces Th17 polarization through activation of endothelial cells

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ECs express the nucleotide oligomerization binding receptor 2 (NOD 2), which recognizes the bacterial derivative muramyl dipeptide (MDP). ECs produce IL-6 following MDP stimulation and, thus, may contribute to skin immune and inflammatory activities by influencing the cutaneous cytokine microenvironment. Whether ECs influence the polarization of a T helper (Th)-type immune responses remains unknown. We examined whether dermal microvascular ECs (DMECs) participate in the generation of a Th17-type immune response. We utilized a transformed murine DMEC line (bEND.3, from BALB/c) for these experiments. Langerhans cells (LCs) from BALB/c mice were cocultured with T cells from DO11.10 transgenic mice in the presence or absence of bEnd.3 cells pretreated with MDP. DO11.10 mice have T cell receptor transgenes that allow for recognition of a fragment of chicken ovalbumin ($OVA_{323-335}$). After 48 hrs incubation with $OVA_{323-335}$, supernatants were harvested for ELISA analysis. bEnd.3 cells treated with MDP increased IL-6 and IL-17 production in the mixed culture while inhibiting IFN-γ, IL-12 and IL-4, suggesting induction of a Th17 immune response at the expense of Th1 and Th2 responses. IL-22 production was not influenced by MDP-treated bEND.3 cells, illustrating differential regulation of IL-22 from IL-17. Experiments using siRNA to knock down IL-6 production by bEnd.3 cells showed that IL-6 produced by activated bEnd.3 cells is at least partially responsible for the increased IL-17 production observed. Additional analysis demonstrated that the increased IL-17 observed corresponded to an augmented number of IL-17 producing T cells and an increased mRNA level of the Th17 transcription factor RORγt as assessed by FACS and real-time PCR. Our data suggest that activated ECs are capable of influencing LC antigen presentation to T cells and they appear to induce a Th17 polarization. These results are important for the understanding of skin Th17 related pathologies such as psoriasis.

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PD-1 expression is a marker of a tumor-reactive but functionally impaired lymphocyte population infiltrating human melanoma

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PD-1 is a marker of functionally impaired CD8+ T cells in viral and tumor immunology. Tumor antigen specific CD8+ T cells infiltrating human melanomas express high levels of PD-1 and are functionally impaired. However, adoptive cell therapy (ACT) using *in vitro*-expanded autologous tumor infiltrating lymphocytes (TIL) can be highly effective therapy for patients with advanced melanoma, with high response rates and some durable complete responses. This discrepancy led us to further analyze the PD-1+ tumor specific T cells infiltrating melanomas. In this report, we found that the percentage of PD-1 expressing CD8+ T cells was higher in the tumor digests that generate tumor reactive TILs after long term *in vitro* culture in IL-2 (P=0.0007). Also we found that sorted and expanded CD8+ PD-1+ T cells in tumor digests showed much higher tumor specific IFN-γ production compared with CD8+ PD-1- T cells. These results suggested that tumor specific CD8+ T cells in melanoma tumor digests are largely PD-1+, and this population can recover function after *in vitro* culture. However, PD-1 is reported as a receptor of inhibitory signal for T cells, and expression of its ligand, PD-L1 on melanoma cells *in vivo* is also reported. Therefore, we then examined the functional effect of PD-1 expressed by TILs, on their tumor recognition. Tumor recognition by PD-1 expressing TILs was suppressed by PD-L1 when over-expressed on tumor cells, however this suppression was minimal from native levels of PD-L1 expression on fresh melanomas. Moreover, the expression level of the PD-1 on CD8+ tumor specific TILs decreased during *in vitro* culture in IL-2 rendering them less susceptible to PD-L1 inhibition. These results confirm and extend the finding that tumor specific T cells in tumor are inhibited by a PD-1-related mechanism and this can be reversed by *in vitro* culture in IL-2. As a consequence, the PD-1 receptor can be a useful biomarker for enriching tumor specific T-cells from fresh melanomas.

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Mast cells are essential to establish contact hypersensitivity by stimulating cutaneous dendritic cells revealed by a newly generated mast cell-specific conditional depletion model

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The role of mast cells in acquire cutaneous immune response remains controversial since some studies showed that mast cell-deficient W/Wv mice were intact in contact hypersensitivity (CHS) while others demonstrated defective elicitation in CHS. However, W/Wv mice are anemic and deficient in melanocyte as well as mast cell deficiency. Therefore, to evaluate the role of mast cells in cutaneous immune response, mast cell specific deficient mice are required. We have recently reported that conserved noncoding sequence 2 (CNS-2) is an essential enhancer for interleukin 4 gene transcription specific to mast cells. Taking advantage of this system, we have newly generated mice in which a diphtheria toxin receptor is knocked-in to the CNS-2 region. In this MaS TRECK mouse, mast cells in the skin were completely depleted after injection of diphtheria toxin, while other cells, such as CD4, CD8 NKT cells, were intact. In this study, we used MaS TRECK mice to address the roles of mast cell in the CHS response. The CHS response was attenuated when MCs were depleted during sensitization phase. In addition, maturation and migration of skin dendritic cells (DCs) were attenuated when MC were depleted *in vivo*. Consistently, bone marrow derived-MCs promoted maturation, chemotaxis, and survivability of bone marrow derived DCs *in vitro*. The effects of MCs on DC functions were abolished prominently by the inhibition of intercellular adhesion molecule-1 and partially by neutralizing antibody to tumor necrosis factor-α. We further observed that MCs and DCs interact each other *in vitro* and *in vivo*. Therefore, using newly generated MasTRECK mice, we have demonstrated that MCs interact with DCs in the skin to enhance their functions, which might be an essential process to establish the sensitization phase of CHS.

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Desmoglein 3-specific TCR transgenic CD4$^+$ T cells that escape from central tolerance induce autoreactive dermatitis

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Pemphigus vulgaris (PV) is an autoimmune blistering disease induced by IgG autoantibodies against desmoglein 3 (Dsg3), which is expressed in stratified squamous epithelium. We previously isolated pathogenic Dsg3-reactive T cell clones, which could induce anti-Dsg3 IgG production and PV phenotype after adoptive transfer with Dsg3$^{-/-}$ B cells into Rag2$^{-/-}$ mice. To analyze tolerance mechanism against cutaneous antigen, here we generated Dsg3H1 mouse, a transgenic mice of Vα8$^+$Vβ6$^+$ TCR specific for Dsg3 using cDNA isolated from the T cell clone. Bone marrow transfer experiments demonstrated existence of Dsg3-dependent central tolerance and Dsg3-specific CD4$^+$ T cells from Dsg3H1-Rag2$^{-/-}$ mice were clonally deleted in the presence of Dsg3. In contrast, Vβ6$^+$CD4$^+$ T cells from Dsg3H1 mice were partially deleted in thymus but escaped into peripheral lymphoid organs without losing Dsg3 reactivity *in vivo* in the presence of Dsg3. To evaluate helper activity of Vβ6$^+$CD4$^+$ T cells from Dsg3H1 mice, the T cells were transferred with Dsg3$^{-/-}$ B cells into Rag2$^{-/-}$ mice. The recipient mice unexpectedly developed inflammation in the skin and mucous membranes with T cell infiltration in a similar pattern to interface dermatitis. Those transgenic T cells dominantly differentiated into Th1 and Th17 subsets in the periphery. However, no anti-Dsg3 IgG production or acantholysis was found. Furthermore, Vβ6$^+$CD4$^+$ T cells from Dsg3H1 mice, which developed in the absence of Dsg3, acquired an additional ability to differentiate into Th2 subset and induced anti-Dsg3 IgG production with combined phenotype of acantholysis and T cell infiltration upon transfer to Rag2$^{-/-}$ mice. Thus, Dsg3-specific TCR Tg mice provide a valuable tool to unveil fundamental mechanisms for both central and peripheral tolerance to Dsg3 under a physiological condition and will shed light on unclarified pathophysiological mechanisms of inflammatory skin diseases with T cell infiltration.

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Both the elicitation phase of contact hypersensitivity response and irritant dermatitis are suppressed by administration of Thioredoxin

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Thioredoxin, a redox regulating protein that scavenges reactive oxygen species, appears to show an excellent anti-inflammatory effect in treating animal models of various human inflammatory diseases. The aim of this study was to clarify whether thioredoxin is useful for treating inflammatory skin diseases such as contact dermatitis caused by epicutaneous exposure to environmental and occupational antigens. The allergic contact hypersensitivity response was suppressed in thioredoxin-transgenic mice accompanied by a rapid release of thioredoxin into circulation. This suppressive effect of thioredoxin appeared to exert via the inhibition of the efferent limb of contact hypersensitivity because administration of recombinant thioredoxin in the elicitation phase but not in the induction phase suppressed the inflammatory response. Adoptive transfer studies revealed that the host environment, but not donor leukocytes, was critical in this suppressive effect. In thioredoxin-transgenic mice, the infiltration of neutrophils in the elicitation site was diminished, while the migratory function of cutaneous dendritic cells and hapten-specific cell proliferation were not disturbed. Thioredoxin-transgenic mice and the administration of recombinant thioredoxin had also an attenuated inflammatory response to croton oil. These findings suggest that thioredoxin suppresses skin inflammatory responses and could be a suitable candidate for the treatment of skin inflammatory diseases.

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A novel polyclonal peripheral B cell tolerance model (Liver specific Ig-κ SuperAntigen)

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Compared to central B cell tolerance, the rules regulating peripheral B cell tolerance are poorly understood. The fate of autoreactive B cells against a given peripheral antigen varies considerably in different models. To clarify this mechanism, and to allow a convenient polyclonal analysis of wild type B cells, we developed a liver-specific Ig-κ superantigen transgenic mouse. Anti-κ single chain antibody was fused with Rat IgG1 Fc domain and expressed on hepatocyte cell surfaces using MHC ClassI transmembrane domain. In contrast to the ubiquitously expressed anti-κ Tg mice model in which B cells undergo receptor editing in the bone marrow, in this model κ positive (CD93+ CD21- CD23-) B cells were detected in the spleen. Tg mice had 50% fewer B220+ B cells in the spleen. Intracellular κ+, λ+ double positive cells were rare and we also did not see Rag gene upregulation in splenic κ positive cells, using Rag2-GFP reporter mice, indicating B cell development was blocked at an editing incompetent transitional B cell stage. Small number of κ + B cells were detected in the lymph nodes. K+ B cells in the spleen expressed CD86 and higher MHC ClassII, indicating an activated phenotype. These data suggest that immature B cells sensed the liver expressed autoantigen. In this Tg mouse, serum κ Ig was not detectable. If Bcl2 was overexpressed in B cells of liver specific Tg mice, κ Ig was detected in sera; κ+ B cells developed into mature B cells, and immunoglobulin deposition was observed in the liver. Interestingly, Tg mouse peripheral blood analysis showed that a larger fraction of escaped cells were detected in breedings in which the mothers were non-Tg mice compared to those in which the mothers were Tg mice. It was considered that maternal IgGs covered the autoantigen expressed on the liver. Our data showed peripheral B cell tolerance exists and that maternal IgGs levels affect the strength of peripheral tolerance. This mouse model is powerful tool for genetic screening to maintain peripheral tolerance and for understanding B cell mediated autoimmune diseases.

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Intratumoral injection of a novel STAT3 inhibitor elicited strong antitumor effects by provoking innate and acquired immunity

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STAT3 plays a key role in down-modulating immunostimulatory effects. GRIM-19 expressed in normal cells has been reported to interact with STAT3 and to inhibit STAT3-dependent signal transduction. Polyarginine (R9) peptides have multiple biological effects such as protein transducers (protein transduction-domain; PTD) and chemoattractant reagents. Previously, we have demonstrated that overexpression of R9-PTD containing GRIM19 (rR9-GRIM19) in phoshorylated-STAT3 (pSTAT3) cancer cells suppressed STAT3-mediated signal transduction in vitro by forming a complex with pSTAT3. Intratumoral (i.t.) injections of rR9-GRIM19 elicited complete rejection of pSTAT3-expressed A20 tumor (murine B cell lymphoma) mass, but not pSTAT3 (-) tumor (EG.7), and provoked tumor-specific acquired immune responses. Therefore, local injections of STAT3 inhibitors like rR9-GRIM19 are an attractive strategy for the cancer therapy without severe side effects. The purpose of this study was to investigate the mechanism of these strong antitumor effects elicited by i.t. injections of rR9-GRIM19. Although rR9-GRIM19-mediated antitumor effects were not observed in A20-bearing nude mice, these effects were completely restored by adoptive transfer of CD8+ T cells, but not CD4+ T cells. Interestingly, i.t. injections of rR9-GRIM19 in A20-bearing (normal) mice prolonged local inflammation with abundant infiltrations of Gr-1 (Ly6G) high/ CD11b+ neutrophils in A20 tumor site, and these antitumor effects were abolished by depletion of Gr-1- and CD8-positive cells. I.t. injections of rR9-GRIM19 also induced many kinds of chemokines and G-CSF productions in tumor sections by cytokine array analyses. These results indicated that rR9-GRIM19 is an unique immune modulator by suppressing STAT3-mediated signal cascades in tumor microenvironment, resulted in abundant infiltrations of neutrophils and CD8+ T cells. These antitumor effects were unique in inhibiting oncogene and provoking both innate and acquired immunity.

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The effect of clonal mesenchymal stem cells on IgE production of activated B cells

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Mesenchymal stem cells(MSCs) can interact with both the innate and adaptive immune systems, leading to the modulation of several effector functions. However, their effect on B lymphocytes remains unclear. The purpose of this study was to know the immunomodulatory effect of MSCs on activated B cells, especially on their production of IgE. Clonal mesencyhymal stem cells(cMSCs) from C3H mouse bone marrow were isolated and expanded. Splenic T cells of C57BL/6 were cocultured with cMSC. Splenic B cells of BALB/c were purified and stimulated by lipopolysaccharide(LPS)/IL-4 or anti-CD40/IL-4. For cell culture methods, co-culture system and transwell culture system were used. In the T/cMSCs cocultures, T-cell proliferation was suppressed and IFN-γ production from T cells was decreased. In the B/cMSCs co-culture system and transwell culture system, IgE and IgG1 production, down regulation of IgD and cellular division of activated B cells were suppressed. In addition, cMSCs could not induce apoptosis of activated B cells, and could suppress the expression of ε germ-line transcripts(εGLT) of activated B cells. These results suggest that cMSCs could exert a suppressive effect on IgE and IgG1 production of activated B cells. The suppression might be mediated through the inhibition of immunoglobulin class switch recombination and unknown soluble factors derived from cMSCs might play an important role.

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Discovery of a new dendritic cell subset, termed "gr-DC", derived from a granulocyte precursor

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Working with our newly generated transgenic mice expressing DsRed gene under the control of IL-1β promoter, we observed that DsRed+ cells became detectable in 24 h in GM-CSF-supplemented bone marrow (BM) cultures. The DsRed+ cells were CD11b+/Ly6G+/CD11c-/MHC II- and exhibited a characteristic morphology of neutrophil precursors, known as "band cells". Surprisingly, a band cell population purified from WT C57BL/6 mice (CD45.2) began to exhibit features of dendritic cells (DCs) when cultured for 6 days with GM-CSF and BM feeder cells from B6-SJL mice (CD45.1); these features included expression of CD11b, CD11c, MHC II, and CD205, inclusion of oval-shaped nuclei, extension of long dendrites, and a potent ability to present OVA peptides to both OT-I CD8 T cells and OT-II CD4 T cells. Importantly, they retained surface expression of a granulocyte marker Ly6G (which is not detectable on any of the currently known DC subsets) and were, thus, termed "gr-DCs". Moreover, we confirmed surface expression of Ly6G in small fractions of CD11b+/CD11c+/MHC II+ DCs isolated from the spleen (2.5%) and the peritoneal cavity (1%) of WT mice, indicating the in vivo presence of gr-DC populations. Markedly increased (>100-fold) numbers of gr-DCs were found in peritoneal exudates after i.p. injection of thioglycolate (TG). When band cells purified from C57BL/6 mice were i.v. transferred to B6-SJL mice, significant numbers of CD45.2+/CD11c+/MHC II+ gr-DCs were recovered from the TG-inflamed (but not untreated) peritoneal cavity. Affymetrix Genechip analyses further revealed a cluster of genes (170 in total including cathelicidin and CD62L) that are expressed by gr-DCs, but not by monocyte-derived DCs purified in parallel, and some of these findings were confirmed at protein levels. Thus, under inflammatory conditions, band cells can give rise to a novel DC subset while retaining some features of granulocytes, thereby perhaps participating in adaptive immune responses.

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***In vivo* immunomodulatory functions of Abcb5$^+$ dermal mesenchymal stem cells**

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Stem cell-based immunomodulatory strategies represent a new therapeutic frontier in allotransplantation. However, identification of specific molecular markers for the isolation and expansion of effective immunomodulatory stem cell subsets, and dissection of the molecular mechanisms involved, require further investigation. Here, we have isolated and characterized a novel mesenchymal stem cell (MSC) subset from the murine dermis based on expression of the ATP-binding cassette transporter, Abcb5, the murine homologue of human ABCB5, which marks progenitor cell subsets in human skin. *In vivo*, both syngeneic or allogeneic Abcb5$^+$ dermal MSC suppressed T cell activation. In addition, in fully MHC-mismatched murine heterotopic cardiac allotransplantation models, treatment with donor- or third party-strain purified Abcb5$^+$ dermal MSC resulted in significantly ($P<0.001$, respectively) prolonged cardiac allograft survival compared to untreated controls (median survival time: 19.0 vs. 24.5 vs. 9.0 days, respectively). *In vivo* fluorescent cell tracking revealed that fully MHC-mismatched Abcb5$^+$ dermal MSC evade immune rejection and localize to recipient immune tissues, including bone marrow, spleen, and blood. Flow cytometric analysis demonstrated expression of the negative costimulator Pd-1 on Abcb5$^+$ dermal MSC. Furthermore, *in vivo* administration of donor- or third party-type MSC specifically activated expression of the Pd-1 ligand Pd-l2 on 12.5±3.8% of Cd4$^+$ ($P<0.01$) and 12.9±2.4% of Cd8$^+$ recipient T cells ($P<0.01$). In addition, histopathological analysis of cardiac allografts dissected from recipients of either donor- or third party-strain Abcb5$^+$ dermal MSC revealed markedly increased levels of intragraft Pd-l2 expression and enhanced presence of Cd4$^+$FoxP3$^+$ regulatory T cells. Our results establish immunomodulatory functions of Abcb5$^+$ dermal MSC and point to promising roles of this novel skin stem cell subset in cell-based immunotherapeutic strategies.

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Abscopal effect in a patient with metastatic Merkel cell carcinoma following radiation therapy

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Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine carcinoma of the skin which preferentially affects older persons and patients with severe immunosuppression, such as those with CLL, HIV, and post organ transplantation. We present a provocative case of an abscopal effect where remote untreated skin metastases regressed after localized radiation treatment to other sites of cutaneous disease. A 74 year old man developed multiple in transit and retrograde metastases on the right lower extremity several months after definitive surgical excision and adjuvant radiation for a single lesion on the right upper medial calf. Surface-mold high-dose-rate brachytherapy was administered to eleven lesions on the right knee and proximal calf. No treatment was given to two lesions that were both palpable clinically and FDG-avid by PET/CT located on the lateral ankle and plantar aspect of the right foot. All lesions, and notably the 2 untreated lesions, resolved clinically and radiographically within several weeks of radiation treatment. The observation that MCC preferentially affects those with severe immunosuppression, coupled with the recent identification of Merkel cell carcinoma polyomavirus in a large subset of MCC patients, highlight the protective role of the immune system in this disease. In this instance, we propose that radiation treatment of the proximal lesions activated an immune response that mediated the destruction of both untreated lesions. While there have been a handful of case reports describing this rare phenomenon in hematologic and solid tumors, this is the first reported case of an abscopal effect in MCC. Further study of the role of immune modulators in the treatment of this aggressive malignancy may be warranted.

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Heat-killed BCG and *Mycobacterium kansasii* Ag85b combined vaccination ameliorates Th2 cytokine-mediated dermatitis in AD model mice by inducing regulatory T cells

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by Th2-cytokine dominance. The Ag85b is one of the immunogenic proteins expressed by mycobacterium species, which induces Th1 cytokine production especially in BCG-vaccinated hosts. We investigated the effects of heat-killed BCG (hkBCG) and *Mycobacterium kansasii* Ag85b (Ag85b) vaccination on the inflammatory skin lesion of AD model mouse. As an AD model mice (K14/caspase-1 transgenic mouse: KCASP-1Tg) spontaneously develops recalcitrant itching dermatitis with overproduction of Th2 cytokines. Mice were divided into three groups: 1) KCASP-1Tg is treated with Ag85b intraperitoneally twice a week from 4 to 14 weeks, 2) KCASP-1Tg initially treated with hkBCG at week 4, is followed by Ag85b injection intraperitoneally twice a week from 4 to 14 weeks. 3) KCASP-1Tg treated with normal saline. The involved skin lesions are evaluated clinically and histopathologically. Cytokine mRNA expression in skin lesions, cervical lymph nodes, and spleen cells are also performed. FOXP3$^+$CD25^{high}CD4$^+$ regulatory T cells, IL-10 producing CD4$^+$ T cells, and IFN-γ/IL-4/IL-17 producing CD4$^+$ T cell are analyzed by flow cytometry. Saline treated mice spontaneously developed marked dermatitis with mast cell infiltration at week 8. However, the hkBCG and Ag85b combination therapy significantly suppressed the skin lesions and mast cell infiltration compared to those in controls. The number of regulatory T cells in spleen is increased, and IL-4 producing CD4$^+$ T cells are decreased in hkBCG and Ag85b-treated mice. A combination vaccination of hkBCG and Ag85b controls the skin lesions of AD model mice inducing regulatory T cells. This suggests hkBCG and Ag85b combination vaccine is a potent new therapy for AD.

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Immune evasion in human Merkel cell carcinoma: a case of T cell narcolepsy

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Merkel cell carcinoma (MCC) is a rare but highly malignant skin cancer. Spontaneous remissions occur, suggesting immunity can control these tumors in some cases. We found that primary MCC contained CD45RO$^+$ skin homing T cells but the activation of these T cells was suppressed. 50% of T cells resident in noninflamed human skin, gut and cervix express the early activation antigen CD69 and expression is higher in inflamed sites. T cells from MCC expressed markedly lower levels of CD69 than T cells from normal skin (18% vs. 56%, $p<.005$). MCC contained more CD8 T cells than normal skin (33% vs. 6%) and expression of CCR7, a molecule that allows T cells to exit tissues, was increased in MCC. Regulatory T cells were not significantly increased. Addition of IL-2 and IL-15 to cultured MCC resulted in a marked activation and expansion of CD8 T cells and loss of viable tumor cells from cultures. CD137, a marker for antigen specific activation, was increased on expanded TILs (79% vs. 19%) and the TCR repertoire of expanded CD8 T cells was markedly skewed. MCC treated with IL-2 and IL-15 had elevated levels of inflammatory cytokines (IFNγ, IL-3, IL-17 & IL-22) and chemotactic factors (IP-10, MCP-3, I-TAC, lymphotactin, MCP-2, MIG, MIP-1α, & MIP-1β). Addition of imiquimod to MCC had no effect. To determine if tumor specific T cells are present but held in an inactive state, we layered expanded TILS over frozen sections of MCC tumors. 22% of expanded TILS produced IFNγ when exposed to autologous tumor, suggesting tumor recognition. In summary, we find evidence that tumor specific T cells are present in MCC but activation is suppressed. Treatment with IL-2/IL-15 activates T cells and clears viable tumor cells from cultures. Our work supports a possible role for intralesional administration of T cell activating agents in MCC. Ongoing studies focus on identifying immunosuppressive factors in MCC tumors and studies of intralesional IL-2 and IL-15 in human MCC grafted to mice.

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Chemokine requirements for epidermal T cell trafficking

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Epidermal T cell trafficking likely involves multiple steps, including extravasation into dermis from peripheral blood, and migration into epidermis from the dermis. CCR4, CCR6 and CCR10 are chemokine receptors regarded as important players in one or both of these events. We undertook to precisely assess the involvement of each receptor in each process. We established murine *in vivo* conditions that required direct competition between WT and receptor-deficient cells for access to inflamed cutaneous sites, and used MHCII-peptide tetramers to identify Ag-specific endogenous CD4 T cells. CCR4-deficiency caused a ~20-fold reduction in Ag-specific CD4 T cell accumulation within inflamed skin, but neither CCR6- nor CCR10-deficiency caused detectable effects. Further skin homing studies in a TCR-transgenic adoptive transfer model revealed no differences in dermal vs. epidermal localization among CCR6-/- or CCR10-/- OT-II donors. Thus, two independent and highly sensitive *in vivo* assays demonstrate that only CCR4-deficiency affects CD4 T cell accumulation and localization within inflamed skin. We propose that CCR6 and CCR10 play (as yet) unknown roles in cutaneous immunology, unrelated to skin-specific T cell trafficking.

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Abnormal barrier function and allergic skin inflammation in mice overexpressing Th2 cells via a constitutively active Stat6 gene

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Recent studies have indicated that treatment of keratinocytes with Th2 cytokines such as IL-4 that are involved in the pathogenesis of atopic dermatitis (AD) can inhibit the expression of epidermal differentiation complex (EDC) genes including filaggrin. The present studies examined the ability of overactivity of the Th2 pathway to affect EDC genes and cutaneous barrier function *in vivo*. Mice were generated that expressed a constitutively active Stat6 (Stat6VT), which resulted in the overproduction of Th2 cells. Almost 40% of Stat6VT mice spontaneously developed allergic skin inflammation that clinically and histologically resembled AD. Of interest, Stat6VT mice expressed significantly lower levels of EDC genes including filaggrin, loricrin and involucrin in comparison to control C57BL/6 mice. Topical treatment with the irritant 5% sodium laurel sulfate resulted in an increased barrier disruption in Stat6VT mice over similarly treated control mice as measured by transepidermal water loss measurements. Crossing Stat6VT mice onto an IL-4-/- background to remove endogenous IL-4 resulted in the normalization of EDC gene expression and inhibited the development of allergic inflammation, indicating a critical role for IL-4 in both these processes. These studies demonstrate that inappropriate Th2 allergic inflammation inhibits epidermal barrier function and results in mice prone to allergic dermatitis. Our results provide a mechanism for the AD-like disease that develops in several transgenic models where there is increased expression of Th2 cytokines in the immune system.

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Langerhans cells induce expansion of skin resident tregs in the absence of exogenous antigen, but activate T effector memory cells in the presence of microbial antigen

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Normal adult human skin contains abundant effector memory T cells (Tem), which represent memory cells that have previously responded to antigens encountered through skin. It has been hypothesized that resident skin dendritic cells, when appropriately activated, are poised to re-activate these skin resident Tem. We tested this hypothesis by extracting both Tem and dendritic cells (DC) from the same samples of human skin. Dendritic cells were isolated both from the dermis (DDC) and the epidermis (LC). Autologous Tem and DC were then co-cultured, with and without heat-inactivated Candida albicans (CA). Proliferation and cytokine production were measured. LCs were more powerful than DDCs in the induction of proliferation of skin Tem in presence of heat-inactivated CA. CD4 T cells proliferated preferentially, and this could be completely blocked by antibodies to CD80/86 or MHC Class II, and partially blocked by antibodies to CD1a. CA specific activated CD4 Tem produced IL-17, and were largely Vβ8 positive. Surprisingly, significant (albeit lower) proliferation of CD4+ T cells was observed when CA was omitted from cultures. The cells that proliferated under these conditions, however, were CD25+FoxP3+CD127- regulatory T cells that produced neither IL-17 nor IFNγ, and this could be blocked by antibodies to CD80/86 and Class II MHC, but not CD1a. DDC's induced the proliferation of CD4 Tem in the presence of CA, but not in its absence. Thus, while both autologous DDC and LC induced proliferation and IL-17 production of skin resident CD4 Tem, only LC could induce the proliferation of skin resident CD4 Tregs. These data suggest a role for LC in the maintenance of peripheral tolerance through their interactions with resident Tregs.

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Comparison of flow cytometry and ELISA in the evaluation of the skin sensitization by non-radioactive murine local lymph node assay using bromodeoxyuridine

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The murine local lymph node assay (LLNA) using H3-thymidine has been developed to detect chemical allergens, replacing the traditional guinea pig maximization test. Recently, non-radioactive local lymph node assay employing bromodeoxyuridine(BrdU) incorporation has been developed to extend its utility further. International validation studies have been conducted to evaluate the performance of the LLNA by BrdU incorporation using ELISA method and OECD has recently presented a draft guideline. Flow cytometry(FACS) method has also received a large interest due to its high sensitivity towards BrdU incorporated lymph node cells. In this study, the irritation and skin sensitization potential of 7 reference materials (5 sensitizers, 1 false positive and 1 negative control) were investigated with non-radioactive LLNA using ELISA and FACS methods in a same animal to compare the performance of respective tests. When 3 or greater SI values were adopted as threshold for sensitizers, LLNA:BrdU-FACS method identified DNCB, PPD, Isoeugenol, HCA and Eugenol as sensitizers, which are classified as sensitizers in the traditional LLNA. In contrast, among these 5 sensitizers, LLNA:BrdU-ELISA defined HCA and Eugenol as negative and sensitivity and specificity of LLNA:BrdU-ELISA were determined to be 60% and 100%, respectively. When 2.0 or greater SI values were adopted as threshold for sensitizers and 1.3 or lower SI for negative according to OECD draft guideline, LLNA:BrdU-ELISA defined as sensitizer, 4 out of 5 sensitizers which resulted in sensitivity 80% but failed to define 1 negative control (specificity 0%). Comparison of FACS and ELISA with traditional LLNA revealed that FACS has a higher sensitivity and more importantly, it produced comparable sensitivity and performance to traditional LLNA. These results suggest that the non-radioactive LLNA by BrdU incorporation using FACS is more useful and better method for evaluating sensitivity than ELISA method although further studies with more chemicals would be necessary to confirm it.

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A controlled aquaporin-3 expression in T lymphocytes regulate their migration and trafficking in cutaneous immune reaction

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Water constitutes ~60% of our body and is essential for maintaining its homeostasis. Aquaporin (AQP, named AQP0 to AQP12) water channels are expressed in various epithelial and endothelial cells involved in fluid transport, such as urinary concentrating. However, it is unknown if aquaporin-mediated water transport is utilized in other biological system, such as immunity, in which specific cell types at different developmental stage are involved. Here we report that AQP3, a water/glycerol transporter, is expressed in T lymphocytes (T cells) and regulates cell-mediated immune reaction. AQP3 protein was abundantly stored in intracellular vesicles of naïve T cells, while it was found on the plasma membrane of effector/memory T cells. We found intracellular AQP3 in naïve T cells was rapidly translocated to the plasma membrane upon stimulation with T cell chemokines, where it facilitated water intake in T cells, suggesting that AQP3 localization is differently regulated during T cell activation. AQP3 deficiency resulted in impaired water transport, actin polymerization and cell migration in response to chemotactic stimuli, indicating the involvement of AQP3 mediated water transport in T cell chemotaxis. Analysis of AQP3 knockout (AQP3-/-) in lymphoid tissues revealed normal cellularity and subpopulations of T cells in the steady state. However, AQP3-/- mice showed marked impairment to an induction of hapten-induced contact hypersensitivity (CHS). Their defect in establishment of CHS was attributed to a severe failure of primed T cell migration to the site of secondary challenge. Thus, AQP3 localization is precisely controlled in T cells to fine-tune the cutaneous immune response, mainly by controlling their migration and trafficking to the inflamed tissue.

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***Propionibacterium acnes* vaccination improves mouse atopic dermatitis inducing regulatory T cells and Th1 immune response**

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Atopic dermatitis (AD) is a chronic inflammatory skin disease based on the activation of T cells by exogenous antigens. In the acute phase, the cytokine profile in AD skin lesions is Th2 dominant including interleukin (IL)-4, IL-5, and IL-13 but less IFN-γ, and therefore AD is categorized as one of Th2 disorders. This Th2 shift is resulted from excessive immune responses to some specific exogenous allergens. Regulatory T cells (nTreg), IL-10 producing regulatory T cells (Tr1), and Th17 cells are also involved in this immune balance. On the other hand, some infectious agents including *Propionibacterium acnes (P. acnes)* elicit strong Th1-type responses including IFN-γ and IL-12p40 production. Acne formation and severity in AD is controversial in clinical fields. However, the precise relationship between AD and *P. acnes* is still unclear. K14/caspase-1 transgenic mouse (KCASP1Tg) develops recalcitrant itching dermatitis with overproduction of Th2 cytokines, and is used for an AD mice model in the present study. Intraperitoneal administration of *P.acnes* twice a week successfully controlled the development of the skin lesions clinically and histopathologically. This therapy induced the cutaneous and systemic Th1 type cytokine expression. Interestingly, the number of IFN-γ+T cells, FoxP3+CD4+CD25+nTreg, and IL-10+ Tr1 in spleen cells was significantly elevated. No significant effects in Th2-cytokine expressions were observed. The present study revealed *P. acnes* vaccination is a potent new therapeutic tool for AD by altering the cytokine milieu.

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Serum- and glucocorticoid-inducible kinase 1 is critical for early but dispensable for delayed type mast cell responses

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Mast cell activation determines IgE mediated immediate type allergic reactions and also some prototypic T cell mediated contact hypersensitivity (CHS) responses. It was still undefined whether early and delayed mast cell activation are consecutive events or independently regulated. Among others, crosslinking of FcεRI receptors activates phosphoinositol-3 (PI3)-kinase. PI3-kinase is known to activate the serum- and glucocorticoid-inducible kinase 1 (SGK1). We aimed to investigate the role of SGK1 for early and delayed mast cell responses. SGK1 knockout (sgk1-/-) and wild-type mice were sensitized with DNP-specific IgE and challenged with DNP to elicit anaphylaxis. Anaphylaxis with a fast decline in body temperature (1,74±0,41°C) was detected in sgk1+/+ mice while sgk1-/- mice showed no anaphylactic reaction. Degranulation as detected by release of β-hexosaminidase was significantly reduced in bone marrow derived mast cells (BMMC) from sgk1-/- mice. With patch clamping we identified reduced early BMMC cell membrane hyperpolarisation following allergen specific activation exclusively in sgk1-/- BMMC. Next, mast cell dependent CHS response to TNCB was investigated. Ear swelling was significantly impaired in sgk1-/- only during the early response (4 and 8 hours) whereas no difference in CHS responses was detected at 24 hours. Thus, these data show that SGK1 is crucial for early cytokine release but not for delayed type cytokine secretion by BMMC, demonstrating for the first time that consecutive early and delayed type mast cell activation pathways can be regulated independently even following the same stimulus. Based on this specificity, targeting SGK1 may be highly promising to treat immediate type allergic responses while sparing critical delayed type mast cell responses.

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Role of HERV-K dUTPase in immune dysregulation and psoriasis disease

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Psoriasis is an inheritable chronic inflammatory and hyperproliferative disease of the skin and joints. The current view of psoriasis pathogenesis is that a complex interaction between genetic and environmental factors confers susceptibility to the disease. While significant progress has been made in identifying genetic loci associated with psoriasis susceptibility and the specific cytokines/chemokines involved with the disease, the agent(s) responsible for initiating the cytokine/chemokine amplification cascade remains unknown. It was suggested based upon haplotype analyses that a human endogenous retrovirus K (HERV-K), which encodes for a deoxyuridine triphosphate nucleotidohydrolase (dUTPase) may be a candidate gene for the psoriasis susceptibility 1 (PSORS1) mutation. However, there have not been any functional studies performed to determine the role of HERV-K dUTPase in psoriasis. For this purpose, we constructed a consensus sequence encoding the HERV-K dUTPase as well as specific mutations reflecting the genotype characteristic for high and low risk haplotypes of psoriasis, cloned them and purified the recombinant proteins for functional studies. Using stable cell lines expressing select toll-like receptors (TLR) and luciferase gene reporter assays, we have recently demonstrated that the wild-type and the mutant HERV-K dUTPase proteins induced the activation of NF-κB (range 3.0 to 51-fold) through TLR2. Over-expression of the consensus HERV-K dUTPase in TLR2-expressing HEK293 cells also resulted in a 57-fold increase in NF-κB activity. Interestingly, treatment of human primary T-cells, Langerhan/dendritic cells (DC) and keratinocytes with consensus and mutant HERV-K dUTPase proteins for 24 h triggered the production/secretion of eight key cytokines/chemokines, including IL-23, IL12p40 and TNF-α, all of which are known to be involved in the formation of psoriatic plaques, in DC and to a lesser extent in keratinocytes. These data support the HERV-K dUTPase as a crucial player in the pathophysiology of psoriasis.

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Modulation of CD86 expression in skin dendritic cells: what consequences on immune functions?

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Because a reduction of CD86 expression in dendritic cells (DC) may affect their ability to be activated, migrate and mature, we investigated the relation between the level of CD86 expression in Langerhans cells (LC)/dermal DC (DDC) and their ability to move/migrate and induce T-cell proliferation following treatment either with dexamethasone (DX) or with Cestrum latifolium (a CD86 expression-modulating plant extract) in comparison to untreated cells. In this study, LC/DDC were generated from monocytes. Results show that DX treatment markedly decreased CD86 expression and allostimulatory properties. Furthermore, DX did not disrupt 2D trajectories and migratory behavior of cells. On the other hand, Cestrum latifolium moderately decreased CD86 expression and did not affect allostimulatory properties, 2D trajectories and migration of cells. Regarding the recovery of CD86 expression in cells after treatment, whereas Cestrum latifolium-treated LC/DDC were able to rapidly and fully recover their basal CD86 expression, DX-treated LC/DDC still displayed a decreased CD86 expression. Even after TNFa/LPS stimulation, differences in CD86 expression between DX- and Cestrum latifolium-treated LC/DDC persisted. Whatever the treatment, when LC/DDC were stimulated afterwards with TNFa/LPS neither 2D trajectories nor migration of cells appeared to be disrupted compared to untreated LC/DDC. In contrast, DX-treated LC/DDC could not recover their allostimulatory properties after TNFa/LPS stimulation whereas Cestrum latifolium-treated LC/DDC could. Our data suggest that migration may be uncoupled from activation of skin DC and that their activation may no longer be a prerequisite for emigration of DC out of the skin. This hypothesis could reinforce the prevailing paradigm of "immature" DC serving a tolerogenic purpose. Differences in the inhibitory effects of drugs may thus be explained by variations in the intensity of immediate/delayed inhibition of CD86 expression in skin DC but not by their disrupted motility or migration properties.

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Interleukin-31 directly regulates neuronal function in inflammation and itch

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The recently discovered cytokine Interleukin-31 (IL-31) appears to play an important role in atopic dermatitis and pruritus. The expression of the functional receptor subunits for IL-31, IL-31RA and OSMRβ, in murine dorsal root ganglia (DRG) neurons and the dorsal horn of the spinal cord suggest that IL-31 may play a specific role in peripheral as well as central role in itch sensation, and possibly for pain. Therefore, we investigated the role of IL-31 in itch and itch sensitization. In a model of oxazolone-induced chronic atopic dermatitis (application for 3 weeks), IL-31 was injected into the nape neck skin of wild-type (WT) and IL-31 receptor deficient (IL-31RA KO) mice. The impact of IL-31 on itch was assessed by analysis of scratching events and duration. Scratching bouts were more prominent in WT than in IL-31RA KO mice after single subcutaneous IL-31 injections. The pruritogenic effect of IL-31 was significantly enhanced after topical oxazolone treatment in WT mice. Without IL-31 injection, oxazolone-sensitized IL-31RA KO mice scratched significantly more frequently than WT mice. Treatment of cultured murine DRG neurons with IL-31 at pH 5.8 indicates that IL-31 directly activates sensory neurons, regulates intracellular Ca^{++} signaling and sensitizes TRPV1. In sum, activation of neuronal IL-31R by IL-31 activates and sensitizes sensory neurons to transmit itch. Thus, IL-31 may be involved in cross-talk with important neuronal receptors and contributes to pruritus and neurogenic inflammation. Targeting IL-31 or IL-31R may be beneficial for the treatment of inflammatory and pruritic skin diseases.

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Sustained skin specific inflammation elicits increases in circulating Ly-6C monocytes and spontaneous atherosclerotic plaque formation in a murine model of psoriasis

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Considerable evidence now suggests that psoriasis patients are at significant increased risk for developing and dying of cardiovascular disease (CVD) compared to people without inflammatory skin disease. Although this increased CVD risk has been attributed to the presence of systemic inflammation and an increase in the prevalence of risk factors, the precise molecular mechanism(s) by which psoriasis promotes CVD is unknown. Using a validated murine model of psoriasis in which transgene expression is confined to keratinocytes; we report the spontaneous development of atherosclerotic plaque in the aortic roots of 33% of KC-Tie2 mice by 12 months of age on a wild-type background and fed a standard chow diet compared to 0% of control mice. Mechanistically, atherosclerotic lesion formation was preceded by changes in pro-inflammatory cytokines and CVD predictors in both the skin and serum including: increases in IL-17 (4.4-fold), IL-12 (4.3-fold), TNFα (2.1-fold), VEGF (1.6-fold), MCP-1 (1.7-fold), myeloperoxidase (8.6-fold) and S100A8/A9 (>100-fold) coupled with decreases in paraoxonase (50-fold) and HDL (1.7-fold; all $p<0.05$). In KC-Tie2 mice that developed atherosclerotic plaque, a 1.6-fold increase in CRP levels ($p=0.05$) was observed compared to non-atherogenic KC-Tie2 and control mice. Most interesting was the identification of a ~4-fold increase in a population of circulating CD11b+Ly-6Chi monocytes in the peripheral blood of KC-Tie2 mice which have been previously associated with atherosclerotic plaque initiation and infiltration. Importantly, because the KC-Tie2 murine model confines Tie2 transgene expression to keratinocytes, these findings suggest that sustained skin inflammation on its own is sufficient to drive atherogenesis and that long term inhibition of skin inflammation or differentiation and/or migration of the circulating monocytes may reduce or prevent the development of atherosclerotic plaque in psoriasis.

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Langerhans cells require cognate interaction with CD4 T cells and secrete IL-10 to suppress CHS responses

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Mice lacking epidermal Langerhans cells (LC) develop exaggerated contact-hypersensitivity (CHS) responses due to the absence of LC during sensitization. To further understand the details of LC-mediated regulation, we examined hapten-specific T cells using an in vitro DNBS restimulation assay. LN cells from DNFB sensitized WT and LC-deficient (Langerin-DTA) mice were re-stimulated in vivo with DNBS-haptenized spleen cells isolated from Rag1-/- mice. We found that the absence of LC led to increased numbers of hapten-specific CD4 and CD8 T cells but did not alter cytokine expression or development of T regulatory cells. Since CHS responses involve both CD4 and CD8 T cells, we generated mice in which MHC-II is selectively ablated only in LC (Langerin-Cre x I-Ab-flox). CHS responses and Ag-specific T cells were increased in these mice demonstrating that direct cognate interaction between LC and CD4 T cells is required for LC-mediated suppression. LC-derived IL-10 is also required for optimal inhibition as Langerin-Cre x IL-10-flox mice also develop increased CHS. Finally, we observed that both LC-derived IL-10-mediated suppression and full LC activation require LC expression of MHC class II. These data support a model in which cognate interaction of LC with CD4 T cells enables LC to inhibit expansion of Ag-specific responses via elaboration of IL-10. We hypothesize that LC have evolved to limit effector responses against epithelial antigens which may function to prevent deleterious immune responses against commensal organisms and innocuous environmental antigens.

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Acute ablation of epidermal Langerhans cells enhances skin immune responses

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Although initially thought to be required for the development of contact hypersensitivity (CHS) responses, recent data obtained from two Langerhans cell (LC)-deficient mouse models has cast this role in doubt. We have previously described huLangerin-DTA mice in which LC are constitutively ablated but other Langerin+ DC are unaffected. In these mice, the absence of LC leads to the development of increased CHS responses. In the second model, muLangerin-DTR mice, LC and Langerin+ dermal dendritic cells (dDC) are inducibly ablated by administration of DT. LC, unlike Langerin+ dDC, repopulate slowly after diphtheria toxin (DT) administration. By comparing early and late time-points after DT administration, it appears that Langerin+ dDC are required for optimal CHS responses and efficient epicutaneous immunization but that LC are redundant. One major difference between these two models that could account for the disparate results is the timing of LC ablation: inducible (muLangerin-DTR) vs. constitutive (huLangerin-DTA). We now report the development of a new line of mice (huLangerin-DTR) in which administration of DT leads to inducible ablation of LC without affecting Langerin+ dDC. The acute and selective ablation of LC in these mice results in increased CHS responses as well as enhanced epicutaneous immunization. Thus, LC-mediated suppression does not require their absence during ontogeny or during the steady state. Rather, LC appear to actively limit cutaneous immune responses. We hypothesize that LC function to terminate ongoing cutaneous immune responses and/or prevent responses to epidermal antigen such as commensal organisms.

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Homoisoflavanone prevents allergic responses by degranulation of mast cells through the inhibition of FcεRI signaling pathways

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Homoisoflavanone isolated from Cremastra appendiculata Makino is known to be involved in anti-angiogenesis and cell cycle arrest. However, other functions of the homoisoflavanone have not been investigated. In this study, we determined the anti-allergic effects of homoisoflavanone. For *in vitro* experiments, two mast cell lines were used. HMC-1 was activated by phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187, and RBL-2H3 was sensitized with dinitrophenyl (DNP) – specific IgE overnight and then challenged with DNP - human serum albumin (HSA) to induced mast cell degranulation. Treatment of homoisoflavanone inhibited the release of histamines and β-hexosaminidase. In addition, the production of leukotriene B4, prostaglandin D2, interleukin (IL)-8, IL-6 and tumor necrosis factor-alpha (TNF-a) was also inhibited. To analyze the anti-allergic effects of homoisoflavanone *in vivo*, we induced ear swelling by compound 48/80 and anti-IgE antibody in mice and attempted to demonstrate whether homoisoflavanone suppressed ear swelling. Homoisoflavanone inhibited ear swelling up to 53%. Furthermore, an IgE-mediated passive cutaneous anaphylaxis reaction was also prevented. Finally, we showed that the anti- allergic effects occurred *In vitro* and *in vivo* anti-allergic effects of homoisoflavanone imply the possible therapeutic application of homoisoflavanone in allergic diseases.

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The spatio-temporal analysis of T cell behavior in contact hypersensitivity response

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The skin is not merely a physical barrier but an active immune organ. Previously, the evaluation of the inflammatory status of the skin was based on the histological analysis; however, three-dimensional or chronological evaluation was difficult in that traditional way. In the present study, we aimed to analyze the spatio-temporal behavior of T cells in contact hypersensitivity (CHS) response by two-photon microscopy. CD4+ and CD8+ T cells were isolated from the spleen of non-treated mice and incubated in the presence of IL-12 *in vitro* to differentiate into Th1 and Tc1, respectively. These effector T cells were labeled and transferred into DNFB-sensitized mice and the ear was challenged. On the following day, T cell behavior in the ear skin was visualized and the cell kinetics was analyzed. T cells rolled on and attached to the vascular wall of deep dermis. Once they transmigrated to the dermis, they started to move around rather rapidly (6-9 um/min). We couldn't find any significant difference between CD4+ and CD8+ T cells on cell distribution, whereas the mean velocity of CD8+ T cells was slower than that of CD4+ T cells. We also analyzed the cell behavior of antigen-specific T cells prepared from DNFB-sensitized mice and observed the arrest of cell migration and reduction of mean velocity of T cells in an antigen-specific manner. Similar results were obtained from a delayed-type hypersensitivity model with ovalbumin (OVA) and OVA-specific TCR transgenic mice. Our study is the first *in vivo* live-imaging of T cells in the elicitation phase of CHS model. Our findings have revealed that even the non-specific effector T cells readily transmigrate to the inflamed-skin and that they can move rapidly in the dermis for antigen scanning. Moreover, we revealed that once T cells meet specific-antigens, they stop migration and exchange signals via long-time cell-to-cell contact. These observations introduce a new concept that T cells perform similar motile activities upon specific antigen exposure in the skin as well as in the lymph nodes.

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Murine beta defensin-14 induces regulatory T cells probably via interleukin-10

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Ultraviolet radiation (UVR) suppresses the adaptive immune response in an antigen-specific fashion via the induction of regulatory T cells (Treg). In contrast, the innate immune response appears to be induced by UVR. Recently we demonstrated that UVR induces the release of antimicrobial peptides (AMPs) in the skin, thereby fostering the defense against microbial attacks. Since most dermatoses are T cell-driven, suppression of the adaptive but induction of the innate immune system by UVR may present a protective mechanism in the skin. Hence, we asked whether AMPs can further contribute to this mechanism by mediating immunosuppression through the induction of Treg. The AMP murine beta defensin-14 (mBD-14) was injected intravenously into naïve C57BL/6 mice before sensitization with dinitrofluorobenzene. 5 days later ear challenge was performed and the ear swelling measured 24 hours later. The contact hypersensitivity (CHS) response was significantly reduced upon injection of mBD-14. The suppression could be adoptively transferred into naïve recipients, indicating that Treg were induced in the donors. To study whether this effect is mediated via interleukin (IL)-10, serum levels of IL-10 in donor mice were measured. This revealed a significant upregulation of IL-10 after mBD-14 injection compared to untreated mice. In contrast to wild type mice, CHS was not reduced upon injection of mBD-14 in IL-10 knockout animals. These findings indicate that mBD-14 induces Treg and that IL-10 is involved in this process. Since mBD-14 is induced by UVR, mBD-14 may contribute to UVR-induced immunosuppression and protect the skin from bacterial infections on the one hand, but tone down exaggerated T-cell responses on the other hand.

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IRF8 as a potentially key determinant in the activation or death of CD8T cells

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We previously reported that IL-15 regulates a critical checkpoint in the generation of disease in a CD8T cell-induced mouse model of GvHD. Using microarrays we found that at this critical checkpoint interferon regulatory factor 8 (IRF8) is persistently upregulated in fully functional CD8T cells compared to partially activated CD8T cells in which IRF8 is only transiently expressed. IRF8 is a member of IRF family, expression is restricted to immune cells and is induced by IFN-**γ**.IRF8 is required for differentiation of CD8αDC, pDCs, and macrophages, supports B cell development and promotes Th1 differentiation. Because little is known about the role of this transcription factor in CD8T cells we sought to determine the role of IRF8 in CD8T cell activation. We first assessed the effect of constitutive IRF8 expression by retroviral transduction of activated OT-I cells. Cell growth curves show that the cumulative cell number of mock transduced OT-I cells is 3 times more than that of IRF8 transduced OT-I cells, indicating that IRF8 suppresses the growth of OT-I cells. IRF8 transduced OT-I cells are 20-50% Annexin V positive, while mock transduced OT-I cells are only 5-10% positive, demonstrating that the IRF8-overexpressing cells undergo apoptotic cell death. Interestingly, the deletion mutant of IRF8 which lacks the interaction domain also induces apoptosis, indicating that the pro-apoptotic action of IRF8 is sufficiently mediated by the DNA binding domain. Furthermore, IRF8 transduced OT-I cells express cleaved forms of caspase 9 by western blot. This result suggests that IRF8 induces apoptosis through a mitochondrial pathway. Surprisingly, immunoprecipitation using an IRF8-expressing EL4 cell clone shows that IRF8 may bind to Akt. Since Akt is a central player of cell survival, cell growth, and anti-apoptotic signals, IRF8 may be a critical determinant in whether activation or apoptosis ensues. Since overexpression of IRF8 may not reflect its physiological role, we are currently generating OT-I cells on an IRF8KO background to assess whether they are able to induce autoimmunity or tolerance in our GvHD mouse models.

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Deletion of microRNAs mediated by Langerin-Cre impairs epidermal Langerhans cell development

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Langerhans cells (LCs) are skin-resident DCs that express the C-type lectin Langerin and have a life cycle distinct from many other types of DCs. Even though LCs were first described more than 100 years ago, their development and immunological functions still remain enigmatic. MicroRNAs (miRNAs), a class of 21–25 nt single-stranded non-coding small RNAs, are increasingly being recognized as important regulators of gene expression through the inhibition of effective mRNA translation. The ribonuclease III enzyme Dicer is required for the processing of mature and functional miRNAs. Using Cre-loxP tissue-specific Dicer deletion, our laboratory and others have reported that deletion of miRNAs significantly affects the development and function of B, T, and NKT cells. To test the roles of miRNAs in the development of LCs, we generated a mouse strain with tissue-specific disruption of Dicer in the late stage of LC development mediated by Langerin-Cre (cre expressed in epidermal LCs). We found that Dicerfl/fl.langerinCre+ mice had significantly reduced number of epidermal LCs compared to WT control mice and the expression of Langerin was significantly reduced in miRNA-deficient I-Ab positive LCs. Furthermore, morphological analysis by confocal microscopy revealed that the miRNA-deficient LCs exhibited significant reduction of the number of dendrites and an increase in dendritic length. miRNA-deficient LCs have a defect on the maturation *in vitro*, but have normal capacity for antigen uptaking. Using BRDU staining, we found that increased LC proliferation and apoptosis appear in Dicerfl/fl.langerinCre+ mice compared to WT mice. Thus, our findings highly suggest that miRNAs are required for the LC lineage development and LC homeostasis.

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Effect of extracorporeal photopheresis on dendritic cell populations in patients with Sézary syndrome and graft versus host disease.

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Dendritic cells (DC) are antigen-presenting cells involved in induction and modulation of T-cell mediated immune responses. Defective blood DC populations are reported in patients with advanced stage Sézary syndrome (SS) and graft versus host disease (GvHD) and may contribute to impaired cell-mediated immunity. Extracorporeal photopheresis (ECP) is an effective treatment for both SS and GvHD, but its ability to restore host immunity is poorly understood. In this prospective study, we investigated the effects of ECP on two types of human DCs: HLA-DR+CD11c+ myeloid DCs (mDC) and HLA-DR+CD123+ plasmacytoid DCs (pDC). Flow cytometry was used to assess the percentage and ratio of mDCs and pDCs in peripheral blood mononuclear cells from SS (n=12) and GvHD (n=5) pts at baseline and 1, 3, 6 month post-ECP. Five of 5 GvHD pts had lower numbers of mDCs (0.10±0.13%, $p<0.01$) and pDCs (0.04±0.07%, $p<0.01$) at baseline than normal donors' (ND) (0.65±0.15%, 0.76±0.49%). Ratios of pDC/mDC were significantly decreased (0.29±0.19, $p<0.05$) compared to ND (1.35±1.08). Three of 4 GvHD pts had an increase in both mDC and pDC numbers post-ECP and 4 of 4 had significantly increased ratios of pDC/mDC (0.919±0.092, $p<0.01$). Among 12 SS pts, 6 had low mDCs and pDCs pre-ECP (0.18±0.12%, $p<0.01$; 0.05±0.02%, $p<0.05$) and six were in the normal range (0.58±0.13%, $p=0.53$; 0.75±0.68%, $p=0.89$). Six SS pts with lower numbers at baseline had continuous increasing mDC counts post-ECP, and only 3 of 6 had increased pDCs. Pts with normal cell counts had variable changes. The ratio of pDC/mDC were in the normal range (0.79±0.76, $p=0.66$) at baseline and tended to decrease over the treatment course while the ratio of mDC/pDC increased. These data suggest the absolute number and ratio of mDCs and pDCs are influenced by ECP in both SS and GvHD patients. ECP may correct defects in DCs, restoring imbalanced immunity in GvHD and SS pts. Further studies on DC maturation and function are ongoing.

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Retionoid Orphan Receptor-gamma (ROR-γ) deficient mice generate potent Th9 responses, but not Th17 responses, under Th17 polarizing conditions

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RORγ is thought to be a pivotal transcription factor for the generation of Th17 cells. Th17 cells are a recently discovered T cell subset that does not produce IFNγ or IL-4, and mediates defense against bacterial and fungal infections. In the present study, naïve T cells (CD4+CD62Lhigh) from RORγ-/- mice and control mice were cultured under optimal Th0, Th1 and Th17 polarizing condition, and polarized cells were assessed for cytokine production by flow cytometry, ELISA and real-time RT-PCR after activaiton. RORγ-/- T cells efficiently generated IFNγ producing T cells under Th1 conditions. However, RORγ -/- did not induce the generation of IL-17 producing cells under Th17 polarizing conditions. Instead, a population of CD4 T cells that produced IL-9, IL-10 and IFNγ, were generated, a profile similar to that of the recently described Th9 T cells. To test this finding in another fashion, we used the LXR agonist T0901317, which has been shown to block ROR-element activation by RORγ in a dose-dependent manner. Naïve T cells were cultured under optimal Th17 polarizing conditions, with or without T0901317 at various doses. The addition of T0901317 completely abrogated the generation of IL-17 producing cells and, similar to RORγ -/- T cells, induced a population of T helper cells that produces IL-9, IL-10 and IFNγ. Finally, transcriptional profiling experiments were performed on RORγ and normal T cells after Th17 polarizing conditions. One of the most abundant transcripts in normal T cells was the IL-23R; this was completely absent in RORγ-/- T cells. In conclusion, the absence of RORγ activity blocks the generation of IL-17 producing cells but induces the generation of a novel population that produces IL-9, IL-10 and IFNγ.

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Deficiency of retinoid related orphan receptor–gamma (ROR–γ) results in impaired contact hypersensitivity (CHS) responses, but enhanced melanoma tumor immunity

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ROR–γ is a transcription factor that is critical for the generation of Th17 cells. Because ROR–γ -/- mice lack organized secondary lymphoid tissue, including peripheral lymph nodes, they have not been a useful model for studying the role of ROR–γ in immune responses *in vivo*. In this study, we generated bone marrow chimeric mice, by transferring bone marrow cells from ROR–γ -/- or wild type (WT) mice in to sub-lethally irradiated C57BL/6 Rag1-/- recipients. After 6 weeks, ROR–γ -/- chimeric mice developed comparable numbers of T cells, and lymph nodes as WT controls. To investigate the role of ROR–γ in skin inflammation, we utilized an oxazolone mediated CHS assay. There was markedly diminished skin inflammation in mice bearing ROR-γ -/- T cells, measured by ear thickness and histopathology. Moreover, ROR–γ -/- T cells from draining lymph nodes of hapten immunized mice secreted negligible IL-17 and comparable levels of IFN-γ compared to WT controls. In the tumor immunity model, syngeneic B16 F10 cells were injected in C57BL/6 mice bearing WT or ROR–γ -/- T cells, and tumor growth was monitored over time. Mice bearing ROR–γ -/- T cells had significant delay in tumor development and demonstrated reduced tumor size (weight and volume). In conclusion, ROR–γ appears to be important for the generation of robust CHS responses, but appears to have a negative effect on tumor immunity. We propose that these differences are a result of the absence of Th17 cells in ROR–γ -/- chimeric mice.

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Effector memory T cells persist long term in the skin, and their recirculation into blood is limited

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T cells with an effector memory (Tem) phenotype populate normal, non-inflamed skin in both humans and mice. Little is known about the in vivo trafficking patterns of these cells. To address this question, we created parabiotic mice by joining wild-type mice with GFP-transgenic mice to assess T cell trafficking over time; after two weeks these mice shared a circulatory system. We found that the ratio of GFP+ to GFP- CD3+ T cells in blood and lymphoid tissues, but not in lung, liver, or skin, reached equilibrium 2 wks post-surgery. Complete equilibration of T cells was not noted in skin even 8 wks post-surgery, suggesting that their migration was restricted. To test this possibility, we scarified OT-1 cell-bearing mice with VACV expressing the OT-1 relevant ovalbumin peptide. After 12 weeks, expanded populations of OT-1 memory cells could be detected in skin, blood, and LN, as well as gut, liver and lung. We then joined these VACV-OVA immunized OT-1 mice parabiotically 12 weeks or later after immunization, with normal naïve mice. At 2 wks post-surgery, central memory (Tcm) OT-1 cells had equilibrated in blood and all lymphoid tissues. However, OT-1 cells were detected only in the skin of the immunized parabiont, and not the naïve parabiont, even at 20 weeks of parabiosis. We analyzed the phenotype of these skin resident OT-1 cells and found that they expressed high levels of E- and P-selectin ligands, but were CCR7 negative, consistent with a Tem phenotype. In vitro stimulation with PMA/Ionomycin or peptide/APC did not change the expression pattern. Stable expression of these trafficking molecules may be an important mechanism by which effect memory T cells persist long-term in skin. Activation via TCR triggering of resident OT-1 cells in skin, gut, and lung, showed they were functional by secretion of IFN-γ and TNF-α. We conclude that antigen specific skin resident Tem retain expression of skin trafficking molecules, produce cytokines upon activation, and do not readily exit skin, even long after antigen encounter.

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Vaccination through epidermis with Vaccinia Virus (VACV) generates skin resident T cells, central memory T cells and lung resident T cells which protect the host independent of antibody

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Vaccines against viral illness generate optimal neutralizing antibody responses. However, $CD8^+$ T cell responses are critical to the control of viral infection, and recently new emphasis has been placed on this protective parameter. We tested the efficacy of vaccines at inducing protective $CD8^+$ responses against viral infection using mice that cannot produce antibodies (μMT-/-). Using a VACV vaccine, we immunized μMT mice by different routes, including skin scarification (ss), subcutaneous injection (sc), and intramuscular injection (im). 6 weeks later, we re-challenged mice with VACV to injured epidermis, and measured viral load in skin after 6 days. Mice immunized by ss had complete viral clearance, while sc- and im- immunized mice had viral loads that were more than 5 logs higher. We next treated ss- immunized mice with FTY720 prior to epidermal challenge. FTY720 blocks egress of T cells from LN, and thus blocks mobilization of memory cells from blood and lymph node. FTY720 treatment had no effect on viral clearance, indicating that skin resident VACV- specific Tem generated by ss alone were responsible for viral clearance. We next examined the response of VACV immunized mice to a lethal respiratory challenge (100% mortality in UI mice) with VACV. Mice immunized with ss experienced negligible weight loss and no mortality, while sc- and im- immunized mice showed 100% morbidity and partial mortality. When mice were treated with FTY 720 before lethal respiratory challenge, ss immunized mice still showed partial protection against mortality, indicating that ss immunization generated a population of respiratory mucosal epithelial resident Tem whose protective activity after challenge was augmented by central memory T cells. These data indicate that ss with VACV generates, in order of protective efficacy, skin resident Tem, Tcm, and lung resident Tem. Collectively, these VACV specific T cells can protect the host against VACV challenge by both skin and respiratory routes independent of antibodies.

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T-cell immunosenescence is associated with the presence of Kaposi's Sarcoma in antiretroviral treated human immunodeficiency virus infected persons

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We reported an atypical cohort of patients with Kaposi's sarcoma (KS) with undetectable viral loads and CD4 count>300 cells/mm^3. Patients in this KS cohort had an indolent disease course resembling elderly or classical HIV- KS. This atypical KS cohort provides opportunity to examine whether immunosenescence, or accelerated "aging" of the immune system, could occupy a significant role in development of KS in the absence of advanced immunosuppression. We hypothesized that CD57, a marker of immunosenescence, would be elevated in this atypical cohort of patients with KS. All KS cases and controls had CD4 counts >300 cells/mm^3, were male and were on antiretroviral therapy (ART) with viral loads<75 copies/mL. Peripheral blood was collected from HIV+KS+ cases (n=19) and HIV+KS- controls (n=47) with undetectable viral loads. CD57 was examined in peripheral blood via flow cytometry. Our results showed that KS+ cases had a higher proportion of CD57+CD8+ T cells vs. KS- controls (median of 41.5% vs. 27.7%, age-adjusted $p=0.005$). There was a trend suggesting that KS+ cases had a higher frequency of CD57+CD4+ T cells than KS- controls (median of 7.4% vs 3.7%, age-adjusted $p=0.07$). In conclusion, the indolent cutaneous KS increasingly observed among patients on ART is associated with a higher frequency of immunosenescent T cells. These observations are consistent with a model whereby HIV infection results in premature and irreversible immunosenescence, which then contributes to development of cutaneous KS. This association of immunosenescence in well-controlled HIV and an AIDS-defining malignancy carries implications for adequacy of current immunologic HIV monitoring, and the potential for immune-related morbidity in aging HIV+ populations.

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Langerhans cells induce IL-10-/TGF-beta-producing regulatory T cells after glucocorticosteroid treatment *in vivo* and *in vitro*

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Although glucocorticosteroids (GCS) have been used for many decades in autoimmune diseases and transplantation, the exact mechanisms responsible for their immunosuppressive properties are not fully understood. We therefore undertook a clinical study with 24 nickel-allergic patients, performed epicutaneous patch tests (EPT) prior and after oral GCS or placebo treatment and took skin biopsies after EPT application. Expectedly, oral GCS treatment led to a reduction of clinical symptoms that was paralleled by a sizeable decrease of infiltrating T cells. Interestingly though, we observed increased numbers of dermal $FoxP3^+CD25^+CD4^+$ T cells and epidermal LC in post-GCS lesions as compared to the placebo group. These cellular alterations were associated with upregulated expression of TGF-beta. In order to investigate this phenomenon further, we exposed purified dendritic cells / LCs to GCS and co-cultured them with T cells. When LCs were incubated with GCS, they exhibited, in contrast to GCS-negative LCs, i) a more immature phenotype, ii) decreased levels of HLA-DR, iii) higher intracellular amounts of TGF-beta and iv) increased RANK expression, conditions that supposedly favor the generation of regulatory T cells. Indeed, we observed an induction of functionally suppressive $FoxP3^+$ T cells when $CD3^+$ cells were incubated with GCS-pretreated LC. This suppressive activity was neutralized by anti-TGF-beta and anti-IL-10 antibodies. Our data show that GCS endow LCs with regulatory T cell-inducing properties and, thus, shed new light on the mechanisms of glucocorticosteroid-mediated immunosuppression.

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A role for IL-5 in promoting increased IgM at the site of disease in leprosy

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Leprosy is an infectious disease, in which the clinical manifestations correlate with the type of immune response mounted to the pathogen, Mycobacterium leprae. To investigate which biological pathways or gene sets are overrepresented in lepromatous (L-Lep) vs. tuberculoid (T-Lep) patients that might be relevant in disease pathogenesis, we compared the gene expression profiles of L-lep vs. T-lep skin lesions using knowledge-guided bioinformatic analysis, incorporating data on likely biologic functions, including gene ontology information and regulatory data. Analysis of probesets comparatively increased in expression in L-lep vs. T-lep revealed multiple pathways and functional groups involving B cell genes (p values all < 0.005) relevant to the dataset. Further pathways analysis of B cell genes comparatively increased in expression in L-lep vs. T-lep lesions revealed a potential network linking the expression of IgM and IL-5. Analysis of the leprosy lesions by immunohistology indicated that there was approximately 8% more IgM positive cells in L-lep compared to T-lep lesions. Furthermore, IL-5 synergized with M. leprae *in vitro* to enhance total IgM secretion from peripheral blood mononuclear cells. This pathways analysis of leprosy in combination with our *in vitro* studies implicates a role for IL-5 in the increased IgM at the site of disease in leprosy.

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Psoriasis lesions are enriched in IL-22+ and IL-17+ T cells with an associated increase in the IL-17+/IL-13+ T cell ratio

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Despite many recent advances, the composition of effector T cells in psoriatic vs. normal skin remains incompletely understood. Measurement of T cell function is often performed after a period of in vitro culture to expand numbers of cells for analysis. However, the phenotypes and relative proportions of T cells can change during culture, especially in the presence of exogenous cytokines. To avoid this artifact, we developed a method where immune cells are isolated directly from mechanically and enzymatically digested psoriatic plaques(PP), unaffected psoriatic skin(PN), or control skin(NN). Without intervening culture, we performed standard intracellular cytokine staining and flow cytometry. Using this direct ex vivo analysis, we confirmed our previous findings that IL-17+ CD4+ and CD8+ T cells were increased in PP and PN skin compared to NN skin or blood. However, no differences were observed between blood of psoriasis patients or controls in production of IFN-γ, IL-17, IL-22, IL-13, or IL-4. In contrast, the proportion of T cells producing IFN-γ in PP was decreased 1.7-fold compared to psoriatic blood T cells ($P<0.05$). A greater proportion of T cells produced IL-22 in NN, PN, and PP skin compared to blood($p<0.01$), and compared to NN skin there were 1.7 fold increased CD4+IL-22+ T cells in PN($p<0.05$), which was seen to a lesser extent (1.2 fold) in PP skin,. Furthermore, most IL-22 producing T cells did not produce IL-17 or IFN-γ, confirming the distinct phenotypic identity of IL-22-producing T cells. IL-13 can negatively regulate RORγt expression and thereby decrease T cell IL-17 production. Interestingly, our study revealed that the proportion of both CD4+IL-13+ and CD8+IL-13+ T cells is decreased in PP compared to PN or NN skin. Consequently, the ratio of IL-17+/IL-13+ T cells is significantly increased in PP compared to NN or PN skin. Improved understanding of the balance of effector T cell function in psoriasis lesions will have profound implications for the natural course of disease and for optimized approaches to therapy.

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Topical genetic immunization and engineered dendritic cell-based vaccines raise CD8 T cell specific responses against a carcinogen-induced point mutation of the H-ras oncogene

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We have found that development of a T-cell-mediated immune response senstized by polyaromatic hydrocarbons (PAHs) is associated with resistance to tumorigenesis by those agents. Topical application of the PAH carcinogenic 7,12-dimethylbenzanthracene (DMBA) results in a characteristic point mutation in the H-ras oncogene. We hypothesize that a vaccine against the mutation Q61L epitope (H*ras) would induce sufficient T cell immunity to elliminate cells with DMBA-induced mutations and protect against carcinogenesis. We observed that mutant but not wild type (WT) H-ras peptide, injected s.c., induced DTH responses following challenge with mutant but not WT peptide in A/J and C3H/HeN mice. Because hapten-specific responses are mediated by CD8 T cells but suppressed by hapten-specific CD4 T cells, we generated chimeric genes of mutant or WT H-ras sequences flanked by ubiquitin (Ub) and EGFP to favor loading into MHC I. H*ras-specific DTH responses could be ellicited following topical genetic immunization (GI) and boost with plasmid DNA encoding Ub/H*ras/EGFP, but not control Ub/ WT H-ras/EGFP delivered by occlusion patch. GI induced responses were comparabe or superior to responses induced by peptide patch vaccines in C3H/HeN and A/J mice, respectively. Specific CD8 T cell responses were supported by in vivo cytotocity assays. Genetically engineered LC-like XS106 (XS) lines expressing WT or mutant fusion proteins similarly induced H*ras-specific DTH responses and increased the numbers of IFN gamma producing CD8 T cells. Ellicitation of DTH responses by DMBA was detected in mice vaccinated wth engineered XS-Ub/H*ras/EGFP lines. This observation suggests that vaccination against H*ras may provide protection against DMBA-induced skin carcinogenesis.

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Effect of local hyperthermia on Langerhans cells in normal and HPV infected skin

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Langerhans cells (LCs) are professional antigen presenting cells of the skin. Direct heating of the skin causes immigration of LCs away from the epidermis, while the fate and role of immigrated LCs remains largely unknown. We found *in vivo*, that local hyperthermia could cause the decrease in number of LCs in the epidermis of mouse skin, reaching the lowest in number at day 3, and regain homeostasis at day 7. Moreover, the decrement in number of LCs was temperature dependant, in that higher local hyperthermia tent to reduce the epidermal LCs more efficiently. Immigrated LCs could be recovered from regional lymph nodes. Hyperthermia- induced immigration of LCs was orchestrated with up-regulation of CCR7 and down-regulation of CCR6, a pre-condition for LCs movement to lymph nodes. By employing an organotypic culture system, we again found that hyperthermia could promote the immigration of LCs to the culture media, in a temperature dependant manner. There was a higher percentage of LCs that expressed CD83 molecule, a marker for mature LCs, in emigrates from tissue subjected to higher local hyperthermia. The above observation alluded that local hyperthermia could promote migration and maturation of epidermal LCs. In a similar organotypic culture system, we tested the effect of local hyperthermia on human papillomavirus (HPV) infected skin. Again, we found that local hyperthermia could promote the migration and maturation of epidermal LCs in a temperature dependant manner. Moreover, the emigrated number, as well as the percentage of CD83+ LCs, was more prominent than that from the normal skin. We thus postulated that hyperthermia- induced migration and maturation of epidermal LCs might have an enhanced potential to present HPV antigens to T cells in regional lymph nodes, and subsequently aid in establishing a specific immune response against HPV infection. Our hypothesis was partly supported by our randomized controlled clinical observation that local hyperthermia was effective in the treatment of warts caused by HPV infection.

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The human T cell repertoire contains CD1a autoreactive T cells that home to the skin and modulate keratinocytes through interleukin-22

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CD1a is an antigen presenting molecule that is structurally homologous to MHC class I, but instead of peptides it presents self and foreign lipid antigens to T cells. Because CD1a is highly expressed on Langerhans cells in human epidermis, it is often used as a marker to identify these cells, yet the functional role of CD1a in skin immunology and the frequency and natural functions of CD1a-restricted T cells are almost entirely unexplored. To study the human CD1a-restricted T cell repertoire, we developed a system to measure CD1-reactive T cells in *ex vivo* T cell populations. We used K562 cells with low to absent surface expression of MHC proteins, so that they might be used as universal antigen presenting cells without causing alloreactivity from MHC-mismatched donors and transfected these cells to express high levels of CD1. By screening a large panel of blood bank donors we found unexpectedly strong T cell responses to CD1a, not to other CD1 isoforms. CD1a-autoreactive T cells in the blood were enriched in memory T cell populations and expressed skin homing markers. Indeed, CD1a-reactive T cells were recovered from the dermis and were potently activated by epithelial Langerhans cells. In contrast to conserved T cell receptor alpha (TCRalpha) and Th1-like cytokine profile of CD1d-restricted NKT cells, CD1a-autoreactive T cells express diverse TCRs and secrete Interleukin-22, a cytokine involved in epithelial homeostasis and defense. Thus, CD1a-reactive T cells represent a normal part of the T cell repertoire in humans and play a role in skin immunity and homeostasis

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Platelet induction of monocyte-to-dendritic cell maturation

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Activated platelets express and release a broad range of proteins with known immunologic roles. These elaborated products include proteins normally encountered on the activated endothelium, extracellular matrix, and activated leukocytes; such as p-selectin, fibronectin, and CD40L. Because platelets abundantly accumulate in wound healing and inflammatory sites, often in close association with monocytes and dendritic cells, we hypothesized that platelet-monocyte interactions may contribute to monocyte differentiation to dendritic antigen presenting cells (DC). To test this hypothesis, a parallel-plate flow chamber was used to pass mononuclear cells over plastic-surface-immobilized platelets. Using digital microscopy, video analysis, and FACS, we determined that: (1) monocytes transiently adhere to and "jump" from platelet to platelet as they flow across the field of view; (2) the number of adhesions are dependent on flow dynamics, especially wall shear stress, with maximal adhesions occurring between 0.3-0.6 dyne/cm^2; and (3) a high correlation exists between the number of adhesions observed and the yield of new DC, as assessed by induction of the DC CD83+/DR+ phenotype. To maximize platelet-monocyte interactions, parallel-plates were then coated with high concentrations of autologous platelets, and mononuclear cells were passaged at wall shear stress of 0.5 dyne/cm^2 for 75 minutes. FACS analysis of the monocyte population confirmed an average conversion of 71% of the processed monocytes to the DC pathway, in a single day, in the absence of added cytokines. These findings reveal that platelets can directly contribute to rapid, and perhaps physiologic, monocyte-to-DC maturation. By maximizing platelet-monocyte interactions *in vitro*, a four-fold increase in cells phenotypically-consistent with mature DC was obtained. The rapidity and efficiency of our observed platelet induction of new DC suggests an *in vivo* role for the mechanism. This system may also facilitate the *in vitro* production of large numbers of DC for both experimental and clinical use.

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Antimycotics enhance the production of human β-defensin-3 in human keratinocytes: Another possible mechanism for antimicrobial effects

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Objectives: Antimycotic agents improve cutaneous symptoms of atopic dermatitis. These patients are susceptible to the infection with Staphylococcus aureus, partially due to the reduced production of antimicrobial peptides like human β-defensin-3 (hBD-3) in the epidermis. We recently found that prostaglandin D2 (PGD2) enhances hBD-3 production in human keratinocytes by stimulating activator protein-1 (AP-1) via chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2). We examined *in vitro* effects of antimycotics on hBD-3 production in human keratinocytes. Methods: hBD-3 secretion and mRNA levels were analyzed by ELISA and RT-PCR, respectively. AP-1 activities were analyzed by luciferase assays. Results: Itraconazole and terbinafine hydrochloride increased hBD-3 secretion and mRNA levels in keratinocytes in parallel to the enhancement of AP-1 activity, c-Fos expression and phosphorylation while fluconazole was ineffective. These effects of antimycotics were abrogated by CRTH2 antagonist. Prostaglandin H2, a precursor of PGD2 can be converted to thromboxane A2. Itraconazole and terbinafine hydrochloride increased PGD2 release from keratinocytes and reduced that of thromboxane B2, a metabolite of thromboxane A2. Conclusions: Itraconazole and terbinafine hydrochloride may enhance c-Fos expression and phosphorylation, AP-1 activity and hBD-3 production by increasing PGD2 release from keratinocytes. These antimycotics may suppress thromboxane A2 synthesis and redirect the conversion of PGH2 toward PGD2. The induction of hBD-3 in keratinocytes may be another possible mechanism for the fungicidal effects of itraconazole and terbinafine hydrochloride. These antimycotics may restore the compromised defense activity against Staphylococcus aureus in the patients with atopic dermatitis via hBD-3.

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Staphylococcus aureus hijacks a skin commensal to intensify its virulence: Vaccination targeting β-hemolysin and CAMP factor

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The need for a new anti-Staphylococcus aureus (S. aureus) therapy, which can effectively cripple bacterial infection, neutralize secretory virulence factors and lower the risk of creating bacterial resistance, is undisputed. Our data demonstrated that the hemolysis and cytolysis by S. aureus were noticeably augmented when S. aureus was grown with Propionibacterium acnes (P. acnes), a key member of human skin microbiome. The augmentation was significantly abrogated when the CAMP (Christie, Atkins, Munch-Peterson) factor of P. acnes was neutralized or β-hemolysin of S. aureus was mutated. In addition, the hemolysis and cytolysis of recombinant β-hemolysin were markedly enhanced by recombinant CAMP factor. These results strongly suggest that S. aureus β-hemolysin reign over P. acnes CAMP factor to amplify its virulence. P. acnes also exacerbated S. aureus-induced skin lesions in mice. The combination of CAMP factor neutralization and β-hemolysin immunization cooperatively suppressed the skin lesions caused by co-infection of P. acnes and S. aureus, providing a novel immunotherapy targeting the interaction of S. aureus with a skin commersal. P. acnes enhanced the hemolytic activities of both hospital-acquired and community-acquired methicillin-resistant S. aureus (MRSA) bacteria, suggesting the novel immunotherapy can be extended for treatment of MRSA infection in the future. Here, we propose a novel infectious mechanism by which S. aureus may commandeer P. acnes to spread its invasion and highlight two secretory virulence factors (S. aureus β-hemolysin and P. acnes CAMP factor) as potential molecular targets for immunotherapy against S. aureus infection. Anti-S. aureus therapy targeting S. aureus alone without considering its interaction with human commersals may exhibit a cutoff in potency for the treatment of S. aureus/MRSA infection.

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N-acetyl-S-farnesyl-L-cysteine (AFC) suppresses ATPγS-induced CXCL8, CCL2 and CXCL1 production in a human dermal microvascular endothelial cell line (HMEC-1) and primary human dermal microvascular endothelial cells (pHMDECs)

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AFC is a small-molecule isoprenylcysteine analog that inhibits G-protein coupled receptor (GPCR) signaling. Topical application of AFC inhibits local skin inflammation in a TPA-induced contact irritation mouse model. AFC also inhibits macrophage chemotaxis and neutrophil aggregation. However, the mechanism of these anti-inflammatory effects is not well understood. Endothelial cells (ECs) can secrete chemokines that recruit inflammatory cells from blood into tissue. We have previously shown that both adenosine-5′-triphosphate (ATP) and ATPγS, a hydrolysis-resistant ATP analogue, increase secretion of CXCL8, CCL2 and CXCL1 from HMEC-1 cells and pHMDECs. We have utilized this system to examine whether AFC can modulate ATPγS-induced production of CXCL8, CCL2, and CXCL1 by HMEC-1 cells and pHDMECs *in vitro*. Subconfluent cultures of HMEC-1 cells were pre-incubated for 2 hrs with various concentrations of AFC and then co-treated with 100 μM ATPγS for 6 hrs to induce chemokine secretion. Then, supernatants were harvested and assayed for CXCL8, CXCL1, and CCL2 content by ELISA. AFC dose-dependently inhibited production of all 3 chemokines while cell viability was not affected under these conditions as assessed by Trypan blue exclusion. Similar results were observed with pHDMECs. Real-time PCR for CXCL8, CCL2 and CCL1 mRNA suggested that AFC inhibition is occurring at the mRNA level. N-acetyl-S-geranyl-L-cysteine (AGC) is an AFC analogue that is inactive at blocking GPCR signaling. AGC did not inhibit stimulated chemokine release from HMEC-1 cells. Upon treatment of HMEC-1 cells with ATP or TNFα as alternative stimulators, AFC also dose-dependently decreased induced chemokine release, suggesting possible activities in addition to GPCR inhibition. AFC appears to exert anti-inflammatory effects, at least in part, through inhibition of chemokine production by stimulated ECs.

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Staphylococcal LTA suppresses TLR3-dependent inflammation following skin injury

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Keratinocytes have microbial recognition systems such as TLRs, but commensal microflora such as Staphylococcus epidermidis (SE) normally do not initiate inflammation despite contact with keratinocytes. We therefore hypothesized that SE might produce a molecule to evade or suppress inflammation and confirmed this by observing that a <10-kDa product of SE suppressed inflammation after skin injury in mice and decreased IL-6 (-53.04%, p=0.0401) and TNF-α (-53.23%, p= 0.0015) Nature Medicine 2009 15(12) 1377. SE also suppressed IL-6 and TNF-α mRNA/protein release by human keratinocytes (NHEK) stimulated by a TLR3 ligand [poly(I:C)], but not by other stimuli. The suppressive molecule from SE was identified as a unique lipoteichoic acid (LTA) with D-alanine, and confirmed by use of synthetic LTAs. This inhibitory effect was specific as other synthetic LTAs failed to suppress TNF-α, while staphylococcal LTA had the opposite effect (induced TNF-α) other cell types. The mechanism for suppression of TLR3 signaling was through the activation of TLR2 to induce the negative regulator TRAF1, as SE LTA failed to suppress IL-6 and TNF-α in Traf1-/- and Tlr2-/- mice. These findings also suggested that TLR3 participates in stimulating inflammation after normal injury, a hypothesis confirmed in Tlr3-/- mice that were shown to have less production of IL-6 and TNF-α at the wound edge compared to controls (-66.47% IL-6, p=0.0022; -69.78% TNF-α, p= 0.0311), and in NHEK shown to respond to RNA released from necrotic cells in a TLR3-dependent manner. The physiological relevance of this system was further seen by observing decreased expression of the negative regulator TRAF1 in the wounds of antibiotic-treated mice (-47.49%, p=0.2256) and in the skin of germ-free mice (-23.56 fold ±16.37) compared to controls. These findings show for the first time that skin requires TLR3 for normal inflammation after injury and that the microflora can modulate inflammatory responses. Our findings emphasize the potential negative consequences of complete depletion of microflora from skin by indiscriminate use of topical and systemic antibiotics.

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Dietary vitamin D3 protects against bacterial skin infection

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In skin infection vitamin D metabolism is activated to induce expression of the antimicrobial peptide cathelicidin, as well as CD14 and TLR2, thus enabling increased response and recognition of microbes. The aim of this study was to assess if exposure to vitamin D3 is physiologically relevant for increasing resistance to infection. Human keratinocytes (NHEK) stimulated in culture with 100 nM 25-D3 for 24 h showed 150-fold higher cathelicidin mRNA and 25-fold increased CD14, but when co-stimulated with a low MW (<10kDa) fraction of S.aureus (SA113), expression further increased for cathelicidin (510-fold) and CD14 (190-fold). There was no increase with the low MW SA fraction alone, indicating a synergistic effect of 25-D3 and SA. Functional relevance was seen in a bacterial killing assay in which lysates from NHEK co-stimulated with 25-D3 and SA most efficiently killed S. aureus deltaMPRF (3-fold decrease in SA growth). Based on these observations we wished to develop a mouse model to test the importance of vitamin D in vivo. Mice lacking the 1α-hydroxylase enzyme CYP27B1 were fed a special chow devoid of vitamin D3 for 3 weeks to achieve vitamin D deficiency (n=7), and were then compared to CYP27B1-/- mice receiving vitamin D (2200 IU/kg) in their diet (n=6). 3 days after injection sc. with 10^7 CFU of Group A streptococcus NZ131 (GAS), mice lacking vitamin D in their diet developed significantly larger skin lesions (30.4 ± 14.95 mm vs. 5.72 ± 1.12 mm, p<0.05) and had more bacteria (2 fold increase, p<0.05) than when receiving vitamin D. Interestingly, wild-type mice (C57Bl6/J) on the vitamin D3 deficient diet developed smaller lesions than CYP27B1-/- but larger than the CYP27B1-/- mice on the high vitamin D diet. Our results show that vitamin D3 intake may protect from bacterial infection in vitro and in vivo. Vitamin D3 supplementation should be evaluated in the prevention and therapy of bacterial skin infections.

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Sustained and dose-dependent induction of cathelicidin and defensins in human skin following UV irradiation

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Antimicrobial peptides (AMPs) are small, evolutionarily conserved, peptides involved in innate immunity. Aberrant expression and processing of AMPs, such as cathelicidin (LL-37), have been implicated in promoting inflammation in rosacea. Additionally, compared with psoriasis, a relative deficiency of AMPs in atopic dermatitis may predispose atopic patients to frequent microbial infection. Interestingly, ultraviolet (UV) irradiation is thought to have a pathogenic role in rosacea, whereas UVB phototherapy alone or in combination with UVA is used to treat atopic dermatitis. To investigate the potential relationship between AMPs and UV irradiation, normal human skin was irradiated with 2 MED UVB (N=12) and punch biopsies were obtained at baseline, 6, 24, 48, and 72 hours post-irradiation. RT-PCR was used to quantify mRNA expression of DEFA1, HBD-2, HBD-3, and LL-37. Expression of DEFA1 was maximally increased 23-fold at 6 hours; LL-37 was maximally increased 4-fold at 48 hours; and HBD-3 and HBD-2 were maximally increased 180 and 1290-fold, respectively, at 72 hours, (all $p<0.05$). Both DEFA1 and LL-37 remained elevated at 72 hours. Immunohistochemistry revealed that HBD-3 protein levels were increased 2190-fold in the epidermis at 72 hours post-irradiation, compared with no treatment (N=4, $p<0.05$). Additionally, AMP mRNA levels were measured in human skin at 24 hours post-irradiation with 20, 40, and 80 J/cm^2 UVA1 (N=10). HBD-3, LL-37, DEFA1, and HBD-2 mRNA expression increased in a dose-dependent fashion, and maximal levels of 8, 17, 78, and 335-fold, respectively, were seen following 80 J/cm^2 (all $p<0.05$). These findings indicate that AMPs are rapidly induced in a dose-dependent manner following UV irradiation, with sustained induction for at least 72 hours post-irradiation. Such an increased AMP profile following UV irradiation may contribute to flares of rosacea that can occur following sunlight exposure and illustrates one potential mechanism of UV irradiation as a treatment modality for atopic dermatitis.

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Staphylococcus aureus derived monomeric peptidoglycan is a NOD2 ligand and aggravates TLR mediated inflammation by amplifying Th1 and Th17 responses

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Staphylococcus aureus causes severe acute infections and is a co-factor of chronic inflammatory diseases such as atopic dermatitis (AD). Innate immune sensing of *S. aureus* by pathogen recognition receptors (PRR) may cause harmful inflammation. Important *S. aureus* PRR ligands are peptidoglycan (PGN), lipoteichoic acid (LTA), and lipoproteins (LPP). To characterize their exact role, polymeric PGN was purified from wildtype *S. aureus* and from *S. aureus* deficient in either LPP or LTA. NFκB reporter assays revealed that PGN activity depended on LPP or LTA. Therefore, PGN was digested into fragments that are also naturally released from bacteria, and purified monomeric PGN devoid of LPP or LTA activity was analyzed. In contrast to stimulation with *S. aureus* TLR ligands, dendritic cells (DC) remained immature and produced no cytokines after incubation with monomeric PGN alone. However, monomeric PGN enhanced IL-12p70 and IL-23 levels induced by different *S. aureus* TLR ligands. To define the coactive pathway utilized by monomeric PGN, DC deficient in NOD2 proteins were analyzed. Strikingly, amplification of IL-12p70 and IL-23 production by monomeric PGN was completely abolished in NOD2-/- DC identifying *S. aureus* monomeric PGN as a natural NOD2 ligand. To investigate consequences on adaptive immune responses DC-T-cell cocultures were set up. Dual activation of DC with TLR and the NOD2 ligand monomeric PGN lead to several fold upregulation of IFN-γ and IL-17 production by Th cells and diminished IL-4 levels. Thus, combinative innate immune sensing of *S. aureus* preferentially induces Th1 and Th17 while suppressing Th2 cells. Our data demonstrate that monomeric PGN is an active and potent *S. aureus* PAMP and acts in a newly discovered amplifying circuit of *S. aureus* innate immune sensing that finally boosts pro-inflammatory Th1 and Th17 responses and suppresses Th2 cells leading to sustained inflammation as also seen in chronic AD.

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IL-23 and Th17 cytokines control cutaneous infection with *Candida albicans* as well as *C. albicans*-induced epidermal hyperplasia

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IL-23 and Th17 cells play an important role in controlling systemic infections with extracellular bacteria and fungi, although their roles in limiting infections in skin have not been well characterized. Consequently, we established a cutaneous infection model of *Candida albicans* to explore the contributions of IL-23 and Th17 cells in combating infection. Wild-type (WT) and various knockout (KO) mice were injected in the dermis at a dorsal site with *C. albicans* pseudohyphae ($5x10^6$ cells) and nodule size was measured 3x weekly by an investigator blinded to the mouse genotype. WT mice healed *Candida* lesions efficiently (median=13 days). IL-12-deficient (*IL-12p35* KO) and *IL-22* KO mice healed *Candida* lesions in a similar manner ($p>0.15$ vs WT for both). IL-23-deficient mice (*IL-12/23p40* KO and *IL-23p19* KO mice) and *IL-17A* KO mice, however, demonstrated delays in resolving infections ($p<0.02$ vs WT for all 3). In WT mice, *Candida* induced 1) expression of *IL-12/23p40* and *IL-23p19* mRNAs within 6 hours post-infection; 2) expression of *IL-17A* and *IL-22* mRNAs 24 hours post infection; and 3) epidermal hyperplasia overlying infected skin 4 days post-infection. In skin of IL-23-deficient mice, neither *IL-17A* nor *IL-22* mRNAs were expressed following infection, and these mice demonstrated minimal epidermal hyperplasia. In skin of IL-12-deficient mice, IL-22 mRNA and protein levels were higher and epidermal hyperplasia was more pronounced following *Candida* infection relative to WT mice. These results establish that IL-23 is critical in controlling cutaneous candidiasis. Additionally, these results indicate *Candida* is a potent inducer of IL-23 in skin, and that *Candida* infection stimulates IL-23-dependent epidermal hyperplasia. In this model, IL-12 does not directly influence infection healing rates, but can limit epidermal hyperplasia mediated by IL-23. We speculate that cutaneous *C. albicans* may act as a trigger for psoriasis by stimulating local production of IL-23, and that blockade of IL-23 in psoriasis may predispose individuals to *Candida* infections.

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Orchestration of atopic dermatitis inflammation by Staphylococcus aureus: Toll-like receptor 2 ligands exaggerate dermatitis and Th1 responses

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Atopic dermatitis (AD) is a chronic inflammatory skin disease induced by infiltrating Th cells. Th2 cells dominate acute flares of AD as seen in atopy patch test lesions. Chronic AD lesions are often indistinguishable from other forms of eczema and dominated by Th1 cells. The mechanisms underlying this change of inflammatory pattern in AD remained elusive. Colonization with Staphylococcus aureus, a gram-positive bacteria providing potent TLR2 ligands, is seen in >90% of AD patients. However, the impact of TLR2 ligands on AD inflammation is still unclear. To this end, we established a model for acute AD inflammation by adoptively transferring and activating OVA-specific Th2 cells in the skin of naive mice. The increase of ear thickness in this model correlates with antigen-specific inflammation. Th2 cells or OVA alone only lead to minor changes. In contrast, Th2 cells plus OVA provoked inflammation and strong ear swelling after 24h. One important pathogen associated molecular pattern of Staphylococcus aureus is the cell wall component and TLR2 ligand lipoteichoic acid (LTA). Importantly, LTA or TLR2 ligand Pam2Cys provoked prolonged and increased dermatitis, a pattern very similar to the OVA-specific dermatitis following transfer of Th1 cells. Cross-over experiments with TLR2-deficient mice and Th cells revealed that TLR2 on accessory cells but not on T cells is responsible for TLR2 mediated exacerbation of cutaneous inflammation. In addition, we could show that different TLR2 ligands stimulate dendritic cells to produce Th1 inducing IL-12p40 and IL-12p70 as well as IL-10. However, IL-10 is completely downregulated by IL-4 and cutaneous inflammation most pronounced in the absence of IL-10. Thus, we identified IL-10 as key regulator of TLR2 mediated inflammation.These data indicate that S. aureus derived TLR2 ligands shift Th2 cell dominated cutaneous inflammation towards chronic and persistent dermatitis through a concerted activation of TLR2 and IL-4R.

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Heat-treated vaccinia virus induces type I IFN production in dendritic cells and macrophages through distinct pathways

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Vaccinia virus is a prototypal poxvirus that belongs to the *Orthopoxvirus* genus. Poxviruses are large cytoplasmic DNA viruses that cause significant human and veterinarian diseases. They have been investigated as potential vaccine vectors and agents for oncolytic therapy. We have previously shown that vaccinia infection in dendritic cells (DCs) attenuates innate immune responses and inhibits the ability of the infected DCs to activate antigen-specific T cells. Here we report that heat treatment of vaccinia virus by incubating the virus at 55°C for 1 h converts an immune suppressive virus to an immune stimulating virus. Infection with heat-treated vaccinia induces type I IFN and pro-inflammatory cytokine and chemokine induction in plasmacytoid dendritic cells (pDCs), conventional dendritic cells (cDCs) and macrophages. It also induces DC maturation. Using various genetic knock-out mice that have deficiencies in pathogen recognition receptors, we determined that heat-treated vaccinia induction of type I IFN in pDCs is dependent on TLR7/MyD88, IRF5/IRF7 and IFNAR. However, the induction of type I IFN by heat-treated vaccinia in cDCs is TLR-, RLR-, and IRF3 independent, whereas in macrophages, the induction effect is TLR-, RLR-independent, but IRF3-dependent. Vaccinia E3 is an important immunomodulatory protein that subverts host antiviral immune responses. Here we show that the Z-DNA binding domain of vaccinia E3 is required for suppressing IFN induction by heat-treated vaccinia in various cell types. Our study reveals cell-type specific poxviral sensing pathways that lead to type I IFN production. Heat-treated vaccinia has the potential to developed as a novel vaccine adjuvant.

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UVB triggered induction of vitamin D3 metabolism differentially affects antimicrobial peptide expression in keratinocytes

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Antimicrobial peptides (AMPs) are important effectors in cutaneous innate immunity as antibiotics and signaling molecules in the defense against microbes. The regulation of AMP expression and function in the skin is not completely understood. UVB irradiation of human keratinocytes induces the expression of human beta-defensin 2 (HBD2) *in vitro* and *in vivo*. In contrast, expression of another AMP, cathelicidin, was found to be induced in skin *in vivo* upon UVB irradiation, *in vitro* no induction in cultured keratinocytes was observed. In this study we investigated the effect of UVB irradiation on AMP expression in primary human epidermal keratinocytes (NHEK) in the presence or absence of the 1α,25-dihydroxyvitamin D3 (1α,25D3) precursor 7-dehydrocholesterol (7-DHC). Generation of active 1α,25D3 from 7-DHC requires UVB irradiation. Indeed, UVB irradiation of human keratinocytes supplemented with 7-DHC triggered the synthesis of hormonally active 1α,25D3 by primary keratinocytes which then differentially affected the expression of cathelicidin and HBD2: UVB irradiation alone induced HBD2 in keratinocytes, whereas cathelicidin expression was not affected. Addition of 7-DHC before UVB-treatment led to elevated cathelicidin but reduced HBD2 levels. Inhibition of CYP27B1 – the enzyme that activates vitamin D3 by 1α-hydroxylation - by ketoconazole or siRNA techniques reversed this effect. These findings might provide an explanation for the differences observed in cathelicidin regulation upon UVB treatment *in vitro* and *in vivo*. We propose that these differences might be due to high levels of 7-DHC in epidermal keratinocytes *in vivo* compared to low levels in cultured keratinocytes.

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Heat-treated vaccinia virus induces autophagy in macrophages and dendritic cells

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Autophagy is a process of "self-eating" through lysosomal degradation of long-lived proteins and organelles. It is characterized by the formation of double membrane-bound autophagosomes *de novo* to sequester cytoplasm and then fusion with lysosomes to form autophagolysosomes. Autophagy has been implicated in many diverse processes including development, differentiation and tissue remodeling. It is also involved in sensing pathogen associated molecular patterns (PAMPs) and the induction of host immune responses. Autophagosome protein LC3 translocation from the cytosol to newly formed autophagosomes which appear as cytoplasmic puncta is a hallmark of autophagy. We generated RAW264.7 macrophage cells expressing green fluorescent protein (GFP)-LC3 through lentivirus transduction and drug selection. Infection of macrophages with heat-treated vaccinia but not with untreated live vaccinia induced LC3 puncta formation. Electron microscopic (EM) images of macrophages infected with heat-treated vaccinia revealed the formation of many double membrane-bound vesicles indicative of autophagosomes. We observed that treatment with endosomal/lysosomal acidification inhibitors chloroquine and bafilomycin A1 blocked the induction of type I IFN by heat-treated vaccinia in macrophages, indicating that endosomal/lysosomal digestion of viral particles might be important for the release of viral DNA to be detected by cytosolic DNA sensors. Similarly, treatment with a PI3K inhibitor, 3-methyladenine, resulted in the reduction of type I IFN induction by heat-treated vaccinia, which is consistent with the role of PI3K involvement in autophagy induction. Similar results were observed with bone marrow-derived dendritic cells. Taken together, our results indicate that heat-treated vaccinia induces autophagy in macrophages and dendritic cells and autophagy formation is required for detecting cytosolic viral nucleic acids by host sensors.

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***Propionibacterium acnes* induces an inflammatory response in keratinocytes via crosstalk of the p38 MAPK and NF-κB pathways**

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Acne vulgaris is a common disorder of the skin which manifests in the pilosebaceous unit, and affects around 40-50 million people. The pathogenesis of acne is multifactorial, and includes follicular hyperkeratinization, increased sebum production, and inflammatory mediators. In recent years, there has been a greater understanding of the role of p. acnes in the etiology of acne; however, molecular mechanisms that potentiate the inflammatory component of this disease are still being investigated. Towards this end, we evaluated the signaling cascade involved in upregulation of pro-inflammatory markers by p. acnes. Exposure to viable, as well as heat-killed p. acnes for 24 hr led to a prominent increase in the NF-κB promoter in keratinocytes as compared to the untreated control. Having shown the activation of NF-κB promoter by p. acnes, we then investigated if the p38 MAPK pathway was involved upstream of NF-κB. Primary keratinocytes were pre-treated with pharmacological inhibitors of NF-κB and p38 MAPK, BAY11-7082 and SB203580, respectively, followed by exposure to p. acnes. Treatment with SB203580 led to a reversal of the effects of p. acnes on NF-κB promoter, suggesting that p38 MAPK might be involved in p. acnes mediated signaling, working upstream of NF-κB. Treatment of keratinocytes with BAY11-7082 also led to substantial inhibition of p. acnes-induced NF-κB luciferase activity, confirming previous results. These observations were further corroborated by western blotting results from keratinocytes showing that p. acnes activates p38 MAPK and p65 NF-κB, which is eliminated by pre-treatment with the inhibitors. Furthermore, inoculation of keratinocytes with p. acnes also led to induction of the pro-inflammatory mediator, IL-8 and use of BAY11-7082 and SB203580 mitigated the release of this chemokine. Taken together, our results show that p. acnes-mediated inflammation is regulated through crosstalk of the p38 MAPK and NF-κB pathways, leading to upregulation of inflammatory markers.

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Reduced expression of dermicidin, a peptide active against Propionibacterum acnes, in the sweat of acne vulgaris patients

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Dermcidin (DCD) is an antimicrobial peptide that exhibits a broad spectrum of activity against bacteria. This peptide is expressed constitutively in sweat in the absence of stimulation due to injury or inflammation. DCD may play an important role in the onset of skin diseases associated with resident or environmental microbes, or with increased susceptibility to bacterial infection. To better understand this role, the relationship between DCD expression and acne vulgaris, associated with Propionibacterum acnes (P. acnes), was elucidated. Recombinant (r-) DCD was cloned. A histidine-tagged r-DCD was then constructed from the DCD gene region, without the signal sequence, and expressed in Escherichia coli using the pTKK19ubinew vector. r-DCD was partially purified by affinity chromatography and the histidine-tag was removed with ubiquitin hydrolase. The antimicrobial activity of r-DCD against P. acnes was evaluated with a colony forming unit (CFU) assay. An IgG monoclonal antibody (10C3) was developed by immunization with r-DCD. The amount of DCD in the sweat of patients with acne vulgaris (AC) was compared with healthy subjects using an original monoclonal antibody raised against r-DCD. Human sweat, induced by physical exercise on a bicycle ergometer, was collected from the back of 14 healthy volunteers and 15 AC patients. The DCD concentration in sweat samples was determined by solid-phase ELISA using 10C3. Two key findings were made. By CFU assay, r-DCD demonstrated a microbicidal rate of 83% against P. acnes. Furthermore, the DCD concentration in the sweat from AC patients was significantly lower than that in the sweat from the healthy subjects. P. acnes, thriving as a resident microbe, is linked to the onset of acne vulgaris. The antimicrobial activity of DCD against P. acnes and the reduced DCD secretion in AC patients provides novel insights into the incidence of acne vulgaris.

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Long-term ICAM-1 and transient PDGF-B expression on PMA-activated THP-1 cells harboring early-onset sarcoidosis/Blau syndrome-associated *NOD2* mutations

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Sporadic early-onset sarcoidosis and familial Blau syndrome form a distinct class of autoinflammatory syndromes showing early-onset systemic granulomatosis. These diseases are associated with *NOD2* mutations causing constitutive nuclear factor-κB activation. Although intracellular signaling pathway through Nod2 has been intensely investigated, it is still unclear how *NOD2* mutations result in granuloma formation. To clarify the precise mechanisms of *NOD2* mutation-associated granuloma formation, human monocytic THP-1 cells expressing disease-associated *NOD2* mutations were generated and analyzed. Without stimulation, no significant difference of cytokine expressions was observed among THP-1 derivatives. Therefore, PMA was added to induce differentiation of THP-1 cells into macrophage-like cells. Morphologically, THP-1 cells spread pseudopods and attached to the culture plate for several days after PMA addition, then again floated in medium to proliferate. Interestingly, mutant THP-1 cells spread more and longer pseudopods and attached to the culture dish for longer period. By RT-PCR, expression of PDGF-B was specifically induced in mutant THP-1 cells after short exposure to PMA. Furthermore, by flow cytometric analysis, although similarly upregulated ICAM-1 expression was observed on both control and mutant THP-1 cells at short period after PMA addition, following downregulation of its expression was specifically inhibited in mutant cells. Considering the previously-reported involvement of PDGF-B and ICAM-1 in ordinary sarcoidosis, these effects should contribute to the *NOD2* mutation-associated granuloma formation.

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Antimicrobial and anti-inflammatory effects of newly designed synthetic Cecropin A (1-8)-Magainin 2 (1-12) hybrid peptide CA-MA analogue P5 against Malassezia furfur

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The lipophilic fungus Malassezia furfur (M. furfur) is a commensal microbe, which is associated with several chronic diseases such as pityriasis versicolor, folliculitus, and seborrheic dermatitis. Antimicrobial peptides are promising new antifungal candidates due to an action mechanism distinct from previously known antifungal agents. These small cationic peptides also function as effectors of innate immunity in skin. In this study, we investigated the antimicrobial and anti-inflammatory effects of the newly designed synthetic antimicrobial CA-MA analogue peptide P5 on M. furfur. The minimal inhibitory concentration (MIC) value of P5 against M. furfur was 0.39 μM making it 3 to 4 times more potent than commonly used antifungal agents such as ketoconazole or itraconazole. P5 exhibited no cytotoxicity against normal human keratinocytes (HK) as determined by MTT assay at the concentration found to be fungicidal. P5 efficiently inhibited the expression of IL-8 mRNA and the secretion of IL-8 protein upon incubation of M. furfur with normal HK. Additionally, the peptide significantly inhibited the M. furfur-induced NF-kappaB translocation from the cytoplasm to the nucleus. These results demonstrate for the first time that the newly designed synthetic antimicrobial CA-MA analog peptide P5 negatively regulates the activation of NF-kappaB, which is closely related with enhanced expression of inflammatory genes induced by incubation of M. furfur with normal HK. These results demonstrate that the newly designed synthetic peptide P5 may have significant potential as a pharmacological agent for the treatment of human skin disease associated with fungi such as M. furfur.

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Production of LL-37 during herpes simplex virus types 2 infection in human keratinocytes enhances HIV susceptibility in Langerhans cells

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Recent studies have shown that patients who were infected with herpes simplex virus types 2 (HSV-2) had three times the risk of acquiring HIV, however biologic mechanisms responsible for enhanced HIV acquisition are unclear. Since Langerhans cells (LC) were thought as initial cellular targets in sexual transmission of HIV, we tested whether HSV-2 affected HIV susceptibility in LC directly or indirectly via keratinocytes. To assess the direct effects, monocyte-derived LC (mLC) were exposed with HSV-2 before HIV infection. HIV infection in mLC was analyzed by HIV p24 intracellular staining in FACS. Interestingly, HSV-2 did not affect HIV susceptibility in mLC. As for the indirect effects, we tested whether antimicrobial peptides (AMPs), including αDefensin 5, 6 and βDefensin 1, 2, 3, 4 and LL-37 affected HIV susceptibility in LC because it has been reported AMPs affected HIV susceptibility. Surprisingly, only LL-37 dramatically enhanced HIV susceptibility in mLC, whereas other AMPs did not. On the other hand, HSV-2 infection in human keratinocytes increased the production of LL-37, but not other AMPs. To assess the physiological effects of HSV-2 on LC, we used *ex vivo* skin explant model. Epithelial sheets were incubated with HSV-2 before HIV infection, and then floated. The emigrating LC were collected 3 days after the HIV exposure. HIV-infected LCs were assessed using HIV p24 staining. LC from HSV-2 treated epithelial sheets significantly enhanced HIV susceptibility about three times compared with LC from no treated epithelial sheets. Regarding mechanisms underlying increased HIV infection by LL-37 in LC, we found that LL-37, but not other AMPs, induced significant up-regulation of cell surface CD4 and CCR5 on mLC. Furthermore, LL-37 significantly decreased mRNA expression of TRIM5α, but not APOBEC3G/3F, HIV suppressor factors. Given these data, LL-37 produced from HSV-2 infected keratinocytes enhances HIV susceptibility in LC.

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The contact allergen 2,4-dinitrochlorobenzene has minimal effects on microRNA-155 expression in human monocyte-derived dendritic cells and in a skin explant model

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Allergic contact dermatitis is a considerable clinical problem, accounting for a significant proportion of occupationally-related dermatoses and causing substantial morbidity for affected individuals. The main approach to treatment is through limiting further exposure to the offending allergen, hence there is a need to identify and understand mechanisms which allow certain individuals to develop allergies to relevant compounds. There is evidence that microRNA-155 plays an important role in many aspects of immunity, including antigen presentation, and it has been reported that this microRNA can be upregulated by TNF-alpha and IL-1beta, both of which play a role in Langerhans cell (LC) migration during allergic contact sensitisation. In this study, the expression of microRNA-155 was investigated following the application of the potent contact allergen 2,4-dinitrochlorobenzene (DNCB) in cultures of human monocyte-derived dendritic cells (MoDCs) and in a human skin explant model. Quantitative PCR demonstrated some variation in microRNA-155 expression by MoDCs after DNCB application. However, there was no significant overall increase following exposure to this agent at 6 hours; n= 8 subjects. Despite the migration of LC out of the epidermis after topical exposure to DNCB (observed by a reduction in epidermal LC in all cases at 18 hours; n=8 subjects), variable expression of microRNA-155 was noted in a skin explant model, with no evidence of a consistent increase in this microRNA at 1, 6 and 18 hours; n=2, 4 and 3 subjects respectively. The results indicate that the levels of microRNA-155 are not routinely elevated in MoDCs and in human skin following exposure to the potent allergen DNCB, thus the data suggest that microRNA-155 is unlikely to play a role in allergic contact sensitisation to DNCB.

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TLR2-activation turn mast cells in skin sentinels against viruses

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Mast cells are well known effectors of allergic reactions and are considered sentinels in the skin and mucosa. Their localization and capacity to produce and release antimicrobial peptides (AMPs), such as cathelicidin, protects against bacterial infections when the epithelial barrier is breached. We therefore hypothesized that mast cells could also act as sentinels in the skin against viral infections. This hypothesis was supported using a mouse model of viral infection. Mast cell-deficient ($KitW^{-sash/-}$) mice were more susceptible to vaccinia virus (VV) infection than the wild-type animals, while $KitW^{-sash/-}$ mice reconstituted with mast cells in the skin showed a normal response to VV. Using mast cells derived from mice deficient in cathelicidin, we showed that antimicrobial peptides were the most important anti-viral granule components in mast cells. The mechanism of cathelicidin induction during VV infection in mast cells was through the activation of Toll-like receptor 2 (TLR2) by bacterial byproducts (lipotechoic acid, LTA) at the epithelial surface. Signaling through TLR2 increased the production and expression of cathelicidin in mast cells thereby enhancing their capacity to fight VV. Taken together, our findings reveal one possible mechanism by which mast cells transition from a histamine storage container to a well-organized weapon to fight viral infection. Furthermore, here we suggest that the expression and control of AMPs and TLR signaling on mast cells is likely the key to this transformation. Our findings also provide new insights into the pathogenesis of skin infections and suggest potential roles for mast cells and TLR2 ligands in anti-viral therapy.

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Claudin-1 defect in atopic dermatitis may enhance susceptibility to HSV-1 infections.

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Atopic Dermatitis (AD) subjects are recognized for their susceptibility to recurrent, widespread cutaneous infections with HSV-1, called eczema herpeticum (EH). The mechanism for this enhanced HSV infectivity is unknown. We have recently shown that AD subjects have a selective reduction in claudin-1 (CLDN1), a key component of tight junctions (TJ) in the human epidermis. We hypothesized that this TJ defect may also contribute to the development of EH. To determine if reductions in epidermal CLDN1 contributed to HSV-1 spreading, we infected confluent control and claudin-1-deficient primary human keratinocytes (KC) monolayers with the highly virulent HSV-1 strain F (MOI 0.1). CLDN1-specific siRNA induced a dose-dependent reduction in CLDN1 expression compared to scrambled siRNA-transfected cells. Importantly, expression of other TJ (occludin and ZO-1), AJ (e-cadherin and nectin-1) or SC (filaggrin) proteins were unaffected. CLDN1 knockdown significantly reduced transepithelial electrical resistance (P=0.007; n=4) and increased sodium fluorescein flux (P=0.026; n=4). In CLDN1-depleted KC we observed significantly increased number and size of HSV-1 focus forming units (FFU; 4.8 ± 0.7 FFU/field) as compared to siRNA-transfected cells (2.5 ± 0.5 FFU/field; P=0.05, n=6). Furthermore, the diameter and area of FFU were significantly greater in CLDN1-depleted KC (207.1 ± 21.5 μm and 15649.2 ± 4367.0 pixels, respectively) compared to controls (151.8 ± 39.6 μm and 9901.9 ± 4943.2 pixels; P=0.03 and P=0.04; respectively, n=6). Our data demonstrates that reductions in CLDN1, which reduce TJ integrity enable greater HSV-1 entry into surrounding epidermal cells. This observation lends further support for our hypothesis that defects in epidermal claudin-1 expression predispose AD subjects to greater HSV spread characteristic of EH. Funding:The Atopic Dermatitis and Vaccinia Network NIH/NIAID (N01 AI40029)

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Activating Transcription Factor 3 (ATF3)-mediated protection against endotoxins causes severe susceptibility to bacterial infections

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Systemic bacterial infections such as cellulitis or fasciitis generate severe oxidative stress and activate the innate immune system and may result in severe shock. These symptoms result from TLR-signals like lipopolisacharide (LPS) released during bacterial infections. Recent data revealed that activation of the negative transcription factor ATF3 provides protection against the toxicity resulting from innate immune activation by LPS and LPS-induced death, by initiating a negative feedback loop in the NFkB signal pathway. Here, we found that oxidative stress (ROS), as it occurs during sepsis, strongly enhances ATF3 expression by LPS-triggered dendritic cells (DC) or peritoneal macrophages (PM). *In vivo*, ROS resulting from glutathione depletion enhanced LPS-induced ATF3 4-fold, inhibited IL-6 protein production >90% *in vivo*, and significantly decreased the risk of LPS-induced lethality. This protection was fully reverted by the ROS scavenger N-acetyl-cysteine and strictly dependent on ATF3-induction, as glutathione depletion affected neither cytokine production nor survival in ATF3-/- mice. In sharp contrast, the increased awareness of ATF3-/- mice to innate signals such as LPS might, established solid protection against systemic infection: >90% of ATF3-/- mice survived conditions of bacterial infection after cecal perforation that were lethal within 2 days in wild type (wt) mice. In wt mice bacterial infection caused glutathione depletion, high ATF3 mRNA levels, low IL-6 and high mortality; glutathione depletion further enhanced suppression of IL-6 and mortality. In sharp contrast, ATF3-/- mice produced normal amounts of IL-6, even after glutathione depletion, and were largely protected from sepsis-induced mortality. These insights are essential for the management of bacterial and fungal infection, especially in the ever increasing community of immune compromised patients.

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The Activating Transcription Factor 3 (ATF3) determines the state of postseptic immune suppression in humans

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Following severe systemic infections, such as cellulites, patients are at high risk of severe secondary infections of bacterial, fungal or viral origin. This phenomenon is defined as postinfectious immune paralysis; however the molecular mechanisms underlying this phenomenon are elusive. ATF3 is has recently been identified as the first transcription factor in the NF-kB signaling pathway that appears after innate immune stimulation. ATF3 negatively regulates the transcription of IL-6 and TNF. Analysing the role of ATF3 in human disease, we first show a close correlation of severely suppressed glutathione-levels with the strong induction of ATF3 and the loss of activation induced IL-6 in postseptic patients. We therefore speculated that ATF3 might be the key transcription factor responsible for the postseptic immune suppression and the increased susceptibility to opportunistic infections. To test this hypothesis we used CLP (cecal ligation and puncture), one of the best-established models of bacterial sepsis. We first induced sublethal CLP in wild type (wt) and ATF3-/- mice, to closely imitate the clinical conditions. Subsequently we challenged the mice during the postseptic immune suppressive phase with the fungal pathogen Aspergillus fumigatus, at doses that are non pathogenic to healthy mice. Post-septic wt-mice rapidly succumbed to this sublethal pulmonary Aspergillus fumigatus infection. In sharp contrast, ATF3-/- mice had not only a significantly prolonged survival, 20% of these mice even survived the lung infection lethal to 100% of wt mice. Thus, ATF3 is an important regulator of postseptic immune suppression, that critically determines susceptibility to and the course of opportunistic infections. By showing, that this immune suppression directly results from glutathione-mediated ATF3 induction, we provide the basis for a rational treatment that counteracts this dangerous immune suppression.

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Survey of bacterial diversity on infant skin over the first year of life

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Infant skin has been shown to differ from adult skin in structure and function and continues to develop during the first years of life. Similarly, the bacterial communities on infant skin were found to change over the first year. Skin swabs were taken from skin of 35 infants (grouped into 1-3, 4-6, 7-12 month age groups). Three body sites were investigated: arm, forehead, and buttocks. Collected samples were analyzed for DNA markers of over 800 species of bacteria using a pyrosequencing approach. Analysis of the bacterial phyla in infant skin showed a predominance of Firmicutes, Actinobacteria and Proteobacteria. Bacilli were found to be one of the most abundant classes within the baby skin samples, along with Actinobacteria and Clostridia. At the genus level, the most prominent bacteria from forehead samples were Streptococci, Staphylococci, and Propionibacteria, from arm samples were Streptococci, Staphylococci, and Corynebacteria, and from buttock samples were Clostridia, Streptococci, and Ruminococci. Finally, the similarity in bacterial genera detected among the samples within an age group increased with age (or equivalently the variability decreased). Principal Component Analysis revealed that from all regions of sampling, those taken from the oldest infants, ages 7-12 months, had the highest degree of similarity, followed by the 4-6 month group, while the youngest group were least clustered (or less similar). This indicates that the infant's microbiome develops over time and certain taxa are able to dominate over others as the infants grow older.

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The HPV profile of cutaneous warts in HIV infected patients

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Cutaneous warts in HIV infected individuals are very common, despite anti-retroviral treatment. The problem is stigmatising for patients and consumes dermatology resources. Little is known about the human papillomavirus (HPV) types found in these cutaneous warts. We have profiled them using newly developed Luminex technology (HSL-PCR/MPG). A sensitive reverse hybridisation line probe assay (SPF10-LiPA$_{25}$, version 1) was also used to identify the presence of genital HPV types. DNA was extracted from 64 cutaneous warts from 42 HIV infected patients and 45 warts from 24 healthy individuals. Warts were clinically and/or histologically confirmed. HPV-7 was found in 22% (14/64) of warts from ten HIV infected patients but none (of 45 lesions) from healthy individuals (p=0.002). The commonest types in lesions from HIV infected patients were HPV-57 (38%; 24/64) and HPV-27 (34%; 22/64). HPV-2 was less common in warts from HIV infected patients (3% or 2/64 compared with 29% or 13/45 in healthy individuals; p=0.004). Mixed infections with cutaneous HPV types were seen in 19% (12/64) of lesions from HIV infected patients compared with 7% (3/45) from healthy cases (p>0.05). Genital HPV types were also frequently found in cutaneous warts from both groups; 19/64 (30%) in HIV infected lesions and 10/45 (22%) in lesions from healthy individuals (p>0.05). High and low risk types were equally represented. HPV-7 causes common warts but is also associated with butchers warts in meat and fish handlers. To date, case reports and smaller studies have implied that HPV-7 may be more prevalent in HIV and our data are in accord. Genital types seem common in cutaneous lesions; this phenomenon may be partly explained by the high sensitivity of modern assays.

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The HPV profile of genital warts in HIV infected patients.

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HIV infected individuals are more likely to harbour genital human papillomaviruses (HPVs) and have mixed HPV infections with high risk (oncogenic) HPV types. In contrast to cervical and anal lesions, HPV types in external genital warts from this group have been poorly characterised. The purpose of this study was to investigate the genital HPV profile found in external genital warts affecting patients with HIV. Twenty-one histologically confirmed genital warts from 16 patients were studied. Warts from healthy patients were also investigated (28 specimens from 21 patients). DNA derived from warts was analysed with a genital HPV reverse hybridisation assay (SPF10-LiPA$_{25}$, version 1). The commonest HPV types detected in HIV infected patients were HPV-11 (48%; 10/21), HPV-6 (33%; 7/21), and HPV-40 (10%; 2/21). In warts from healthy patients HPV-6 (82%; 23/28) and HPV-11 (11%; 3/28) were detected most frequently. HPV-6 was significantly less prevalent in HIV infected patients (p=0.002). Oncogenic genital HPV types were detected in one lesion from HIV infected patients and two lesions from healthy individuals. Multiple genital HPV types were found in similar numbers of lesions from both groups (3/21 in HIV infected patients and 2/28 in healthy individuals). Contrary to the detection ratio of HPV-6 : HPV-11 of 2-3:1 in the general population, we found that HPV-6 was much less prevalent in HIV infected persons (ratio 0.7:1). Determination of viral loads using real time PCR may be useful in ascertaining the significance of this.

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Langerhans cell dendrites penetrate through epidermal tight junction barrier during foreign antigen uptake

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The mammalian skin consists of two sets of epidermal barriers: stratum corneum (SC) and tight junctions (TJs). It has been long believed that epidermal Langerans cells (LCs) acquire foreign antigens encountered in skin, but how such antigens penetrate the epidermal barriers is completely unknown. Using 3D visualization method of epidermal TJs in mouse ear skin, we investigated the behavior of LC dendrites in relation to TJs. In resting state, LCs projected their dendrites upward to the skin surface but stopped short of TJs. Surprisingly, once activated by tape stripping or intra-dermal injection of IL-1β or TNFα, elongated LC dendrites docked with TJs, and sometimes penetrated through, to reach to the SC. Next, we investigated whether LC dendrites that penetrated TJ could engage in endocytic activity. To this end, we topically applied a protein biotinylation reagent on the skin. Remarkably, membrane biotinylation revealed endocytic activity that took place at the dendrite tip. Birbeck granule formation was observed at the tip of TJ-penetrated dendrite in electron microscopy, suggesting that Birbeck granules were involved in this process. Of note, TJ formation was observed between LC dendrites and surrounding keratinocytes, and lanthanum penetration assay indicated that the TJ barrier integrity was maintained during this dynamic process. Finally, we successfully visualized human epidermal TJs in 3D and demonstrated the existence of functional TJ barrier in human skin. TJ-docking of LC dendrites was also observed in human, suggesting that this ability of LCs to gain access to extra-TJ environment is conserved among mammals. These findings will provide a new fundamental framework for the immunological behavior of dendritic cells at the body surface.

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Development of a *Staphylococcus aureus* superficial skin infection model in mice that uses advanced *in vivo* imaging to evaluate topical antimicrobial therapy and immune responses in real-time

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S. aureus skin infections represent a rapidly growing epidemic that has been further complicated by the widespread emergence of MRSA and multi-drug resistant strains. To evaluate potential new topical antimicrobial therapies and cutaneous immune responses, a superficial *S. aureus* infection model in mice was developed. A bioluminescent *S. aureus* strain ($2x10^5$-$2x10^7$ CFUs/10 μl) was inoculated into three superficial scalpel cuts on the upper backs of mice and bacterial burden was measured in real-time by using *in vivo* bioluminescence imaging. To determine whether this model could be used to evaluate the efficacy of topical antibiotic therapy, mupirocin versus vehicle ointment was applied at 4 h then twice daily for 7 days. Mupirocin treatment resulted in up to 34-fold lower bacterial counts than vehicle treatment. To determine whether this model could be used to evaluate cutaneous immune responses, bacterial counts were compared between IL-1R-deficient mice and wt mice. IL-1R-deficient mice developed up to 20-fold higher bacterial counts than wt mice, consistent with the known role of IL-1 in immunity against *S. aureus* skin infection. Lastly, this model was used to evaluate the host neutrophilic response in real-time by using lysEGFP mice. The lysEGFP mouse strain is a genetically engineered mouse line that possesses green-fluorescent neutrophils due to a knockin of EGFP into the lysozyme M gene. We found that *S. aureus* burden as well as neutrophil infiltration could be measured in real-time by sequential *in vivo* bioluminescence and fluorescence imaging. Taken together, this superficial *S. aureus* skin infection model in mice may be a valuable tool to evaluate novel antimicrobial topical treatments and cutaneous immune responses.

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TRIF-mediated IgE isotype class switch which are inhibited by TLR9 and MyD88 activation

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The commitment of a B cell to isotype class switch to an IgE-producing cell is tightly regulated process. Lipopolysaccharide (LPS), a ligand for toll like receptor 4 (TLR4) can strongly induce IgE in B cells together with interleukin 4 (IL-4). This induction is inhibited by CpG oligodesoxynucleotides (CpG-ODNs) which are TLR9 ligands. TLR4 signaling is activated by TRIF and MyD88 adaptors whereas TLR9 is mainly activated by MyD88. However, the exact molecular mechanisms how CpG inhibit IgE production are not fully understood. Here, we first showed that CpG inhibit the TRIF-mediated TLR4 signals such as phosphorylation of IRF3 and processing of NF-kB p100 to p52. Consistently, we found that Trif-/- B cells could not produce IgE under LPS and IL-4 stimulation whereas Myd88-/- B cells produced the IgE similar level to wild type B cells. Also, LPS-induced NIK stabilization and p52 expression are impaired in Trif-/- B cells but not in Myd88-/- B cells. These findings may explain why CpG effectively inhibit allergy diseases such as atopic dermatitis and asthma in mouse model system.

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An antimicrobial protein, lactoferrin is expressed in eccrine glands of the human skin and exists in the sweat

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The eccrine gland is one of the major cutaneous appendages and secretes sweat. In addition to thermoregulatory function, antimicrobial peptides such as dermcidin and cathelicidin are secreted in human sweat. Lactoferrin is expressed and secreted by glandular epithelial cells such as mammary glands. It has demonstrated potent antibacterial, antiviral and antifungal activity. In this study, to investigate the hypothesis that sweat glands produce lactoferrin and sweat can play an important part in establishing a skin defense barrier against microbes, we evaluated the expression of lactoferrin in eccrine glands and sweat. By immunohistochemistry lactoferrin was detected in eccrine glands of normal human skin. In addition, lactoferrin was found in sweat obtained from healthy volunteers based on Western blot and ELISA. Interestingly, the specimen obtained from the normal skin surface showed lactoferrin. In the proteomic analyses of sweat, several antimicrobial peptides and proteins including lactoferrin were detected. These findings suggest that lactoferrin may contribute to skin defense against infection through its secretion in sweat.

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Exploration of requirements for the Wnt-signaling pathway in the development of murine epidermal Langerhans cells

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Langerhans cells (LC) are unique dendritic cells (DC) that populate stratified squamous epithelia. Despite extensive study, important aspects of LC development and function remain undefined. TGFβ1 is known to be critical for LC development, but other epidermis-derived influences may also be important. We identified EpCAM as a surface marker for LC years ago. Since EpCAM is a known Wnt-target gene, we hypothesized that epidermis-derived Wnt might regulate LC development. We first characterized effects of modulators of Wnt signaling on bone marrow-derived DC *in vitro*. Addition of Wnt-3a into bone marrow cultures with GM-CSF, M-CSF and TGFβ1 resulted in small increases (~24%, n=5 experiments) in the numbers of EpCAM+ DC that were recovered, but decreased the total numbers of cells in cultures. The Wnt inhibitor DKK1 also decreased the number of cells in cultures, and did not selectively influence DC development. To assess the role of Wnt signaling on LC development *in situ*, we analyzed LC in K5rtTA/tetO-DKK1 mice. These mice produce the Wnt inhibitor DKK1 in skin after treatment with doxycycline, and have previously been used to study effects of Wnt signaling on hair development. Doxycycline was fed to mothers beginning on post-natal day 0 to induce DKK1 in the skin of nursing pups. This avoids the limb and dental defects that result from earlier exposure of developing mice to DKK1. Because LC numbers do not reach adult levels until at least 2 weeks of age, it was anticipated that an effect on LC development might be evident. DKK1 induction resulted in an obvious hair phenotype. Analysis of LC in epidermal sheets from these mice revealed that LC numbers were minimally reduced (~20%; n=5 mice; p=0.018). LC in DKK1-expressing epidermis were MHC class II Ag+ and Langerin+, but EpCAM levels were low. Although our studies are limited both in number and scope, the results do not provide strong support for the concept that epidermis-derived Wnts are essential for LC development.

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Peptidoglycan and related molecules increase tight junction function and expression in human keratinocytes

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Innate immune receptors are important for the immediate response to microbial invasion and injury. We hypothesized that their function in the epidermis may also include barrier repair. To address this we investigated the effect of peptidoglycan (PGN) and related molecules on keratinocyte (KC) barrier function by measuring transepithelial electrical resistance (TEER) and expression of key tight junction (TJ) proteins by qPCR and Westerns. We also measured the relative mRNA expression of PGN-responsive innate receptors in the epidermis from nonlesional skin of subjects with atopic dermatitis (n=6), a disease characterized by S. aureus colonization and infections and compared them to nonatopic (n=6) controls. In the presence of PGN, we observed a dose-dependent increase in TEER, which was maximal at 20 μg/ml (fold control: 1.69±0.4, n=4), and enhanced mRNA expression of TJ proteins, claudin-1 (CLDN1) and occludin in primary huKC (fold control: 1.71; 1.36, n=3; respectively). When KC were treated with PGN-related molecules including PAM3CSK4, PAM2CSK4 and MDP, TEER was also significantly increased (fold control: 1.34±0.2, n=4; 1.52±0.2, n=2; 1.26±0.1, n=5; respectively). PAM3CSK4 enhanced claudin1 and occludin protein expression (fold control: 1.63, n=3; 2.5, n=1, respectively). Interestingly, the mRNA expression of TLR1 and 2 was reduced in AD epidermis (fold control: TLR2 - 0.5±0.3 vs. 0.2±0.01, p=0.05; TLR1 - 3.6±2.2 vs. 1.6±0.3, p=0.059). No differences were observed in NOD2 and TLR6 expression. This work highlights the importance of *S.aureus*-related molecules in epidermal tight junction integrity. This is a new paradigm for innate immune receptors – extending their biological actions beyond antimicrobial actions to barrier repair. We hypothesize that AD subjects have an impaired barrier repair response to *S. aureus*-related proteins that is due to reduced epidermal expression of the TLR1/2 heterodimer

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Sebum free fatty acids modulate skin innate immune responses by enhancing antimicrobial function of sebocytes and attenuating TLR2-mediated inflammatory response of keratinocytes

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Free fatty acids (FFAs) are the major components in human sebum. Herein, we examined the immunomodulatory effects of FFAs on human sebocytes and keratinocytes. Incubation of lauric acid (LA), palmitic acid (PA), or oleic acid (OA), the predominant FFAs in human sebum, with human sebocytes (25 μg/ml) increased human β-defensin (hBD)-2 mRNA by 54, 25170, or 45260 fold, respectively (P<0.01). Western blot data demonstrates that the secretion of hBD-2 peptide from cultured sebocytes was only observed when sebocytes were stimulated with the FFAs, but not the vehicle control. The cultured media of FFA-stimulated sebocytes showed antimicrobial activity against *Propionibacterium acnes* (*P. acnes*) and the enhanced antimicrobial activity was neutralized by an anti-hBD-2 antibody, suggesting that the antimicrobial activity was dependent on hBD-2 production. Epicutaneous application of OA (1%) on mouse ear skin increased the expression of mouse β-defensin 4, a mouse ortholog for hBD-2, in sebaceous glands. FFAs induced hBD-2 via the CD36 and NF-κB pathways, as adding the anti-human CD36 IgG or blocking the NF-κB signaling pathway with BMS-345541, a highly selective inhibitor of inhibitory κB kinase, both dampened hBD-2 induction by FFAs. Pre-exposure of the HaCaT keratinocytes to PA or OA (25 μg/ml) significantly suppressed IL-8 production induced by subsequent stimulation with TLR-2 ligands (LTA, Malp-2 or Pam3CSK4) or *P. acnes* (P<0.005). Pretreatment of mouse skin with PA or OA (1%) suppressed Cxcl2/ MIP-2 mRNA induction by subsequent infection with *P. acnes*, and decreased *P. acnes*-induced migration of Gr-1$^+$ granulocytes into the dermis (P<0.005). These data demonstrate that the sebum FFAs protect skin by inducing the expression of hBD-2 in the sebocytes and by attenuating TLR2-mediated inflammatory response of keratinocytes.

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Epidermal Langerhans cells act as negative regulators of a protective anti-Leishmania response, whereas dermal CD11chigh dendritic cells promote healing

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As there is still no vaccine available against Leishmania infections and considering the critical roles of (skin) dendritic cells (DC) as regulators of the anti-Leishmania immune response, DC are attractive targets for immunotherapy. Defense mechanisms against pathogens are exerted by different DC subsets, including dermal DC (dDC) and Langerhans cells (LC). In this study, we analyzed the role of LC in murine experimental leishmaniasis by their inducible ablation in vivo using knock-in mice expressing a diphtheria toxin (DT) receptor (DTR) under the control of the langerin promoter. Upon infection with L. major (1,000 parasites), mice selectively depleted of LC developed significantly smaller ear lesions and decreased lesional parasite burdens, which correlated with reduced numbers of lesional CD4+Foxp3+ regulatory T cells (Treg) as compared to control mice. This was accompanied by increased IFNγ/IL-4 and IFNγ/IL-10 ratios upon antigen-specific restimulation of lymph node cells prepared from LC-depleted mice. In an independent experiment, we utilized CD11c-DTR (donor) – C57BL/6 (recipient) bone marrow (BM) chimeras to deplete CD11chigh DC, but not the radio-resistant LC. During low dose infection with L. major, these BM chimeras developed exacerbated disease with larger lesion volumes as compared to PBS-treated controls. In conclusion, our data reveal a suppressive role of LC in leishmaniasis by Treg-derived IL-10 leading to attenuated Th1 responses. In contrast, CD11chigh dDC are required to promote the induction of efficient protective immunity. Thus, future immune intervention strategies will aim to circumvent LC and selectively target dDC for the development of vaccines against this important human pathogen.

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Leukotriene A4 Hydrolase (LTA4H) is expressed in leprosy lesions and variants are associated with susceptibility to mycobacterial diseases

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LTA4H activity balances production of pro-inflammatory Leukotriene B4 with anti-inflammatory Lipoxin A4. We hypothesized that susceptibility to pathogenic Mycobacteria is associated with genetic variation in LTA4H and the ensuing changes in inflammation could be directly examined *in vivo* using the immunologically polarized forms of cutaneous leprosy. Utilizing case-control studies of tuberculosis (TB) in Vietnam and leprosy in Nepal, we examined whether single nucleotide polymorphisms (SNPs) in LTA4H were associated with these infections. In addition, skin biopsies from controls or lesional skin of leprosy patients in our cohort were stained for LTA4H. We found that heterozygosity for 2 of 6 SNPs in LTA4H was significantly associated with both reduced susceptibility to TB, as well as with reduced mortality from meningeal TB. This finding was validated in our leprosy cohort in which heterozygosity for these 2 SNPs was significantly associated with reduced susceptibility to multibacillary (MB) leprosy. With immunohistochemistry, we found that LTA4H was widespread within granulomas of leprosy biopsies, unlike normal skin, supporting a role for this gene in disease. By using biopsies of our genotyped Nepalese cohort, we can directly associate polymorphisms with leprosy clinical phenotypes and *in vivo* LTA4H levels. We conclude that heterozygosity for common LTA4H variants is associated with susceptibility to both tuberculosis and MB leprosy in humans and that LTA4H is expressed in lesional skin of leprosy patients.

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IL-22 contributes to disease progression in Leishmania major-infected mice

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Healing of Leishmania major infections in resistant C57BL/6 mice relies on Th1/Tc1 immunity, since IFNγ secretion of CD4+ and CD8+ cells is critical for parasite elimination. In contrast, Th2/Th17 induction leads to systemic disease and susceptibility as observed in BALB/c mice. Here, IL-17A released from CD4+ T cells and neutrophils contributes to parasite persistence. The role of Th17-associated IL-22 in L. major infections is unclear. IL-22 is presumed to play an important role in innate pathogen defence. We now analysed the role of IL-22 in experimental cutaneous leishmaniasis. In draining lymph node (LN) cells of infected C57BL/6 and BALB/c mice, we detected elevated levels of IL-17A from BALB/c LN cells upon antigen-specific restimulation, whereas IL-22 production was significantly higher in LN cells from C57BL/6 mice (~3-fold). C57BL/6 mice deficient for IL-22 exhibited significantly decreased lesion development after intradermal, low dose infection with L. major mimiking natural transmission of the parasite by the bite of a sand fly as compared to wild type C57BL/6 mice. In parallel, lesional parasite burdens at the site of infection were significantly smaller in IL-22-/- mice 9 weeks post infection (p=0.004, n=3), whereas antigen-specific cytokine production (IFNγ, IL-4, IL-10, and IL-17A) of draining LN cells was unaltered. Finally, in Leishmania-resistant C57BL/6 mice, the majority of IL-22 appeared to be secreted by γ/δ T cells after restimulation with dendritic cells pulsed with soluble Leishmania antigen (SLA) or infected with L. major as compared to CD4+, CD8+ T cells or neutophils. Ongoing studies will aim to further characterize the source of IL-22 and the mechanism of action by which IL-22 promotes disease development in C57BL/6 mice. A detailed understanding of the role of the various Th17-derived cytokines in cutaneous leishmaniasis will aid the development of vaccination strategies against this important human pathogen.

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Response to Toll-Like Receptor agonists in human neonatal foreskin

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Toll-Like Receptors (TLRs) play a critical role in the innate immune response. While TLRs have been examined in isolated cell types from skin in culture, there has been limited examination of their roll in intact skin. In order to further characterize these receptors, we examined the response to TLR agonists in skin explants of human neonatal foreskins in transwell cultures. Explants were cultured for 24, 48 and 72 hours under the following conditions: media alone, agonists to TLR2 (peptidoglycan), TLR3 (poly I:C), TLR4 (LPS), TLR5 (flagellin), TLR7 (imiquimod), TLR9 (ODN). Immunostaining for the keratinocyte inflammatory marker K16 was elevated in untreated neonatal foreskin, in contrast to adult skin where it is normally absent. Interestingly, the addition of peptidoglycan led to the loss of the K16 expression. Expression of K10 was unchanged from media alone, while loricrin expression became patchy with TLR agonists to 2,3,4,7 and 9. In addition, neonatal foreskin explants were cultured in transwell for 72 hours with and without the TLR3 agonist poly I:C. Sections were then dual stained with anti-IL-23p19, and anti-CD31 (endothelial cells), anti-CD1a (Langerhans cells), or anti-CD11c (dendritic cells). Increased expression of dermal IL-23p19 was noted in both endothelial and CD11c+ dendritic cells, demonstrating that poly I:C is capable of stimulating a pro-inflammatory response in this explant system, and potentially driving T-cells to a Th17 population.

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Aberrant TNF signaling in FAN-deficient mice leads to enhanced suceptibility to cutaneous leishmaniasis

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Prior work with TNF receptor (R) p55-/-, or p55-/-/p75-/- deficient mice identified TNF-R signalling as important pathway for protection against leishmaniasis. Here, parasite clearance was delayed and non-resolving lesions in p55-/- mice were correlated to impaired apoptosis of inflammatory lesional cells. We now studied the role of FAN (factor associated with neutral sphingomyelinase activation) in L. major infections, a molecule mediating TNF-induced cdc42 activation and actin reorganisation. FAN-/- mice showed defects in their cutaneous barrier, but so far no role for regulation of immune responses was identified. FAN-/- C57BL/6 mice were infected with physiological low dose inocula (1,000) of metacyclic promastigotes of L. major mimicking natural transmission by sandy flies. Interestingly, FAN-/- mice showed significantly worsened disease outcome as compared to wild types with more severe lesion development over the entire course of infection starting early in wk2-3 (e.g. 5.3±0.9 vs. 20.9±2.9 mm3 in wk9, $p<0.0001$). Full lesion resolution was not observed until wk18, whereas lesions in control mice were healed in wk11. Lesional parasite loads were higher in the absence of FAN in wk9 post infection. After antigen-specific restimulation of draining LN cells with soluble Leishmania lysate (SLA), the supernatants contained significantly higher levels of the Th2 cytokines IL-4 and IL-10 compared to wild types. In contrast to prior reports, release of neutrophil-recruiting IL-6 from draining LN cells was normal. In the draining LN, an impaired proliferative capacity of antigen-specific CD8+ T cells was observed upon SLA restimulation. In conclusion, our data suggest that FAN contributes to the development of protective Th1/Tc1 immunity against L. major parasites. Future experiments will elucidate the mechanism of action of FAN for the generation of antigen-specific, protective immunity in infections with this important human pathogen in more detail.

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Induction of specific immune response by single and mixed-species biofilms infection in porcine partial thickness wound healing model

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Infections with opportunistic pathogens such as Pseudomonas aeruginosa and Staphylococcus aureus are widely known for forming biofilms. Both bacterial species have been detected in biopsies from patients diagnosed with chronic venous leg ulcers, and it is believed that their presence participates in impairment of wound healing. We analyzed immune response in a porcine partial thickness wound healing model after inoculation with biofilm forming P. aeruginosa (PA), Methicillin Resistant Staphylococcus aureus (MRSA) or a combination of both bacterial species. Bacteria were allowed 2 days post wounding to form a biofilm and biopsies were taken at days 2 and 4 to examine time-dependent gene expression of pro- and anti-inflamatory cytokines responding to single or mixed-species biofilm. Wound healing rates were assessed by histology. The most significant changes in cytokine expression were detected in wounds at day 2 post infection. Expression of IL-8 was highly induced in wounds infected with mixed-species, while individually, PA and MRSA moderately induced IL-8 expression. Infection with MRSA or mixed-species led to suppression of IL-6 in comparison to uninfected wounds. No difference was found in TNFα up-regulation between infected and uninfected wounds. IL-1α expression was induced in all infected in comparison to uninfected wounds. Surprisingly, IL-10, an anti-inflammatory cytokine known to promote healing, was up-regulated in wounds infected with PA only. Infection with MRSA prolonged induction of all inflammatory cytokines tested. This study showed distinct differences in the host immune responses in porcine deep partial thickness wounds when stimulated with either single-species or mixed-species biofilm and provides evidence that biofilms contribute to prolonging an inflammatory phase. A better understanding of the mechanisms of infection and host response will aid in treatment decisions and development of new therapies.

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Investigating the influence of Langerhans cells on keratinocyte proliferative response to chemical and ultraviolet B (UVB) exposure.

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We previously demonstrated that Langerhans cells (LC) facilitate chemical carcinogenesis by showing higher rates of both processes in LC-deficient (Langerin-diptheria toxin A transgenic) mice relative to LC-intact littermate controls. Thus, we sought to explore the breadth of potential influences of LC on keratinocyte responses (hypertrophy, apoptosis, and clonal expansion) to chemical or UVB exposure. At baseline, LC-intact and LC-deficient mouse skin showed comparable epidermal thickness (7.9±1.8 vs 6.9±2.8 μm, NS) and proliferation rates as measured by Ki-67 immunohistochemistry (IHC) (13.6±2.6 vs 12.7±2.8 Ki-67+ cells, NS). After a single application of mutagen DMBA followed by repeated promoter TPA (x 5 wks), LC-intact skin showed substantially greater epidermal thickness (59.2±28.4 vs 25.8±8.9 μm, $P<.0001$) and proliferation (31.5±7.7 vs 25.7±7.4 Ki-67+ cells, $P=.007$). To address the potential influence of LC on acute UVB-induced keratinocyte damage, mice were irradiated with a single dose of 3360J/m^2. Relative to LC-deficient mice, LC-intact mice showed a subtle but significantly greater rate of sunburn cells (13.9±7.5 vs 9.3±3.9 cells, $P=.02$) and apoptotic cells indicated by cleaved caspase-3 (CC3) IHC (26.4±11.1 vs 21.4±7.6 CC3+ cells, $P=.03$). Chronic low-dose UVB (500-1500 J/m^2, 5d/wk x 5-9wk) induced keratinocyte islands over-expressing p53 (CM5) in both LC-intact and LC-deficient skin (with a statistical trend towards greater rates within LC-intact skin). Surprisingly, epidermal Langerin+ MHC-II+ DC were readily apparent within chronically UVB-irradiated skin of LC-intact, and to a lesser extent, LC-deficient mice. In summary, LC exert diverse, measurable influences on chemically induced keratinocyte proliferation and acute UVB-induced keratinocyte apoptosis. LC effects on chronic UVB-induced keratinocyte p53+ clonal expansion remains to be fully elucidated.

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Isoprenylcysteine (IPC) analogs: A novel topical acne therapeutic possessing antimicrobial and anti-inflammatory activity against Propionibacterium acnes

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Propionibacterium acnes (P. acnes) is a major etiological factor for inflammatory acne. The interaction of P. acnes with follicular keratinocytes leads to an innate immune response via activation of toll-like receptors (e.g. TLR2, TLR4) resulting in the production and secretion of pro-inflammatory mediators. Subsequent recruitment and infiltration of neutrophils by these mediators generates erythematous inflammation. Isoprenylcysteine (IPC) analogs are structural mimics of the lipidated C-termini of the Gγ subunit of all heterotrimeric G proteins and small G proteins, which participate in eliciting inflammatory responses such as the release of pro-inflammatory mediators, and the migration and activation of inflammatory cells. We recently demonstrated that N-acetyl-S-farnesyl-L-cysteine (AFC) showed potent anti-inflammatory efficacy in an *in vivo* TPA-induced acute contact irritation mouse model (Gordon et al, 2008, JID, 128:643). In this study we investigated whether IPC analogs possess anti-inflammatory activity through inhibition of P. acnes-induced cytokine production *in vivo* and *in vitro*. An,i>in vivo *inflammatory acne mouse model using intradermal P. acnes injections, show IPC analogs have strong anti-inflammatory activity inhibiting neutrophil infiltration, edema and erythema.. Moreover, IPC analogs significantly reduced the induction of several pro-inflammatory cytokines (e.g. IL-1β, IL-6, KC, TNF-α) in mouse skin suggesting a potential mechanism involving the reduction of keratinocyte inflammatory mediator production. Human keratinocytes (NHEKs) exposed in culture to P. acnes upregulate the production of several pro-inflammatory cytokines (e.g. IL-6, IL-8 and GM-CSF) which were reduced in the presence of IPC analogs. Furthermore, results demonstrate each IPC analog inhibits P. acnes growth with differing potencies. Thus, IPC analogs represent a novel class of topical anti-acne therapeutics, with dual acting anti-inflammatory and antibacterial properties.*

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TNFα, a critical cytokine for cutaneous immunity to vaccinia virus

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TNFα is a multifunctional cytokine that mediates inflammation, immune response, and apoptosis. In skin, TNFα is the master cytokine regulator in inflammatory diseases such as psoriasis, contact dermatitis, drug eruptions, cutaneous T-cell lymphoma, etc. However, the role of TNF-α in the cutaneous immune response to viral infection is less well defined. We have been using vaccinia virus (VV) in the form of the smallpox vaccine as a model of viral infection in the skin. We applied VV to TNFR1-/-mice by scarification and found that TNFR1-/-mice developed markedly larger lesions and satellite lesions following VV scarification. Consistent with the satellite lesions, virus dissemination to adjacent skin was also dramatically increased in these TNFR1-/- mice. The exacerbated skin lesions in the TNFR1-/-mice could be caused by infection or inflammation. To distinguish between these two possibilities, replication deficient modified vaccinia virus Ankara (MVA) were applied to the mice by scarification, The size of lesions was similar between the TNFR1-/- mice and wild-type (Wt) mice which indicated that the larger lesions in the TNFR1-/- mice was caused by infection, but not inflammation. We investigated the role of TNFα in regulating various facets of T cell response to VV in mice and found that the primary T cell response to VV was not dependent on TNFα while TNFα deficiency attenuated the contraction of VV specific CD8 T cells. The dynamics of T cell homing to skin was also analyzed by skin cell characterization and the results suggested that VV-specific T cell skin trafficking was dispensable of TNF. Since the skin lesions are comprised of both resident of skin cells and recruited bone marrow(BM)-derived cells, we wanted to determine which cell population utilize TNF signaling to localize VV replication and control skin lesions. Lethally irradiated Wt animals were reconstituted with BM from Wt or TNF-/- mice. The two groups of mice exhibit similar skin lesions which suggested that TNF deficiency by recruited BM-derived cells did not lead to the severe skin lesions.

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Protective effects of platinum nanoparticles against UV-light-induced epidermal inflammation

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Intracellular reactive oxygen species (ROS) and apoptosis play an important roles in the ultraviolet (UV)-induced inflammatory responses in the skin. Metal nanoparticles have been developed to increase the catalytic activity of metals, which is due to the large surface area of smaller particles. Platinum nanoparticles (nano-Pt) protected by poly acrylic acid were manufactured by reduction with ethanol. A marked increase in ROS production was observed in UV-treated HaCaT keratinocytes cell lines, while a decrease in ROS production was observed in nano-Pt-treated cells. Pretreatment of the cells with nano-Pt also caused a significant inhibition of UVB- and UVC-induced apoptosis. Furthermore, we found that mice treated with nano-Pt ointment prior to UV irradiation showed significant inhibition of UVB-induced inflammation compared to UV-irradiated control mice. These results suggest that nano-Pt effectively protects against UV-induced inflammation by decreasing ROS production and inhibiting apoptosis in keratinocytes.

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Preventive effects of ultraviolet radiation on autochthonous murine mammary tumor carcinogenesis - Is sun avoidance killing patients?

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Epidemiologic studies supporting an inverse correlation between sun exposure and breast cancer incidence/mortality, have been taken as evidence of an anti-cancer effect of vitamin D. We assessed directly whether ultraviolet radiation (UVR) or dietary vitamin D have any anti-mammary cancer effect in the C3(1)SV40 Tag transgenic mouse. Mice fed diets depleted of vitamin D (+ rescue minerals) were treated with 0 [group A] or 350mJ/cm2 UVR 3x/week without [Group B] or with [Group C] topical 7-dehydrocholesterol, the precursor of cutaneous vitamin D3. We also fed mice a separate diet containing high vitamin D (20,000IU/kg chow) [Group E]. Control mice were fed diets containing normal levels of vitamin D (1,000IU/kg) with [Group D] or without rescue minerals [Group F]. We examined mice and excised all palpable tumors (mastectomies) every two weeks at ages 16-28 weeks. Circulating 25-hydroxyvitamin D3 (25OH D3) levels in 59 mice, measured in ng/mL [median (interquartile range)] at the end of the study in groups A-F were 3.0(1.0), 34.0(26.3), 26.0(18.0), 28.0(13.0), 121.0(82.5), and 42.0(15.5), respectively ($p<0.001$). UV-treated mice had reduced tumor burden and longer tumor free survival than did the vitamin D-depleted mice not so treated. By contrast, there was no significant correlation among groups between 25OH D3 levels and tumor number, mean volume, or total tumor volume/mouse. Thus, tripling the circulating 25OH D3 level by additional dietary vitamin D did not influence mammary tumor number, size, or tumor free survival, when compared to the normal level vitamin D mice. We found lung metastases in 7, 0, 5, 11, 17, and 23% of mice in groups A-F. We interpret these data to suggest that UVR can inhibit development of autochthonous murine mammary tumors, consistent with the idea that differing exposure to UVR may underlie the latitudinal gradient of breast cancer risk. However, failure of tumorigenesis to correlate strictly with circulating 25OH D3 levels suggests that dietary D may not replace UVR's beneficial effects.

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Sun-induced changes in epidermal function vary with gender and extent of exposure

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Previous studies have demonstrated that erythemogenic doses of UVB irradiation alter epidermal permeability barrier and stratum corneum (SC) hydration. Yet, whether regular exposure to ambient levels of sun exposure alters SC permeability barrier function and integrity is largely unknown. In the present study, we assessed the effects of various amount of sun exposure on SC integrity, SC hydration and permeability barrier function. A cohort of Chinese subjects (124 males and 134 females, mean age: 27.90 + 0.58), aged 15 - 50 yearswere enrolled and assessed during the summer months. According to the extent of daily sun exposure, subjects were grouped as normal controls (daily sun exposure time <1 hr), low levels of daily sun exposure (1-2 hr) and higher amounts of daily sun exposure time (4-6 hr). A multifunctional skin physiology monitor (C&K MPA 5) was used to measure SC hydration and basal transepidermal water loss (TEWL) on the dorsal forearms. While TEWL was significantly higher in the skin sites exposed to higher amounts of sun exposure in males, TEWL was higher with lower amounts of sun exposure in females. In females, SC integrity in higher doses of sun exposure was better in comparison with control and lower sun exposure. SC integrity was poorer in females than in males in both control and lower doses of sun exposure. Barrier recovery rates were also faster in females than in males in both control and the lower doses groups. Yet, barrier recovery was delayed in both males and females following higher doses of sun exposure. In males, sun exposure did not alter SC hydration, while in females SC hydration was lower in low sun exposure group than in control and higher doses sun exposure groups. SC hydration was also lower in females than in males in all groups. Together, these results show that sun exposure-induced changes in skin barrier function and SC hydration vary with gender and sun exposure doses.

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The effect of UVB on cytokines, no production; Using *in vitro* co-culture system of monocyte/keratinocyte culture

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Ultraviolet B (290-320 nm) has been reported to modulate the cytokine-mediated inflammatory process in various inflammatory skin conditions, including production of tumor necrosis factor (TNF)-α, IL-1α, IL-6, IL-8, and IL-10. We constructed an *in vitro* model system involving co-culture of different cell types to study the effect of UVB on the inflammatory process using nitric oxide (NO),TNF-α, and IL-1α as markers of inflammation. This study was conducted to quantitatively assess the inflammatory cytokines produced by human epithelial keratinocytes in the presence and absence of macrophages/ monocytes. Primary human epithelial keratinocytes and immortalized keratinocytes cells (hTERT) were treated with lipopolysaccharide (LPS) as stimulator of inflammatory response and exposed to UVB radiation (10-100mJ/cm^2). The expression of nitric oxide (NO) was measured by modified Griess assay and the expression of TNF-α and IL-1α was measured by quantitative ELISA. For the co-culture system, irradiated keratinocytes were seeded in a 24 well Transwell tissue culture plate whereas SC cells (human peripheral blood monocyte cell line) were seeded in the individual baskets subsequently placed on top of the keratinocyte cultures, and samples of culture supernatants were collected at 1-6 days. When keratinocytes were irradiated with UVB, a dose-dependent stimulation of TNFα and IL-1α production was observed (1.5 fold and 1.3 fold increase above respective baselines). In contrast, when LPS-stimulated macrophages were irradiated with UVB, significant inhibition of NO production (40% suppression, $p<0.001$) was seen. TNF α, different from IL-1α production, was not changed significantly in co-culture. The presence of monocyte may modulate the level of inflammatory cytokines selectively in responding to UVB. The data appeared to show that an improved model of cutaneous inflammation could use multiple cells to study the interaction and to offer convenience, reproducibility, and a closer approximation of *in vivo* conditions.

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Alteration of the migratory behavior of UV-induced regulatory T cells *in vivo*

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UV-induced regulatory T cells (UV-Treg) inhibit the sensitization but not the elicitation of contact hypersensitivity when injected i.v, since UV-Treg express lymph node, but not skin homing receptors and migrate into the lymph nodes but not into the skin. The homing receptor expression and the migration of UV-Treg can be altered by tissue-specific antigen presenting cells (APC) *in vitro*. To prove whether this is also possible *in vivo*, we obtained UV-Treg from mice which were sensitized against dinitrofluorobencene (DNFB) through UV-exposed skin. UV-Treg were injected i.v. into mice which were DNFB-sensitized 5 days earlier. To activate the i.v. injected UV-Treg in the lymph nodes with DNFB-bearing skin-derived APC presumably Langerhans cells (LC), recipient mice were boosted with DNFB on the abdomen and ears challenged 24 hours later. Sensitized but unboosted mice were not suppressed in their challenge despite the injection of UV-Treg. When mice were boosted with DNFB 24 hours after injection of UV-Treg, the ear swelling response was suppressed, suggesting that UV-Treg upon the DNFB boost through the skin migrate into the periphery. Accordingly, i.v. injected CFSE-labeled UV-Treg were detected in unboosted mice in the lymph nodes, but in boosted mice in the ears. The presence of LC appears to be essential for the boosting effect, since it was lost upon depletion of LC, as demonstrated in langerin diphtheria-toxin receptor knock in mice treated with diphtheria toxin. This indicates a first option to alter the migratory behavior of Treg *in vivo* and thus may have input on strategies trying to utilize Treg not only for the prevention but also for the treatment of immune-mediated disorders.

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Obesity exacerbates UVB radiation-induced inflammation and cell survival signals in UVB-irradiated mouse skin

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Obesity has been implicated in several inflammatory diseases and in different types of cancer. Chronic inflammation induced by exposure to ultraviolet (UV) radiation has been implicated in various skin diseases, including melanoma and nonmelanoma skin cancers. As the relationship between obesity and susceptibility to UV radiation-caused inflammation is not clearly understood, we assessed the role of obesity on UVB-induced inflammation, and mediators of this inflammatory response, using the genetically obese (leptin-deficient) mouse model. At the age of 18 weeks, obesity was significantly developed in leptin-deficient obese (ob/ob) mice compared with their wild-type C57/BL6 mice. At this stage, ob/ob obese mice and wild-type counterparts were exposed to UVB radiation (120mJ/cm2) on alternate days for 1 month. The mice were then sacrificed and skin samples collected for analysis of biomarkers of inflammatory responses using immunohistochemistry, western-blotting, ELISA and real-time PCR. Here, we report that the levels of inflammatory responses were higher in the UVB-exposed skin of the ob/ob obese mice than the UVB-exposed skin of the wild-type non-obese mice. The levels of UVB-induced biomarkers of inflammation, such as, cyclooxygenase-2 expression, prostaglandin-E2 production, proinflammatory cytokines (i.e., TNF-α, IL-1β, IL-6), and proliferating cell nuclear antigen and cell survival signals (phosphatidylinositol-3-kinase and p-Akt-Ser473) were higher in the skin of the ob/ob obese mice than the skin of their wild-type non-obese counterparts. Compared to the wild-type non-obese mice, the leptin-deficient obese mice also exhibited greater activation of NF-κB/p65 and fewer apoptotic cells in the UVB-irradiated skin. Our study suggests for the first time that obesity in mice is associated with greater susceptibility to UVB-induced inflammatory responses and, therefore, obesity may increase susceptibility to UVB-induced inflammation-associated skin diseases, including the risk of skin cancers.

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Silymarin, a plant flavonoid, suppresses UV radiation-induced apoptosis of epidermal cells by stimulating DNA repair

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Over exposure to solar ultraviolet (UV) radiation is the major etiological factor for over one million new cases of non-melanoma skin cancers in the US each year. One of the hallmark events of exposure to UVB radiation (290-320 nm) is the induction of apoptotic cell death of keratinocytes, the results of which are evident within the epidermis as sunburn cells. The formation of sunburn cells is a protective mechanism for limiting survival of cells with irreparable DNA damage. Because of its protective function, alterations in UVB-induced apoptosis may have a profound impact in the induction of skin cancer. We have shown that topical application of silymarin, a flavonoid from milk thistle, inhibits UVB-induced skin carcinogenesis in mice. By using *in vitro* cell culture and *in vivo* genetically-modified mouse models, here we report that UVB-induced apoptotic cell death of epidermal keratinocytes was suppressed by silymarin, and in this process UVB-induced DNA damage was significantly reduced or repaired by silymarin. Using normal human epidermal keratinocytes (NHEK) as an *in vitro* model, we found that exposure of NHEK to UVB (20 mJ/cm2) radiation induces apoptosis which was suppressed by pretreatment of NHEK with silymarin (5-20 μg/ml) when analyzed by flow cytometry, and the levels of damaged DNA was reduced as determined by comet assay and dot-blot analysis using antibody specific to thymine dimers. Following animal experiments, we observed that UVB-induced sunburn cells were resolved more rapidly in the skin of mice treated topically with silymarin than untreated controls. The suppression or repair of damaged DNA by silymarin was associated with the enhancement in the levels of nucleotide excision repair (NER) genes. Further, the treatment of NER-deficient mice with silymarin did not promote the repair of UVB-induced sunburn cells while promoted the repair of sunburn cells in wild-type mice, indicating that silymarin can protect keratinocytes from apoptosis induced by DNA-damaging UV radiation by inducing DNA repair.

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Investigating the role of langerhans cells in acute UVB-induced epidermal oxidative stress

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Oxidative stress within epithelial cells has been linked to increased rates of mutagenesis, and ultraviolet light has been shown to rapidly induce oxidative stress in exposed cells. To begin to investigate whether Langerhans cells (LC) influence the epidermal stress response to ultraviolet B (UVB) exposure, we utilized LC deficient (Langerin-diptheria toxin A transgenic) mice and compared them to LC-intact mice (in both groups, mice were rendered deficient in all T cells by TCR β-/- and δ-/- intercrossing). Groups of mice were irradiated with single-dose UVB at 3360 J/m2 and euthanized 1 hour later. Epidermal sheets were separated from ear skin and stained for 8-oxoguanine (anti-8oxoG), indicative of oxidative stress-induced nucleic acid damage, as well as for LC. Using a confocal microscope (Zeiss 510 META LSM), total epidermal oxidative stress (8oxoG+) was readily detected in keratinocytes of UVB-exposed skin as punctate, predominantly cytoplasmic staining, consistent with RNA- and/or mitochondrial DNA-8oxoG lesions. In LC-intact skin, oxidative stress was also evident within LC in a predominantly cytoplasmic pattern, including within dendrites. Consecutive high power fields (hpf; 63x) from UVB-exposed skin (10 hpf/ear, N=6/group) were digitally captured and analyzed using N.I.H. ImageJ software and revealed 67% greater oxidative stress levels in LC-deficient vs LC-intact ear skin (965.7±186.8 vs 577.3±102.8 fluorescent units/hpf; $P<0.009$). Within LC-intact epidermis, the degree of oxidative stress attributable to the LC (by dual Langerin+ 8oxoG+ staining) represented 20.9% of the total epidermal level, thus indicating that the majority of observed oxidative stress was within keratinocytes. Taken together, these data indicate that LC are subject to oxidative stress induced by acute UVB exposure, but nonetheless exert a protective influence against overall levels of epidermal oxidative stress. These studies provide a context in which to assess the role of LC in the acute epidermal response to UVB, with potential implications for cutaneous carcinogenesis.

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Photodynamic therapy with the phthalocyanine Pc 4 for T cell mediated diseases: Activated T cells exhibit increased uptake of Pc 4 and increased susceptibility to PDT-mediated cell death

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Photodynamic therapy in combination with the silicon phthalocyanine Pc 4 and red light (Pc 4-PDT) has been gathering acclaim as an emerging treatment for malignant and inflammatory disorders. Activation of the monomeric form of Pc 4 by red light generates singlet oxygen and other forms of reactive oxygen species to induce cell death. We previously reported that Pc 4-PDT elicited cell death in lymphoid-derived (Jurkat) and epithelial-derived (A431) cell lines *in vitro*, and furthermore that Jurkat cells were more sensitive than A431 cells to treatment. In this study, we examined the effectiveness of Pc 4-PDT on primary human $CD3^+$ T cells *in vitro*. Flow cytometric analyses measuring annexin V and propidium iodide demonstrated a dose-dependent increase of T cell apoptosis (6.6-59.9% in average) at Pc 4 doses ranging from 0-300 nM. Following TCR T cell stimulation, activated T cells exhibited increased susceptibility to Pc 4-PDT induced apoptosis (10.6-81.2% in average). In both unstimulated and stimulated T cells, monomeric Pc 4 uptake increased at doses of 0-300 nM Pc 4 as assessed by flow cytometry. Whereas the mean fluorescence intensity (MFI) of Pc 4 uptake measured in unstimulated T cells ranged from 78-6177, Pc 4 incorporation in stimulated T cells was approximately doubled (111-11517 MFI). Fluorometric analyses of lysed T cells confirmed dose-dependent uptake of monomeric and aggregated Pc 4 in unstimulated and stimulated T cells. Additionally, confocal imaging revealed that Pc 4 localized in cytoplasmic organelles, approximately half of which co-localized with mitochondria in T cells. Thus, Pc 4-PDT exerts an enhanced apoptotic effect in activated $CD3^+$ T cells that may be exploited in targeting T cell-mediated skin diseases.

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CpG-ODN protects against UV-induced apoptosis via enhancement of AKT and mTORC1/mTORC2 activation in keratinocytes and dendritic cells

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CpG-DNA and its related synthetic CpG oligodeoxynucleotides (CpG-ODNs) play an important role in immune cell survival and activation. Existing data have shown that AKT activation is involved in this process. However, the potential role of CpG-ODN in protection against UV-induced skin damage has thus far not been tested. In this study, we found that CpG-ODN protects against UV-induced cell death and apoptosis in both skin keratinocytes (HaCaT cells) and cultured dendritic cells (XS106, or DCs). CpG-ODN pre-treatment enhances UV induced activation of AKT (ser473, thr308 phosphorylation) as well as downstream signal mTORC1 (S6K and 4E-BP1 phosphorylation) in both HaCaT cells and DCs. AKT deficiency (by using AKT1/2 double knockout Mouse Embryonic Fibroblasts as well as AKT inhibitor X, a pharmacological inhibitors specific for AKT) or mTORC1 inhibition (by using rapamycin, a pharmacological inhibitor of mTORC1) largely neutralizes the protective effects of CpG-ODN, suggesting that AKT and downstream signaling mTORC1 activation are necessary for CpG-ODN-induced protective effects against UV-induced cell death. Our findings suggest that CpG-ODN may be utilized to prevent from UV-induced skin aging and provide a novel mechanism of protective effects against UV radiation.

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The novel role of G protein αi1 and αi 3 in UV radiation-induced cell signaling

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Activation of AKT/mTOR (mammalian target of rapamycin) and MAPK (p38/JNK/MEK/ERK) pathways is involved in UV-induced skin cell damage, skin aging and skin cancer. However, the molecular mechanism underlying the activation of these pathways by UV radiation remains elusive. It is well established that EGFR trans-activation is involved in UV-induced signaling. However, other signal transducers are not clear. Here, we report a novel and critical role of the G-proteins αi1 (Gi1) and G-proteins αi3 (Gi3) proteins in this activation process. We observed that UV-induced activation of MAPK (p38/JNK/MEK and ERK phosphorylation) and AKT (Ser473 and Thr308 phosphorylation) together with downstream target mTORC1 (p70S6K, S6 and 4E-BP1 phosphorylation) are almost abolished in Gi1 and Gi3 doubly knockout (DKO) MEFs. Importantly, knockdown of both Gi1 and Gi3 using specific siRNAs largely impairs UV-induced AKT/mTOR and MAPK activation in MEFs and skin keratinocytes cell line HaCaT as well as primary keratinocytes. By using DKO MEFs as well as siRNA knockdown strategy, we found that Gi1/3 is required for UV-induced activation of Gab1, which is a key adaptor protein and mediator for UV-induced signaling and Gab1 knockout almost abolishes UV-induced AKT/mTOR and MAPK activation in MEFs. We also observed that after UV radiation, Gi proteins physically associates with Gab1 to form a complex, which is critical for Gab1 activation. Collectively, our results suggest that Gi1 and Gi3 act downstream of EGFR, but upstream of Gab1, mediating activation of AKT/mTOR and MAPK. Our study reveals a novel role of Gαi proteins in UV signaling pathways and possibly in skin aging and cancer.

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Resveratrol sensitizes keratinocytes to UVA-induced apoptosis through increased oxidative stress in mitochondria and mitochondrial permeability transition pore (MPTP) opening

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We investigated the effects of resveratrol, a polyphenol compound derived from the skin of red grapes, on the mitochondria of HaCaT cells in response to UVA. Although many studies have shown that resveratrol exhibits anti-oxidative and protective properties in mitochondria, our data show that the combination treatment of a low dose of UVA (14J/cm^2) and resveratrol (5μM) in HaCaT cells results in a significant increase of apoptosis ($p<0.05$) relative to UVA and ethanol (vehicle) treated cells as analyzed with annexin V and propidium iodide staining. We also measured mitochondria membrane potential (MMP) using the cationic dye, JC-1. The JC-1 assay showed that the MMP is disrupted immediately after combination treatment with UVA and resveratrol when compared to UVA and vehicle ($p<0.01$). In addition, MPTP opening was detected within two hours after combination treatment of UVA and resveratrol when compared to UVA and vehicle ($p<0.05$) as shown by using Mitoprobe MPTP Assay. Furthermore, using MitoSOX Red mitochondrial superoxide indicator, our data show that this increased apoptosis involves significant oxidative stress in the mitochondria of cells treated with UVA and resveratrol when compared with mitochondria of cells receiving only the combination treatment of UVA and vehicle ($p<0.01$). We propose a model of increased sensitivity to UVA-induced cell death mediated through oxidative stress in mitochondria that is specific to the action of resveratrol in the presence of UVA. These results suggest a possible therapeutic avenue for the treatment of non-melanoma skin cancer involving loading of malignant skin tumors with resveratrol and subsequent UVA irradiation.

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Resveratrol acts specifically with UVA and not with UVB to induce apoptosis in HaCaT cells

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Given both the known influence of ultraviolet radiation in inducing nonmelanoma skin cancer and the emerging evidence demonstrating the chemopreventive nature of resveratrol, we investigated resveratrol as a potential modulator of UVA- and UVB-induced apoptosis in HaCaT cells. Other studies indicate that resveratrol may have direct effects on mitochondrial pathways that result in amplified apoptosis. Resveratrol (5 μM), a phytoalexin that occurs naturally in the skin of red grapes, was added to populations of HaCaT cells, which were then irradiated with measured doses of either UVA or UVB. Using Annexin V staining and flow cytometry to detect apoptosis, we observed that resveratrol sensitizes HaCaT cells to UVA-induced cell death. This sensitization of HaCaT cells by resveratrol was not, however, seen with UVB. Additional experiments demonstrated that UVA sensitization by resveratrol required the compound be present during the irradiation period. The presence of resveratrol exclusively before or after the critical UVA treatment window did not result in increased apoptosis. We also observed that the ability of resveratrol to enhance UVA-induced apoptosis was not mediated via a toxic effect; that is, cells incubated with media containing UVA-exposed resveratrol did not show a significant increase in apoptosis relative to cells incubated with media containing non-UVA-exposed resveratrol. These data suggest that doses of resveratrol as low as 5 μM sensitize HaCaT cells to UVA-induced apoptosis, whereas no effect is observed for UVB. These results suggest that resveratrol combined with UVA, but not UVB, may have potential therapeutic value in the treatment of cutaneous malignant tumors.

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No formation of DNA double strand breaks and no activation of recombination repair with UVA

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Longwave ultraviolet light (UVA) is recognized as an independent class I carcinogen. A complete understanding of UVA-induced DNA damage and how this damage is processed in skin cells is therefore of utmost importance. A particular question that has remained contentious is whether UVA induces DNA double strand breaks (DSBs). These are highly mutagenic and carcinogenic lesions that may result in formation of deletions, insertions, or more complex mutations. UVA may generate DSBs through oxygen radicals or through processing of other types of DNA damage. In order to assess formation of DSBs by UVA in primary skin fibroblasts, we studied formation of γH2AX nuclear foci, considered the most sensitive, albeit not specific, marker for DSBs. We found that unlike ionizing radiation (IR) or UVB, various solar available doses of UVA did not induce formation of γH2AX nuclear foci at several different time points after irradiation (10 – 360 minutes). Likewise, formation of DSBs was not detectable using the neutral single cell electrophoresis assay (neutral comet assay) either. We also studied activation of the Fanconi anemia (FA)/BRCA DNA damage response in response to various doses of UVA, as this pathway is well known to mediate recombination repair of DSBs. Unlike IR or UVB, UVA does not activate this pathway, as it did not induce formation of FANCD2 or BRCA1 nuclear foci or ubiquitination of FANCD2 (all well accepted markers of FA/BRCA pathway activation) at two or six hours after irradiation. The lack of evidence for a formation of DNA DSBs by UVA underscores the pivotal role of UVA-induced base damage, in particular of pyrimidine dimer-type photoproducts in UVA-mutagenesis and carcinogenesis. It is also consistent with most published data on UVA-induced mutagenesis, which show single- and tandem base substitution mutations, but hardly deletions or insertions that would be expected if DSBs were a major pre-mutagenic DNA lesion with UVA.

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p300 histone acetyltransferase inhibitor anacardic acid (6-nonadecyl salicylic acid) inhibits UV-induced MMP-13, COX-2, and TNF-alpha expression in hairless mice

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Our previous study reported that p300 histone acetyltransferase (p300HAT) plays a critical role in the transcriptional regulation of MMP-1 gene by UV in primary human dermal fibroblasts. Suppression of p300 histone acetyltransferase (p300HAT) activity by the p300HAT inhibitor anacardic acid (AA) prevented UV-induced MMP-1 expression and inhibited UV-enhanced γ-H2AX, p53 level, and acetyl-H3. In this study, we investigated the cutaneous photoprotective effects of AA in hairless mice *in vivo*. Six-week-old female albino hairless mice (Hos:HR-1) were topically treated with vehicle (ethanol: polyethylene glycol = 30:70) only, 0.1% AA, or 1% AA once a day for 3 successive days after one time UV irradiation (200 mJ/cm2) on dorsal skin. Our data showed that topical treatment with AA reduced UV-induced skin thickening and formation of apoptotic cells. AA reduced the UV-induced mRNA levels of MMP-13 and TNF-alpha genes as determined by quantitative real-time PCR. Also, topical application of AA inhibited UV-induced COX-2 protein expression. Furthermore, the topical application of AA protected UV-induced DNA damage, which is detected by γ-H2AX level, and decreased acetylation of histone H3. Our data suggest that AA may be a potential agent for preventing UV-induced skin responses.

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Visualization of *in vivo* behavioral responses of Langerhans cells to UVB irradiation

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UVB radiation reduces surface densities of Langerhans cells (LCs), although underlying mechanisms remain unknown. By combining two technologies of I-A beta-EGFP knock-in mice and intravital confocal imaging, we recently reported dynamic behaviors of EGFP+ LCs. The purpose of this study was to visualize the impact of UVB irradiation on dynamic movement of LCs in living animals. I-A beta-EGFP mice received local UVB radiation via four TL 20W/01RS lamps only on the left ears and then monitored under a confocal microscopy at different time points. By recording images of epidermal EGFP+ LCs in the same microscopic fields before and after UVB radiation (intermittent imaging), we measured the impact on LC influx versus LC efflux. UVB radiation (3,000 J/m2) elevated the 24 h LC efflux rate from 1.6 ± 0.1% (sham-exposed right ears) to 34.3 ± 2.8% ($p < 0.001$, $n = 3$) without altering the LC influx rate. FACS analyses showed no significant changes in the % of annexin V+ apoptotic LCs. Our results demonstrate for the first time that UVB radiation reduces LC densities by promoting LC emigration from the epidermis. In time-lapse imaging (in which images were recorded every 2 min for up to 60 min), EGFP+ LCs exhibited "dSEARCH" motion characterized by repetitive extension and retraction of dendrites and limited lateral migration of cell bodies. Single UVB radiation (3,000 J/m2) augmented both dSEARCH activity (3.4-fold, $p < 0.0001$, $n = 29$) and lateral migration (3.6-fold, $p < 0.0001$, $n = 352$) of EGFP+ LCs at 24 h post-radiation as compared to the cells in sham-exposed control sites. Importantly, these changes in motile behaviors were already detectable at 6 h post-radiation when LC densities remained unchanged. When the UVB-irradiated skin sites were monitored 60 days later, all the above parameters measured for EGFP+ LCs returned to the baseline levels observed in sham-exposed skin. Thus, we conclude that UVB radiation induces dramatic, but reversible, changes in motile behaviors of LCs. It is tempting to speculate that such changes may contribute to UVB-induced immunosuppression.

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Ginsenoside-Rg1 protects human dermal fibroblasts against 8-Methoxypsoralen plus Ultraviolet A-induced photoaging through telomere mechanism

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Premature senescence of human dermal fibroblasts (HDFs) can be caused by exposures to a variety of oxidative stress and DNA damaging agents. Ginsenoside-Rg1, an active ingredient of ginseng, has been documented to ameliorate tert-butyl hydroperoxide-induced premature aging in WI-38 cells. In this study, we aimed to investigate whether ginsenoside-Rg1 had protective effects against 8-methoxypsoralen (8-MOP) plus ultraviolet A (UVA; 315-400 nm) irradiation-triggered premature senescence of HDFs, and if appropriate, to explore the underlying molecular mechanisms. Notably, pretreatment of HDFs with ginsenoside-Rg1 reduced photoaging as evidenced by reduced G1 accumulation and telomere shortening. Besides, ginsenoside-Rg1 significantly decreased 8-oxo-dG production elicited by 8-MOP/UVA, reflecti0n of prevention of DNA oxidative damage. Further, pretreatment with ginsenoside-Rg1 greatly diminished the expression of senescence-associated genes including senescence-associated β-galactosidase, P53, P21WAF-1 and P16INK-4a. Collectively, these findings suggest that ginsenoside-Rg1 has favorable effects in the prevention of 8-MOP/UVA-induced photoaging of HDFs, which may be, at least partially, mediated through the regulation of telomere mechanism and subsequent P53-dependent signaling pathways.

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Photosensitive and non-photosensitive trichothiodystrophy: specific XPD protein regions play a major role in human photosensitivity

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We studied trichothiodystrophy (TTD) to examine the molecular role of DNA repair genes in skin sun photosensitivity (PS). TTD is a rare, autosomal recessive disorder with a wide spectrum of clinical manifestations and defects in the DNA repair/transcription genes (XPD, XPB, p8/TTDA) or in TTDN1. Mutations in XPD have previously been reported to cause PS TTD. We describe 8 newly diagnosed TTD patients (age 1.5 to 9 yr) from 6 families with defects in the XPD DNA helicase (XPD-TTD). All of the patients were XPD compound heterozygotes. All had diagnostic features of TTD: abnormal hair (brittle hair, hair shaft defects, and tiger tail banding), short stature, microcephaly, low birth weight, and intellectual impairment. In line with most other XPD-TTD patients, 3 patients were PS. Surprisingly, 5 XPD-TTD patients from 3 different families were not photosensitive (NPS). Two of the PS patients (TTD354BE and TTD421BE) had the same p.A725T mutation while the NPS patient TTD412BE had a different mutation at the same site (p.A725P). The other alleles in these PS patients had large insertions or premature stop codons and would be expected to be non-functional. The second allele in NPS patient TTD412BE was p.L461V which was also present in PS patient TTD383BE; thus, this allele does not modify PS. Siblings TTD352BE, TTD353BE, and TTD397BE were NPS and had p.Q726E from one allele and a large deletion from the other allele. Structural studies of the XPD protein indicate that amino acids 725 and 726 lie in a conserved C-terminal region outside the crystallized archeobacterial region of identified structural homology with the human protein. NPS patient TTD409BE had a p.L399F mutation in one allele and a premature termination mutation in the other. Amino acid 399 is in the arch region of the archeobacterial and human XPD protein. These data suggest that specific regions of the XPD protein play a major role in human PS.

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Proteomic identification of epigallocatechingallate on specific protein oxidation in human epidermal keratinocytes

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UV irradiation is the major sources of oxidative stress through the generation of reactive oxygen species (ROS) in human skin cells. And increasing evidences have supported that ROS are involved in all stages of skin cancer development. Green tea polyphenols exhibit multiple antitumor activities. In this study, we aim to identify the cellular targets responsible for anti-oxidant efficiency of epigallocatechingallate (EGCG) by performing 2-DE LC-MS/MS of HaCaT cells before and after EGCG exposure. Though the protein expression profile, We have identified 17 proteins that showed different expression levels(2-fold) in HaCaT cells treated with EGCG in comparison with untreated cells. All of them were identified. These proteins are involved in cell structure and cytoskeleton, cell division, cell proliferation and apoptosis. Focusing on the oxidative modifications occurring at the protein level, we found Thioredoxin domain-containing protein 17 (TXNDC17) that involve in various redox reactions. Our data indicated that EGCG-induced TXNDC17 upregulation appears to be at the transcriptional level. And confirmed by siRNA, downregulation of TXNDC17 results in the increased sensitivity of HaCaT cells to oxidative stress injure induced by UVB irradiation. The result of the study shows that EGCG- induced modulation of protein expression involved in growth, motility and apoptosis, especially against oxidative injure. It may contribute to explain the multiple antitumor activities of EGCG.

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Ultraviolet B radiation of human skin generates platelet-activating factor receptor agonists

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Ultraviolet B radiation (UVB) is a potent stimulator of epidermal cytokine production. In addition to cytokines such as tumor necrosis factor-alpha, UVB generates bioactive lipids including platelet-activating factor (PAF). Our previous *in vitro* studies in keratinocytes or epithelial cell lines have demonstrated that UVB-mediated production of PAF agonists is due primarily to the pro-oxidative effects of this stimulant, resulting in the non-enzymatic production of modified phosphocholines (oxidized glycerophosphocholines). The current studies use human skin to assess whether UVB irradiation generates PAF-receptor (PAF-R) agonists, and the role of oxidative stress in their production. These studies demonstrate that UVB irradiation of human skin explants results in the formation of PAF-R agonistic activity, which is found selectively in the epidermis. These PAF-R agonists are generated in a dose-dependent manner, with as little as 500J/m2 UVB resulting in PAF-R activity. The formation of UVB-mediated PAF-R agonists was blocked by topical pretreatment with the antioxidant vitamin C, indicating the importance of reactive oxygen species in their generation. Topical application of the epidermal growth factor receptor inhibitor PD168393 also blocked their formation, which is consistent with the role of the epidermal growth factor receptor in UVB-mediated production of reactive oxygen species. Inasmuch as UVB-generated PAF agonists have been implicated in animal model systems as being involved in photobiologic processes including systemic immunosuppression and cytokine production, these studies indicate that this novel activity could be involved in human disease.

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Deficiency of Xeroderma Pigmentosum A results in an augmentation of UVB mediated oxidative stress and platelet-activating factor activity

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Xeroderma pigmentosum (XP) is characterized clinically by photosensitivity and increased risk of developing skin cancers due to the defect in nucleotide excision repair of ultraviolet (UV)-induced DNA lesions. However, the underlying molecular mechanisms remain unclear. Recent studies have implicated the lipid mediator platelet-activating factor (PAF) in UVB-mediated skin inflammation and the systemic immunosuppression known to be a major cause for skin cancers. In the present study, we investigated the role of PAF in the photosensitivity of XP. We demonstrate that UVB irradiation of human XP group A (XPA)-deficient fibroblasts, in comparison to XPA gene corrected cells, resulted in increased levels of reactive oxygen species (ROS) and PAF-receptor (PAF-R) agonistic activity, both of which were inhibited by anti-oxidants vitamin C and N-acetylcysteine. Similarly, UVB irradiation of XPA-deficient mice resulted in increased levels of PAF-R agonistic activity in the lipid extracts from irradiated skin over SKH-1 control mice, which was significantly blocked by feeding the mice vitamin C-enriched (10g/kg) chow for 10 days. UVB irradiation of XPA-deficient mice also resulted in increased skin inflammation over control SKH-1 mice. This augmentation of UVB-induced skin inflammation in XPA-deficient mice was suppressed by PAF-R antagonists, indicating that the increased PAF-R agonists generated in XPA-deficient mice upon UVB irradiation were biologically relevant. These studies suggest not only a pivotal role for PAF in the hyperphotosensitivity of XP, but also a novel function for XPA, that it serves to regulate UVB-mediated ROS and PAF-R agonist formation.

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Development and validation of a high frequency ultrasound-guided fluorescence tomography system to improve targeting of photodynamic therapy of skin tumors

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Photodynamic therapy (PDT) of skin cancer is not always fully effective, partly due to inadequate levels of fluorescent drug (photosensitizer, PS) and to a heterogeneous distribution of the PS within tumor tissue. We developed a fluorescence tomography system (FTS) that combines a fluorescence detection array with a high frequency ultrasound (HFUS) transducer to image the distribution of PS within skin tumors. Here, we outline the development process and validation of this new system. Protoporphyrin IX (PPIX) was utilized as the target fluorophore for our system. Single layer and double layer gelatin phantoms containing serial dilutions of PPIX were used to calibrate the system and to test it for accuracy and repeatability. Subsequently, multilayer phantoms were prepared to mimic the three-dimensional geometry of a tumor; different combinations of PPIX concentrations were placed in the layers and modeled using the FTS. Next, *in vivo* experiments were performed using a subcutaneous tumor model; A431 tumor-bearing mice were treated with aminolevulinic acid (ALA) to induce production of PPIX. FTS reconstructions were compared with H&E histology and with the bulk tumor imaged on an *ex vivo* fluorescence scanner. Reconstructed images obtained from the FTS corresponded to the histology and the *ex vivo* scans. Such comparisons confirmed that PPIX fluorescence was increased several-fold in the skin and in the tumor compared to the surrounding tissues. Our results demonstrate the feasibility of the FTS for *in vivo* subsurface imaging of PPIX in skin carcinoma. This device will ultimately be utilized for individualized treatment planning in PDT, to improve overall patient response to therapy.

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Role of neutrophil elastase in extracellular matrix damage and wrinkle formation in chronic low-dose ultraviolet irradiation

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It is well known that wrinkle formation is induced by repeated ultraviolet (UV) irradiation. It is thought that accumulation of imperfectly repaired extracellular matrix (ECM) after exposure to sunlight leads to wrinkle formation, and that matrix metalloproteinases (MMPs) are involved in ECM damage in photoaged skin. We previously reported increased neutrophil infiltration and neutrophil elastase (NE) activity in mouse photoaging model repeatedly irradiated with under minimum erythema dose (MED) of UV for 10 weeks. We also reported that typical photo-induced ECM damages, such as collagen fiber and basement membrane damage, were observed in the neutrophil-infiltrated skin. Although it has been reported that keratinocyte produce neutrophil inflammatory mediators, such as tumor necrosis factor-α and interleukin-8, following UV irradiation at over 2MED, it remains unknown how keratinocyte respond to repeated low-dose UV irradiation. In this study, in order to elucidate how neutrophil was infiltrated in low-dose UV-irradiated skin, we examined inflammatory chemokines. In addition, to determine the effects of NE on ECM damage, we examined whether NE activates pro-MMPs *in vitro*. To this end, several chemokines in low-dose UV-irradiated skin were measured by real-time PCR and ELISA. Furthermore, to examine the activation of some MMPs by NE, we analyzed MMP activity by immunoblotting and gelatin zymography. As results, inflammatory chemokines were found to be increased by low-dose irradiation with UV. Additionally, MMP-1 and MMP-2 were activated by NE in fibroblast culture medium. Taken together, we confirmed that new wrinkle mechanism involved in neutrophil infiltration and NE-mediated MMP activation in chronically low-dose UV-irradiated skin.

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MyD88 mediates UV-induced inflammatory responses and cell apoptosis in mouse skin *in vivo*

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Myeloid differentiation factor 88 (MyD88) is known as an adaptor protein for the Toll-like receptor (TLR) family and participates in signal transduction by binding to the cytoplasmic Toll/IL-1 receptor (TIR) domains of activated TLR. Our previous study showed that MyD88 regulates basal- and UV-induced expressions of IL-6 and MMP-1 in the human epidermal keratinocytes. To investigate the role of MyD88 in UV-induced skin responses in mouse skin *in vivo*, MyD88 knockout (MyD88 KO) mice and their wild-type (WT) counterparts were irradiated by 200 mJ/cm^2 of UV. Skin samples were obtained at 48 hrs after UV irradiation. UV-induced expressions of interleukine-1β (IL-1β), IL-6, Cox-2 and MMP-13 were significantly increased in WT mice, but were not changed in MyD88 KO mice. The number of TUNEL-positive cells in the epidermis of MyD88 KO mice after UV irradiation was less than that of WT mice. Expression of cleaved caspase-3 by UV was also less in MyD88 KO, compared with that in WT mice. However, UV-induced skin thickness and Ki-67 expression level didn't show any significant differences between MyD88 KO mice and WT mice. Taken together, our results show that MyD88 mediates IL-1β, IL-6, Cox-2 and MMP-13 expression, and cell apoptosis by UV irradiation.

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Telomere shortening of cultured human dermal fibroblasts in association with acute photodamage induced by ultravioletA irradiation

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Exposures of the human skin to ultraviolet radiation (UVR) result in inflammation, photoaging, and even skin cancer. The research on the photodamage of dermal fibroblasts is of help in elucidating the mechanism of photoaging which still remains elusive. The telomere length serves as an important indicator in the study of the aging process of the cells. The objective of this study was to investigate the effect of different dosages of ultraviolet A(UVA)on the length of telomere of human skin fibroblasts and to study the mechanism of skin photoaging initiated by UVA irradiation. Primary cultured fibroblasts were irradiated with different doses of UVA light, the same passages of the cells untreated with UVA irradiation being used as the control groups. The viability was assessed with MTT assay, cell cycle was detected by flow cytometry assay, senescence associatedβ-Gal was detected by histochemical stain, and the length of telomere was measured by real time PCR. Inhibited proliferation, enhanced expression of senescence associatedβ-Gal and S phase cell cycle accumulation was observed in cultured fibroblast after UVA irradiation. Compared with the control groups, the length of telomere of UVA-treated cells was shortened in a dose dependant manner. The statistical difference was seen between the high irradiation—dose group and the UVA—untreated group ($P<0.05$). These results showed that telomere length of human dermal fibroblasts can be affected by a single high dose UVA-irradiation, and acute photodamage might contribute to early photoaging process in human skin by rapid telomere shortening. Although further study needs to be conducted, this discovery may provide a basis for deeper understanding the molecular mechanism of photoaging.

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Activators of peroxisome proliferator-activated receptor alpha (PPARα) protect UV-induced changes of MMP-1 and procollagen via catalase induction in human skin fibroblasts

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We have reported that among the antioxidant enzymes, the activity of catalase decreased significantly in the dermis of photoaged and aged skin. Therefore, we suggested that the induction of catalase expression may offer a good strategy for the treatment and prevention of aging and photoaging in human skin. PPARα is a nuclear receptor involved in transcriptional regulation of lipid metabolism, fatty acid oxidation, and glucose homeostasis. In addition, PPARα activation stimulates the expression of antioxidant enzymes such as catalase. In this study, we examined whether PPARα activator modulates the expression of MMP-1 and procollagen through catalase regulation in human skin fibroblasts. We found that PPARα and catalase mRNA levels were significantly decreased by UV irradiation and in the aged skin fibroblasts. Treatment with PPARα agonists, bezafibrate and Wy14643, increased protein, mRNA and activity of catalase in dermal fibroblasts. PPARα activators, bezafibrate and Wy14643, inhibited the UV-induced MMP-1 expression and recovered the UV-induced decrease of procollagen expression. Also, in aged dermal fibroblasts, Wy14643 decreased the expression of MMP-1 and increased the levels of procollagen and catalase. Furthermore, UV-induced ROS was decreased by PPARα activator, Wy14643. These results suggest that up-regulated catalase by PPARα activation might scavenge the UV-induced ROS. Transfection with PPARα siRNA decreased catalase level and abolished all beneficial effects of Wy14643 in dermal firboblasts. In summary, our data indicate that PPARα activator increases the antioxidant enzyme catalase, leading to scavenging ROS, and protects the skin from UV irradiation as well as intrinsic skin aging process. Therefore, we propose that the PPARα activator would be a good candidate to prevent and treat skin aging.

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Microarray analysis of differently expressed microRNA profiles induced by UVB irradiated in mice skin

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UV irradiation can induce to photodamge and aging, it could be a carcinogenic factor in human skin, especially UVB. More and more evidence indicates that microRNAs play an important role in embryogenesis, cell differentiation, cell generation, and pathogenesis of human diseases including skin cancer. In this study, we attempt to identify the targets responsible for cell immunologic response by microarray analysis of differential expressed miRNA profiles after UVB exposured. Though the miRNA expression profile, We have identified 6 miRNAs those showed different expression in mice skin treated with UVB in comparison with untreated, among them, miRNA-188-5p,miRNA-223 and miRNA-22 were up-regulated,and miRNA-125a-5p,miRNA-146a and miRNA-141were down-regulated. By the analysis of bioinformatics, we found that these miRNAs are involved in cell immunologic response, cell proliferation and apoptosis. Focusing on the immunologic response, we found that miRNA-146a was related with NF-κB signal pathway, and its target genes, such as IRAK1 and TRAF6 were related with TNF-α,which was an known activator of NFκB signal pathway. By the research, we found that up-regulation of miRNA-146a resulted in inhibiting the immunologic response in HaCaT cells induced by UVB irradiation. The result of the study suggested that miRNA-146a induced modulation of protein expression involved in growth, motility and apoptosis, especially against immunologic response. It may contribute to explain the miRNA-146a could accelerate the progress of skin cancer initially.

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TLR2 mediates UV-induced epidermal proliferation, inflammation and cell apoptosis in mouse skin *in vivo*

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Toll-like receptors (TLRs) recognize endogenous ligands released from stressed and damaged tissues, activate the immune system and promote tissue regeneration. UV irradiation induces inflammatory responses, cell apoptosis, cancer and photoaging in the skin. To investigate the role of TLR2 in UV-induced inflammation and apoptosis, TLR2 knockout (TLR2 KO) mice and their wild-type (WT) counterparts were irradiated by 200 mJ/cm^2 of UV. At 2, 8, 24 and 48 hrs after UV irradiation, skin samples were obtained. We showed that UV-induced epidermal thickness and Ki67 expression in TLR2 KO mice were significantly increased compared with those in WT mice. Also UV-induced expression levels of interleukine-1β (IL-1β), IL-6 and MMP-13 in TLR2 KO mice were significantly increased compared with those in WT mice. Interestingly, the expression of IL-10 mRNA and Foxp3 mRNA by UV in WT mice skin were significantly increased, but were not induced at all in TLR2 KO mice. UV-induced phosphorylation of ERK was significantly higher in TLR2 KO mice skin than in WT mice. Next, we found that the number of UV-induced TUNEL-positive cells in TLR2 KO mice epidermis was significantly greater than that in WT mice. Cleaved caspase-3 expression by UV in TLR2 KO mice also was significantly increased compared with that in WT mice. Taken together, our results suggest that TLR2 mediates UV-induced skin thickness, IL-1β, IL-6, and MMP-13 expression, and cell apoptosis in mice skin through Treg infiltration and ERK phosphorylation.

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Ultraviolet A radiation causes loss of subcutaneous fat

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Loss of subcutaneous fat is a hallmark of aging found in animal models, human progeroid syndromes like Cockayne syndrome (CS) and in normal human aging. Accordingly, we have previously shown that CSB (CSBm/m) deficient mice which were allowed to chronologically age spontaneously developed an almost complete loss of subcutaneous fat. We now report that loss of subcutaneous fat can be significantly accelerated if CS mice are exposed to ultraviolet A (UVA) radiation. Accordingly, CSBm/m mice were crossed into the hairless background and subsequently subjected to a chronic UVA irradiation protocol (10 J/cm^2 per day, 5x per week). After a cumulative dose of 1200 J/cm^2 UVA, loss of subcutaneous fat was observed in CSBm/m mice, but not in irradiated wildtype controls or unirradiated CSBm/m mice, as was shown by normal histology as well as Sudan Red staining. UVA-induced atrophy of adipose tissue was apparently not caused by increased apoptosis as we did not detect apoptotic adipocytes in the skin of UVA-irradiated CSBm/m mice. Interestingly, loss of subcutaneous fat in UVA-irradiated CSBm/m mice was accompanied by progressing skin fibrosis. In agreement with this histological observation, real time PCR analysis showed increased mRNA expression of collagen 1α1 and 1α2 in skin biopsies obtained from UVA-irradiated CSBm/m mice while no increase was noted in the irradiated wildtype controls. It has previously been reported that MMP-3 (i) is critically involved in the development of fibrosis (in combination with TGFβ-1) and (ii) capable of impairing the development and differentiation of adipose tissue. It was therefore intriguing to see an 8-fold upregulation of MMP-3 mRNA in the skin of irradiated CSBm/m mice, while only a 2-fold upregulation was detected in irradiated wildtype animals. These data indicate that the lack of a functional CSB protein renders the skin sensitive to loss of subcutaneous fat and conversion into fibrotic tissue. They also show for the first time that solar UVA radiation can contribute to the loss of subcutaneous fat, most likely by interfering with adipocyte differentiation.

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Neuraminidase 3 (Neu3) is critically involved in the initiation of the UVA stress response

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Solar UVA radiation exerts detrimental effects on human skin. Previously, we have shown that in primary human epidermal keratinocytes (HNK) UVA-induced signal transduction is initiated in cell membrane lipid rafts where second messenger ceramides are being generated. This leads downstream to activation of Src kinases, transcription factor AP2 and increased expression of UVA-inducible genes such as ICAM-1. Modulation of the lipid composition of rafts on a molar basis revealed that approx. 60% of the ceramides formed as the consequence of non-enzymatic hydrolysis of sphingomyelin (SM). In the present study we have searched for the mechanism responsible for the remaining 40%. Depletion of the raft stabilizing protein caveolin-1 by retroviral knockdown in HaCaT cells in vitro or knockout of caveolin-1 in mice in vivo rendered cells/mice completely unresponsive towards UVA radiation. Interestingly, this unresponsiveness was associated with an altered expression of the raft associated Neu3, capable of degrading the monosialoganglioside GM3. Accordingly, UVA radiation increased raft-associated Neu3 activity 2-fold in HNK and 3-fold in HaCaT cells and this was associated with a 40% degradation of GM3 in rafts. Also, complete inhibition of UVA-induced GM3 degradation with the Neu3 inhibitor DANA caused an approx. 40% inhibition of UVA-induced (i) ceramide formation, (ii) activation of Src, (iii) activation of AP2 and (iv) upregulation of ICAM-1 expression. These results indicate that in addition to non-enzymatic degradation of SM, enzymatic, i.e. Neu3 mediated degradation of gangliosides is an initiating event in the UVA stress response. Accordingly, in caveolin-1 knockdown cells neither degradation of SM nor of GM3 was observed after UVA irradiation. Our studies also show for the 1st time that Neu3 is critically involved in the UV stress response in mammalian cells and thus represents a novel molecular target for the prevention of UV-induced skin damage.

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Epigallocatechin-3-gallate inhibits UVA-induced photoaging in human skin fibroblasts through telomere pathway

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In this study, we aimed to investigate whether Epigallocatechin-3-gallate (EGCG) could protect cultured human skin fibroblasts (HSF) from multiple ultraviolet A (UVA) irradiation induced photoaging through telomere mechanism. The photoaging model was established by UVA irradiation in HSFs with or without EGCG exposure . The cell senescence was determined by histochemical staining of senescence associated β-galactosidase (SA-β-Gal). The changes of cell cycle were detected by flow cytometry. The telomere length, mRNA levels of p53, c-myc and p16 were measured by real-time quantitative PCR. With multiple UVA exposure, the proportion of SA β-Gal positive cells and the percentage of G1 phase cells increased apparently in comparison with untreated cells. UVA irradiation also accelerated telomere shortening and up-regulate the level of senescence associated proteins (p53, p16 and c-myc). Pre-treated with EGCG before multiple UVA exposure, the above changes were inhibited by EGCG. These results suggested that EGCG have the inhibitory effect on the UVA-induced photoaging, and its mechanism may be due to telomere protection and expression of p53, p16 and c-myc genes.

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Potential role of interleukin-11 in photoaging-induced loss of subcutaneous fat

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Interleukin-1 (IL-1) and transforming growth factor-β (TGF-β) are known to stimulate fibroblasts to produce IL-11, and IL-11 inhibits the differentiation of adipocytes *in vitro*. We hypothesize that ultraviolet light may also stimulate the production of IL-11 by fibroblasts, and that this mechanism may at least partially explain the loss of facial subcutaneous fat upon aging. Using cultured fibroblasts, epidermal-dermal equivalents, and explants from human abdominal skins, we have confirmed that the combination of IL-1 and TGF-β stimulates IL-11 production in these culture systems. In addition, we observed that exposure to a solar simulator (2.5 – 10 J/cm^2) also stimulates IL-11 secretion, and that the combination of cytokines and solar radiation results in an approximately additive effect. Epidermal equivalents failed to produce IL-11 in response to solar radiation (5 J/cm^2), confirming that dermal cells are required for IL-11 secretion. A non-denatured soybean extract inhibited both spontaneous and radiation-induced IL-11 secretion by human skin explants. These results support the hypothesis that solar radiation-induced IL-11 may be involved in the photoaging-induced loss of subcutaneous fat. Our data suggest that the inhibition of this process by known agents might slow or reverse age-induced changes in facial contouring.

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Baicalin protects human fibroblasts against Ultraviolet A induced oxidative damage and photoaging *in vitro*

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The human skin is the only line of defense against UV radiation, which induces the generation of reactive oxygen species (ROS) and premature senescence of human skin. In this study, we aim to investigate the photo-protective effects of Baicalin, the active flavonoid component of Scutellaria baicalensis, in human skin fibroblasts (HSFs). Subconfluent fibroblasts were shammed or irradiated with different dosages of UVA irradiation and treated with Baicalin. The cell senescence was determined by histochemical staining of senescence associated β-galactosidase (SA-β-Gal). The changes of cell cycle were detected by flow cytometry. The activity of superoxide dismutase(SOD), glutathione peroxidase (GSH- Px), hydrogen peroxidase(CAT) and the level of malondialdehyde(MDA) were detected with colorimetric methods. The telomere length, mRNA levels of p66 and MMP-1 were measured by real-time quantitative PCR. The results showed that UVA irradiation increased the proportion of SA-β-Gal positive cells and the percentage of G1 phase cells in comparison with untreated cells. With UVA exposure, the activities of SOD and GSH-Px decreased, when the activity of CAT and the content of MDA was ascensive. UVA irradiation also accelerated telomere shortening and up-regulate the mRNA levels of p66 and MMP-1. But under the intervention of Baicalin, the above changes induced by UVA irradiation were inhibited. These data indicated the multiple protective mechanisms of Baicalin against photoaging induced by UVA irradiation, which may be related with strengthening antioxidation and decreasing oxygen radicals, telomere protection and down-regulation of p66 and MMP-1 genes expression.

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Photoprotective effects of ferulic on human keratinocyte HaCaT cells: Proteomic identification of proteins associated with cutaneous cancer

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Solar ultraviolet (UV) irradiation, in particular UVB induces different hazardous effects on the skin, including photoaging and cancer. Protection against sun-induced damage is being investigated as a potential approach for the management of UV damages including skin cancer. In this study, we aimed to investigate the proteomic changes of human epidermal keratinocytes (HEK) induced by UVB and ferulic, isolated from Chinese herbal medicine with anti-oxidant and antitumor activities, and irradiation. We used a proteomic method to study these responses. Keratinocytes were kept on culturing for 24h after that 200μg/ml ferulic was added into the medium and exposure to UVB irradiation. The total proteins were collected and separated by two-dimensional gel electrophoresis(2-DE). Differentially expressed proteins were processed through mass spectrometric analysis and database searches. Among all the protein spots detected, 40 were found to be different between the cells before and after ferulic intervention. Of these spots, 19 were identified by using mass spectrometry analysis, which involved in cytoskeleton, metabolism, proliferation, apoptosis and tumorigeness process. Focusing on the tumorigeness occurring at the protein level, we found heat shock protein beta-1 that involve in anti-apoptosis,cell death, regulation of translational initiation. we examined the protein expression of heat shock protein beta-1 by using Western blot analysis. Our data have shown that ferulic induced heat shock protein beta-1 was down-regulated following UVB exposure. And confirmed by siRNA, downregulation of heat shock protein beta-1 results in the increased apoptosis of HaCaT cells to oxidative stress injure induced by UVB irradiation. These results strengthen the notion that ferulic act as potential agent to reduce risk of tumorigeness effect of UVB.

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The anti-aging effect of Light-Emitting Diodes (LEDs) on human skin cells

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Interest in the anti-aging effects of light-emitting diodes (LEDs) has grown significantly in recent years. LEDs target mitochondria and interact with several cellular mechanisms. Little, however, is known about how they improve skin appearance. In this study, we were interested in evaluating the effects of different LED wavelengths on human fibroblasts and keratinocytes. Cells were irradiated twice a week with three different wavelengths: 590nm for yellow, 630nm for red, or these wavelengths combined (orange). Repeated dose studies showed that 12J/cm2 is the dose of choice for these *in vitro* studies. Morphological studies of fibroblasts and keratinocytes revealed that orange LED led to the greatest improvement. Epidermal and dermal markers were most improved with the combined orange LED. While expression of collagen III and fibronectin in fibroblasts, and involucrin in keratinocytes, was more enhanced with red LED than yellow, yellow LED provided for a better enhancement of collagen I. Moreover, a synergic effect was observed with Ki67, tranglutaminase-1, beta-1 integrin, and the CD44 hyaluronic acid receptor. Thus, orange LED with the accumulation of red and yellow wavelengths is typically preferable for improving cell morphology and function. IBIDI cellular migration assay showed an improvement of fibroblast migration 24h after the first irradiation, and was superior at 48h. In order to check if LEDs have an antioxidative effect, we performed Mitosox assay on keratinocytes that showed a reduction of mitochondrial ROS level when irradiated with orange LED. Moreover, apoptosis studies of P53 expression revealed that these LED wavelengths did not induce P53 expression. These studies confirmed that orange LED upregulates the best, cell proteins, and many ECM proteins, and also improves keratinocyte differentiation. Furthermore, dermo-epidermal junction beta-1 integrin was reinforced by orange LED. These results, demonstrate that orange LED is a stress-free positive stimulator of the anti-aging mechanism in skin.

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Irradiation with visible light induces skin damage

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Daily skin exposure to solar radiation causes skin cells to produce reactive oxygen species (ROS) which are a primary factor involved in skin damage. While the contribution of the ultraviolet (UV) component to skin damage has been well studied, few studies have examined the effect of non-UV solar radiation on skin physiology. Solar radiation is comprised of <10% UV radiation, so the purpose of this study was to examine the physiological response of skin to visible light (VL, 400-700nm). The production of ROS, pro-inflammatory cytokines (IL-1α & IL-8), and expression of matrix metalloproteinases (MMP-1) were analyzed *in vitro* using human skin equivalents which were exposed to increasing doses of VL. Irradiation with VL elicited an increase of ROS production corresponding to increasing doses of exposure. Furthermore, IL-1α, IL-8 and MMP-1 expression also increased in a dose dependent manner to VL. Commercially available sunscreens were tested for the ability to reduce VL-induced ROS production and were found to have minimal effects, suggesting that UVA/UVB sunscreens do not protect the skin from VL-induced damage. A non-invasive *in vivo* method to assess the generation of free radicals from oxidative stress is measuring the chemiluminescence (CL) signal in skin. Using CL we were able to show higher levels of free radical activity after VL exposure. In additional studies, we combined a photostable UVA/UVB sunscreen with an antioxidant combo featuring Parthenolide-Free Feverfew (PFF), a natural extract with potent antioxidant activity. Pretreatment with a photostable UVA/UVB sunscreen containing PFF significantly reduced ROS production and decreased IL-1α, IL-8 and MMP-1 expression *in vitro* and CL signal *in vivo* after VL irradiation. Taken together, these findings suggest that other portions of the solar spectrum aside from UV, particularly VL, may also induce substantial skin damage leading to premature photo-aging of skin.

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Multiple exposures with visible light following a large initial dose induce persistent pigmentation, blue wavelengths are the major contributors

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It has been shown that visible light (400-700nm) is able to induce pigment formation in human skin. The present study had two goals, to determine how a series of visible light doses would increase apparent pigment content in multiple skin types, and to determine the action spectrum for visible light induced pigmentation in human skin. In a first experiment two protocols were used to irradiate skin: 1) skin sites received 3 doses of visible light (150J/cm2 at 150 mW/cm2 x 3 days) and 2) a larger conditioning dose was followed by two lower doses (300J/cm2, 150J/cm2 x 2 days at 150mW/cm2). The resulting pigmentation was assessed clinically and with Diffuse Reflectance Spectroscopy (DRS). The response of the skin to the series of exposures at 150 J/cm2 resulted in no changes in pigmentation. With the second protocol (300+150+150 J/cm2) increase in pigmentation was observed following each exposure. The pigment decreased some in between exposures but never disappeared completely and increased further following each exposure persisting for at least 2 weeks after the last exposure. In a second series of experiments 3 distinct spectral bands of visible light (blue: 440-480nm, green: 500-550nm, red: 620-680nm) were used to irradiate skin sites with a single dose of 150J/cm2 at 150 mW/cm2. It was found that the blue band produced the greatest amount of pigment followed by green, with no pigment formation with red band. These results show that an initial higher conditioning dose of visible light is necessary for significant increase in pigment formation and that the blue part of the visible spectrum is the major contributor to visible light induced pigmentation.

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E-Light as a novel nonablative approach to photoaging

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Photoaging was first described in 1986 as a distinct subset of skin aging due to the effects of chronic exposure to the elements, primarily UV radiation. Nonablative rejuvenation for skin aging has become an important method in laser dermatologic surgery. E-Light, a combination of intense pulsed light (IPL) and radiofrequency (RF), is a novel nonablative treatment for reversing the signs of skin aging based on these combined approaches. The first approach includes light-based technologies (IPL) where discrete chromophores are targeted via photothermal mechanisms; and the second approach uses the creation of stress waves at the skin surface for rejuvenation (RF). In order to assess the effects of E-Light on cutaneous aging we evaluated the connective tissue changes induced by this technology on various structures of the skin via immunohistochemical techniques coupled with computerized morphometric analysis. Volunteers of Fitzpatrick skin type III-IV and Glogau class I–II wrinkles were subjected to three months of treatment (six sessions at 2-week intervals) using E-Light. Standard photographs and skin biopsies were obtained at base line as well as at 3 and 6 months after the start of treatment. We show that E-Light produced noticeable clinical results, with facial skin improvement and corresponding high patient satisfaction. Compared to the base line, there was a statistically significant increase in the mean of collagen types I, III and VII, and newly synthesized collagen, together with increased levels of tropoelastin, while the mean level of total elastin was significantly decreased, at the end of treatment and three months post treatment. In conclusion, E-Light is a promising, effective and valuable treatment for photoaging and contour facial skin laxity. This modality stimulates the repair process, and reverses the clinical as well as the histopathological signs of cutaneous aging, with the advantage of relatively risk-free procedure and avoiding the down time.

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Evaluation of sunscreens photoprotection against cyclobutane pyrimidine dimers on *ex vivo* human skin model exposed to UVB or UVA radiation

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Carcinogenic properties of solar ultraviolet (UV) radiation are mediated by its ability to generate DNA damage. It was recently shown that cyclobutane pyrimidine dimers (CPD) are the main type of premutagenic DNA damage produced not only by UVB but also by UVA radiation. Sunscreen's efficacy is currently evaluated to prevent short-term effects of UV such as sunburn but it is difficult to determine whether they efficiently protect against long-term effects such as photocarcinogenesis. DNA damage can be considered as an early biological marker with regard to solar carcinogenesis. So using a highly accurate and quantitative HPLC-mass spectrometry assay, we investigated the ability of sunscreens to protect human skin against CPD formation after UVB or UVA irradiation. For this purpose, human skins obtained after breast plastic surgery were treated or not with sunscreens (2 mg/cm2) and exposed to increasing doses of UVB or UVA. Firstly, a validation of the method was made with standard products (P2, P3 and JCIA standard). Sunscreen stability was shown by the linear formation of CPD with respect to the applied dose. UVA or UVB DNA protection factors (PF) were then calculated for each sunscreen by determining the ratio between the yields of CPD in unprotected versus protected areas of the explants. For the three standard sunscreens, P3 product demonstrated the highest UVB-PF and the lowest UVA-PF while it was the opposite for JCIA standard. We then applied this assay to the determination of the genoprotective efficiency of a new AVENE sunscreen (SPF 50+). This product demonstrated a very good and very high UVA and UVB protection. Interestingly, we found that the ratio between UVB-PF and UVA-PF was similar to that obtained between SPF and PPD values. In conclusion, CPD quantification can provide an excellent quantitative endpoint for evaluating the photoprotective efficacy of sunscreens against UVA and UVB radiation.

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Photoprotection by Avène 50+ inhibits UVA-activated synthesis of metalloproteases 1, 3, and 9 in keratinocytes: implication of the EGFR/beta-catenin pathway

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BACKGROUND: Photoaging is characterized by dermal matrix remodeling including degradation of collagen, fibronectin, and proteoglycan. These important events are, at least in part, due to UVA-mediated metalloproteinase (MMP) synthesis activation in keratinocytes. The signaling events which result in MMP deregulation are not completely understood. However, in a recent report, we have described for the first time that, in both primary human keratinocytes and HaCaT cells, following EGFR activation, UVA induced beta-catenin tyrosine phosphorylation and subsequent nuclear translocation, as well as stimulation of MMP gene transcription through TCF4 (Jean et al., Cancer Research 2009). OBJECTIVE: The aim of our study was to evaluate the efficiency of the photoprotector Avène 50+ on the modulation of this UVA-induced pathway. METHODS: Avène 50+ or control creams were spread on quartz sheet. UVA irradiation was performed through the sheet, and HaCaT cells were collected for immunostaining or real time PCR analysis. RESULTS: Confocal microscopy revealed that Avène 50+, but not control cream, abrogated UVA-induced beta-catenin relocalization to the nucleus. Moreover, Avène 50+ cream inhibited UVA-mediated increase in MMP 1, 3 and 9 mRNA expressions. CONCLUSION: The photoprotector Avène 50+ is able to inhibit the EGFR/beta-catenin/MMP pathway activated by UVA in keratinocytes. This sunscreen might thus prevent changes in skin matrix components induced by prolonged solar irradiation.

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High levels of serum resistin in psoriasis patients are restored to normal by phototherapy

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In addition to the well-known associations between obesity and diabetes, hypertension, dyslipidemia, sleep apnea, and heart disease, obesity and metabolic syndrome are also associated with psoriasis. We investigated the prevalence of metabolic syndrome in patients with psoriasis and the relationship between obesity and psoriasis, focusing on the role of the adipokines leptin, resistin, and adiponectin. First, to investigate the prevalence of metabolic syndrome in patients with psoriasis, data for 165 adult patients (120 men and 45 women) with psoriasis who visited the Dermatology Clinic of Nagoya City University Hospital during the years 2008-2009 were retrieved from the medical records. Next, to study phototherapy-induced changes in adipokine levels, patients with psoriasis (n=36) were recruited and their body mass index (BMI) and disease severity (Psoriasis Area and Severity Index: PASI) were recorded. BMI was calculated as weight (kg)/height (m2). Serum resistin, leptin, and adiponectin levels before and after bath-psoralen and UVA (PUVA) or narrow-band UVB therapy were examined by enzyme-linked immunosorbent assay. Our results indicated that psoriatic patients in the Japanese population have a high prevalence of metabolic syndrome. Serum leptin levels were positively correlated with BMI, but phototherapy induced no remarkable change in leptin levels. Serum adiponectin levels were inversely correlated with BMI in psoriasis patients. Serum resistin levels were high (9.02±8.83 ng/ml) in psoriasis patients, and phototherapy induced a significant reduction (4.86±3.30 ng/ml) to restore normal levels. Serum resistin levels are thought to be involved in insulin resistance and inflammation and are correlated with disease severity in patients with psoriasis. Therefore, these findings indicate that the clinical efficacy of phototherapy for patients with psoriasis may be due to a reduction of serum resistin levels.

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High-dose UVA1 in combination with narrow-band UVB irradiation completely suppresses UVB-induced Langerhans cell migration and diminishes UVB-induced tolerance.

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The longer wavelengths of ultraviolet (UV) A1 phototherapy (340-400 nm) penetrate dermal layers more deeply than the shorter wavelengths of UVB radiation (290-320 nm), and act to clear atopic dermatitis lesions by inducing apoptosis of skin-infiltrating T helper cells. In addition to the induction of apoptosis in pathogenetically relevant cells, antigen-specific tolerance is thought to be an important mechanism underlying the therapeutic effects of narrow-band UVB (NB-UVB) and psoralen and UVA therapy. Whether UVA1 also induces immune suppression, however, remains controversial. We previously demonstrated that consecutive high-dose UVA1 irradiation treatments suppressed sensitization, but not tolerance, in both contact hypersensitivity (CHS) and delayed-type hypersensitivity. Furthermore, UVA1 irradiation did not induce Langerhans cell (LC) or dermal dendritic cell (DC) migration. To elucidate the mechanisms underlying UVA1-induced immunosuppression, we investigated the effect of combined NB-UVB and UVA1 exposure on CHS. Surprisingly, UVA1 irradiation in combination with NB-UVB diminished the suppression of CHS induced by NB-UVB. The underlying mechanisms of UVB-induced tolerance are thought to involve LC, which have a crucial role in antigen presentation. Therefore, we assessed the effect of UVA1 on UVB-induced LC migration. Whereas an approximately 30% reduction in LC was induced by NB-UVB alone, consecutive high-dose UVA1 in combination with NB-UVB irradiation completely suppressed NB-UVB-induced migration of LC and only partially suppressed that of dermal DC, suggesting that UVA1 suppresses the LC migration induced by NB-UVB. These findings suggest that UVA1 suppresses sensitization in CHS by inhibiting antigen-loaded DC migration, and indicate that UVB-induced migration of LC may be an essential event for UVB-induced tolerance.

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Calpain plays a pivotal role in the UVB-irradiated ROS generation in HaCaT keratinocytes through stimulation by IL-1alpha

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In a previous study, we found a biphasic generation of ROS, at immediately after (early stage) and 7 hours after (late stage), in UVB-irradiated HaCaT keratinocytes. In addition, an elevation of Ca2+ levels in the cells was observed prior to the ROS generation. The purpose of this study was to elucidate mechanism(s) regarding the ROS generation after UVB irradiation (UVB-R). In general, UVB-R stimulates the secretion of pro-inflammatory cytokines such as IL-1alpha and TNF-alpha which are stored in cells and activated by Ca2+-dependent processing enzymes. Thus, we speculated the involvement of a Ca2+-dependent pathway on ROS generation at the late stage as a underlying mechanism. In this study, we specialized to elucidate a contribution of IL-1alpha to the ROS generation and underlying mechanisms focusing on a Ca2+-dependent cysteine protease, calpain. In order to demonstrate the speculation, we firstly examined following points; 1) a time-dependent IL-1alpha secretion, 2) role of IL-1alpha on ROS generation. UVB-R on HaCaT keratinocytes stimulated the secretion of IL-1alpha within 7 hours, which was consistent with the time-course of ROS generation. In addition, an exposure of IL-1alpha to cells increased intracellular ROS level. As further investigations, we examined whether UVB-R activated calpain, a calpain inhibitor, ALLN gave suppression on ROS generation after UVB-R. In results, we found that UVB-R increased calpain activity, and that a calpain inhibitor, ALLN, suppressed the elevation of ROS within 7 hours. Conclusively, we propose a mechanism in which calpain is a pivotal enzyme in the UVB-induced generation of ROS through stimulation by IL-1alpha.

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Effect of TNFα blockade on UVB-induced TNFα-mediated photodamage/photoaging

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UVB exposure induces TNFα expression in both keratinocytes (KCs) and dermal fibroblasts. TNFα stimulates nearby cells to display cell adhesion molecules facilitating inflammatory cells recruitment. Inflammatory cells secrete MMPs that alter collagen and glycosaminoglycans (GAGs), leading to cutaneous damage. We hypothesized that blocking of functional activity of TNFα should block aspects of UVB-induced cutaneous alterations. Etanercept, a soluble TNF receptor, inhibits the binding of TNFα to the cell surface TNFRs. We examined the effect of Etanercept on T-cell and mast cell number, MMPs, alteration of collagen and GAGs in the UVB-irradiated C57BL/6J mice skin relative to those known to occur in human photodamaged skin. Mice were treated with Etanercept (4mg/kg/day) during each of 4 days 1h prior to UVB irradiation (100 mJ/cm^2/day) and on the 5th day mice were sacrificed 3h after the last exposure to UVB. We quantitated the T-cells and mast cells in the skin sections with anti-CD3 antibody and Luna staining. Total GAGs were assayed by HALE staining, and collagen was quantitated by measuring hydroxyproline content. MMP-2 and MMP-9 were assayed by zymography and western blot analysis. UVB + Etanercept-treated mice showed fewer T- cells (101.66±10.4 vs 161.5± 12.6, $p<0.05$) and mast cells (97.5±5.1 vs 141.3± 4.1, $p<0.05$) in the dermis when compared to UVB alone. UVB increased the expression of the active form of MMP-2 by 75% as compared to control ($p=0.04$). UVB + Etanercept-treatment increased the expression of the active form of MMP-2 by 54% ($p=0.075$) over UVB-irradiated mice. There was no change in MMP-9 expression as compared to UVB (p=ns). UVB + Etanercept decreased the expression of GAGs (0.37 ± 0.07 vs 0.86 ± 0.08, $p<0.001$) and increased the collagen (0.34 ± 0.05 vs 0.14 ± 0.01, $p<0.001$) compared to UVB-treated mice, suggesting that TNFα has direct effects in vivo in down-regulating collagen. These results suggest that blockade of TNFα has significant effects on the cutaneous inflammatory and extracellular matrix response of mice to UVB.

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Effects of helium-neon laser on melanoblast differentiation: Focusing on mitochondrial biogenesis and antioxidant enzyme

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Helium-Neon (He-Ne) laser has been demonstrated as a phototherapeutic modality for treating vitiligo, inducing both follicular and perilesional repigmentation in responding patients. This particular laser emitting irradiation at visible light spectrum (632.8nm) imparts biostimulatory effects on exposed cells. We have previously shown that He-Ne laser induces functional development of immature pigment cells. Recently, it has been shown that stem cell differentiation process is associated with coordinated changes of mitochondrial biogenesis and antioxidant enzymes. We set out to investigate the effects of He-Ne laser on immature melanoblasts in terms of mitochondrial and antioxidative parameters at different time points. Cultured melanoblasts were irradiated with He-Ne laser and mitochondrial biogenesis markers, antioxidative enzyme expressions, as well as melanoblast differentiation parameters were determined. He-Ne laser stimulates mitochondrial biogenesis, increases the expression and function of antioxidant enzymes, and induces differentiation markers of immature melanoblasts in a sequential manner. These results demonstrated that He-Ne laser induces melanoblasts differentiation via retrograde mitochondrial signaling pathway and provided further insights on how He-Ne laser induces follicular pigmentation on vitiligo lesions.

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Vitamin loaded nanosomes reduce erythma and tryptophan content changes in skin due to solar simulated ultraviolet radiation induced damage

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The objective of our work is to find an optimum size of nanosomes that deliver the encapsulated vitamins to the UV damaged skin and assess the effects of above treatment with respect to non-invasive methods and known biological assays. These experiments have been performed on an animal model, the SKH1 Hairless mouse. Exposure to Ultraviolet (UV) radiation leads to several skin conditions such as sunburn, erythema, immunosuppression (local and systemic) and chronic exposure can result in photocarcinogenisis. These effects can be quantitated by histology and assays of key biomarkers such as matrix metalloproteinases (MMPs) and tryptophan moieties. We developed a solar simulator with a spectral output very similar to that of solar light and have used it to conduct our experiments. SKH1 hairless mice were exposed to varying doses of simulated solar radiation and their Minimum Erythemal Dosage (MED) was found. There were 4 groups of SKH1 hairless mice in the study – Controls (no treatment, no exposure), Group 1 (treated with vitamin loaded liposomes, exposed to UV), Group 2 (treated with Vitamin A, exposed to UV) and Group 3 (no treatment, exposed to UV). Non invasive optical measurements were made on these animals everyday and at the end of 5 weeks, the animals were sacrificed and the skin was harvested. Oxygenated and deoxygenated hemoglobin changes are measured by means of a Diffuse Reflectance Spectrometer. A special spectrophotometer is used to measure the changes in the tryptophan at the irradiated spots every week. The changes in the non-invasive data and the histology suggest that the treatment with vitamin loaded nanosomes help alleviating damage caused by exposure of the animal skin to solar simulated UV radiation.

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Antiapoptotic, pro-antioxidative protective effects of melatonin against UV-induced damage in a human full skin model *in vitro*

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Melatonin has been implicated in skin functions such as hair growth, fur pigmentation and melanoma control. Additionally, melatonin was identified as a strong antioxidant and cell survival protectant through differentially regulated anti-apoptotic effects in UV-induced damage in human keratinocytes. Here, we used a human full thickness skin model *in vitro* to investigate by immunofluorescence dose- and time-dependent UV-induced events by analysing structural skin damage (HE-staining), pigmentation (Masson-Fontana), apoptotic damage (casp-9/-3, TUNEL, p53), DNA-repair enzymes (PARP), proliferation (Ki67) and antioxidative enzymes (catalase). Melatonin protective effects were tested by pre-incubation with melatonin (10-3 M) 1 h before UV-exposure. Human skin was irradiated with UVB/UVA at increasing doses from 25-600 mJ/cm2 and successively frozen directly (0 h), 24 and 48 h after UV-irradiation. Structural changes (e.g. sunburn cells) occurred already under 25 mJ/cm2 at 24 h with increasing intensity at 50, 75, 100, 200, 300, 400 up to 600 mJ/cm2 and up to 48 h post UV. At the mid-range dose of 300 mJ/cm2 (40% of maximum skin damage at 600 mJ/cm2), there was significant sunburn cell reduction by melatonin. Parallely, there was an extremely fast decrease of catalase reaching approx. 42% and 55% vs. control at 0 h and 24 h, respectively. Melatonin significantly prevented catalase consumption, thus decreasing only by 23% and 24%, respectively. UV-exposure further induced dose- and time-dependent increase of casp-9, casp-3, PARP and p53 expression as well as TUNEL-positivity. Melatonin significantly counteracted these down-stream cascades of UV-induced apoptotic and DNA-damage events as early as 24 h post-UV irradiation with prolonged protective effect up to 48 h. Thus, the relevance of melatonin protective effects was confirmed in a human full skin model, showing significant protection against UV-induced damage mainly by reducing a wide range of apoptosis-related events.

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Apoptosis induced by photodynamic therapy with the silicon phthalocyanine Pc 4 in *Candida albicans*

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The high prevalence of drug resistance necessitates the development of novel antifungal agents against infections caused by opportunistic fungal pathogens such as *Candida albicans*. Manipulation and optimization of programmed cell death or apoptosis in yeast-like fungi may provide a basis for future therapies. In mammalian cells, photodynamic therapy (PDT) has been demonstrated to generate reactive oxygen species (ROS), such as singlet oxygen, that leads to instantaneous oxidative modifications of biological molecules, including lipids and proteins, resulting in apoptotic cell death. In this report, we assess the cytotoxicity of PDT using the silicon phthalocyanine Pc 4, an investigational second-generation photosensitizer in *C. albicans*, *in vitro*. Confocal image analysis confirmed that Pc 4 penetrates *C. albicans* cell membrane and localizes to the cytosolic organelles, including mitochondria, within 15 minutes of incubation at 37°C, CFU (colony formation units) counts showed that 1 uM Pc 4 followed by light at 2.0 J/cm^2 reduced cell survival by ~90% (LD90). We also detected an immediate drop in metabolic activity with the use of the FUN-1 fluorescence probe. Furthermore, we observed changes in nuclear morphology characteristic of apoptosis by confocal microscopy following Pc 4-PDT. These data highly suggest that Pc 4-PDT could serve as an alternative mode of therapy against candidiasis.

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Regulation of ultraviolet (UV) radiation induced cutaneous photoimmunosuppression by Toll like receptor-4 (TLR4)

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UVB radiation is a potent immunosuppressive agent that inhibits cell-mediated immune responses. The mechanisms by which UVB radiation influences cell-mediated immune responses have been the subject of extensive investigation. However, the role of innate immunity on photoimmunological processes has received little attention. The purpose of this study was to determine whether toll-like receptors (TLR) contributed to UV-induced suppression of contact hypersensitivity responses. TLR4-/- and wild type C57BL/6 (TLR4+/+) mice were subjected to a local UVB immunosuppression regimen consisting of 100 mJ/cm2 UVB radiation for 3 days followed by sensitization after 24h with the hapten 2,4-dinitrofluorobenzene (DNFB) on the UV-irradiated skin site. Wild type TLR4+/+ mice exhibited 74% suppression of contact hypersensitivity response, whereas TLR4-/- developed only 22% suppression which was statistically significant. Moreover, CD4+CD25+ regulatory T-cells from the draining lymph nodes of UV-irradiated TLR4-/- mice expressed lower levels of Foxp3 than WT mice. When cytokine levels were compared in these two strains after UVB exposure, T-cells from TLR4+/+ mice produced higher levels of IL-10 (1.05 vs 0.5 ng/ml) and TGF-β (0.81 vs 0.52 ng/ml) and lower levels of IFN-γ (4.5 vs 6.3 ng/ml) than TLR4-/- mice. Thus, signal transduction pathways activated by TLR4 appear to be necessary for UVB-induced immunosuppression to occur and blocking TLR4 may be useful in the prevention of photoimmunosuppression.

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Proteomics-based analysis of the effects of *in vivo* ultraviolet radiation on human skin

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Mechanisms underlying UV-induced photodamage and immune modulation are partially understood. Human *in vivo* assays examining UV effects on skin yield more relevant data than animal *in vitro* assays. A high throughput assay evaluating effects of *in vivo* interventions is needed. We used proteomics-based analysis to determine if human skin samples exhibit relevant protein changes after *in vivo* simulated solar radiation (SSR). 4 subjects were exposed to 1 MED of SSR then a sensitizing dose of dinitrochlorobenzene (DNCB). Epidermis was harvested from the treated area and positive and negative control via a suction blister apparatus. Protein was extracted and quantified before 2-Dimensional Fluorescence Difference Gel Electrophoresis analysis. Significant changes in spot patterns were revealed with Decyder software (GE Healthcare), significantly changing spots excised, the proteins digested and analyzed by MALDI-TOF, and identified by peptide mass fingerprinting. DNCB and SSR each induced significant changes in protein abundance. Additional changes were identified under immunosuppressive conditions (DNCB on UV-exposed skin). Involucrin and Keratin 17 increased in all experimental conditions whereas fibrinogen decreased. Hsp70 and Hsp27 decreased in the UV exposed samples. HSP27 increased in both DNCB conditions, but HSP70 only increased in response to DNCB alone. Keratin 10 increased in UV exposed conditions, but decreased with DNCB and UV plus DNCB. IPA (Ingenuity System, Redwood, CA) analysis of UV-exposed skin changes suggested the involvement of NFκB, MAP kinase, TGFβ, and HSP related pathways. Protein analysis of epidermal samples under various conditions *in vivo* captures changes in relevant physiologic pathways and may be a useful surrogate for the mechanisms underlying the photodamaging and immunity effects of UV.

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Generation of immature dendritic cells by modified Extracorporeal photophoresis

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Extracorporeal photophoresis (ECP) is a widely used immunotherapy for the treatment of cutaneous T-cell lymphoma and prevention and reversal of rejection of transplanted organs and graft-versus-host disease. The procedure involves exposure of patient leukocytes to transiently photo-activated 8-methoxypsoralen (8-MOP) in exposure plate, prior to their reinfusion. Since ECP rapidly generates large-scale conversion of monocytes to dendritic cells (DC), it is likely that the reinfused bolus of these immunologically potent cells contributes to ECP's clinical efficacy. To determine how these new DC may contribute to either immune tolerance in the transplant setting, or immunization in the cancer setting, we developed an *in vitro* modified ECP system to analyze the differential effects of 8-MOP/UVA on DC phenotype and function. Treated cells were incubated overnight in platelet storage bags in RPMI-1640, 15% autologous serum. Exposure of normal peripheral blood mononuclear cells (PBMC), to 8-MOP (100 ng/ml) and UVA (2 J/cm2), during 90 minute controlled passage through exposure plate, preferentially generated immature DC (iDC), as assessed by level of expression of co-stimulatory molecules. The difference between mean pretreatment (2.6%) and post-8MOP/UVA treatment (31.6%) expression of HLA-DR and cytoplasmic CD83 was statistically significant at a p=0.002, with the difference between 8MOP/UVA treatment and LPS treatment (80.3%) expression of HLA-DR/CD83 being significant at p=0.001. There was no significant difference between the percentage of CD11c+cells positive for CD80 and CD86 in pretreated cells (2.9%) verses post-8MOP/UVA treatment (8.7%). In contrast, control LPS treated CD11c+cells (72%) expressed significantly higher levels of CD80 and CD86 than 8-MOP/UVA treated cells (p= 0.0003). Since DC maturity correlates with function, our data support the premise that ECP's recognized tolerizing effects, in the transplant setting, results from the reinfusion of large numbers of dendritic cells, whose maturation is truncated by controlled exposure to extracorporeally activated 8-MOP.

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Oxidative stress elevates cystein-rich protein 61 (CYR61), a novel mediator of photoaged skin, in human skin fibroblasts.

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Solar ultraviolet (UV) irradiation causes skin connective tissue damage, which is a prominent feature of sun-induced premature skin aging (photoaging). These skin connective tissue abnormalities largely result from reduced synthesis and elevated degradation of type I collagen, the most abundant structural protein in skin. Cystein-rich protein 61 (CYR61), a secreted, extracellular matrix-associated protein, is substantially elevated in the dermis of photoaged and acutely UV-irradiated human skin *in vivo*, and in UV-irradiated human skin fibroblasts. Elevated CYR61 functions as a negative mediator of collagen homeostasis by inhibiting type I collagen production and promoting matrix metalloproteinase 1 (MMP-1)-mediated collagen degradation. Cellular responses to UV irradiation are initiated by oxidative stress. We have investigated oxidative stress regulation of CYR61, in human dermal fibroblasts. Exposure of fibroblasts to low levels (100 μM) of the naturally occurring oxidant hydrogen peroxide, significantly impaired dermal fibroblast function characterized by reduced production of type I procollagen (70%, n=3, $p<0.05$) and elevated MMP-1 (10-fold, n=3, $p<0.05$), as observed in photoaged and acutely UV-irradiated human skin *in vivo*. Hydrogen peroxide caused substantial upregulation of CYR61 mRNA (5-fold, n=3, $p<0.05$) and protein (4-fold, n=3, $p<0.05$) in human dermal fibroblasts. Treatment with the antioxidant N-acetyl-L-cystein (NAC) reduced oxidative stress-mediated elevation of CYR61 (50%, n=2, $p<0.05$) and prevented reduction of type I collagen and its degradation (n=3, $p<0.05$). These data suggest that elevated CYR61 in UV-irradiated and photoaged human skin is regulated by oxidative stress. By reducing CYR61 expression, effective antioxidant therapy may be able to correct collagen homeostasis in UV-irradiated and photoaged human skin.

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The GS-nitroxide JP04-39 is a potent topical antioxidant that can mitigate skin damage from ionizing radiation.

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There is considerable need to design mitigating agents to reduce ionizing irradiation-induced cutaneous injury. Cutaneous manifestations of radiation damage are generally divided into acute radiation-induced dermatitis (days to weeks later) and chronic radiation-induced dermatitis (months to years later). Mitochondrial damage plays a critical role in the initiation of ionizing irradiation-induced cellular apoptosis and tissue injury. In an effort to ameliorate ionizing irradiation induced skin damage, we designed a small molecule nitroxide, JP04-39, that combines several important anti-oxidant and electron-scavenging properties in a single mitochondrial-targeted functional moiety. To evaluate the effectiveness of this novel drug, the hind legs of C57/BL6 mice were irradiated with 30-35 GY with or without topical treatment with JP04-39 15 minutes after irradiation and then daily for 5 consecutive days. For clinical evaluation, photos were taken for visual evidence of skin damage and leg contracture was measured as a functional evaluation of tissue damage. Histological comparison, including characterization of skin thickening and cellular infiltrates, was also performed. Further, to evaluate the mechanism of radiation damage mitigation, skin samples were assayed for evidence of apoptosis and oxidative stress. Taken together, these studies revealed that topically applied JP04-39 mitigates clinically evident acute skin damage, and damage mitigation correlates with reductions in cellular infiltrates. The GS-nitroxide also demonstrated potent antioxidant function and prevented radiation-induced apoptosis in the skin. These results suggest that topically applied JP04-39 is a potent antioxidant that can prevent/reverse acute irradiation damage.

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Endogenous and exogenous urocanic acid protects against ultraviolet B-induced DNA damage

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Urocanic acid (UCA) is produced by the enzyme histidase and accumulates in the stratum corneum of the epidermis. To investigate the photoprotective role of UCA in the skin, we determined the effects of ultraviolet (UV) B irradiation on wild-type and histidinemic mice, in which the gene encoding histidase is mutated. Histidase was detected by immunohistochemistry in the stratum granulosum and stratum corneum of normal murine epidermis but not in histidinemic epidermis. The UCA content of the stratum corneum and the UVB absorption capacity of aqueous extracts from the stratum corneum were significantly reduced in histidinemic mice as compared to mice carrying at least one wild-type allele of histidase. When newborn mice and the shaved back skin of adult mice were irradiated with 25 and 250 mJ/cm^2 UVB, histidinemic mice accumulated significantly more DNA damage in the form of cyclobutane pyrimidine dimers than wild-type mice. Topical application of UCA rescued the UVB-photosensitive phenotype of histidinemic mice and increased UVB-photoprotection of wild-type mice. Taken together, these results provide strong evidence for an important contribution of endogenous UCA to the protection of the epidermis against the damaging effects of UVB light.

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Enhancement of protoporphyrin IX and suppression of ferrochelatase levels by Vitamin D in tumor models of nonmelanoma skin cancer: Implications for tumor response to photodynamic therapy

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Non-melanoma skin cancers (NMSC) are more prevalent than all other cancers combined. Aminolevulinic acid based photodynamic therapy (ALA-PDT), involving protoporphyrin IX (PpIX) as a photosensitizer to induce target-specific cell death in the presence of light, is still suboptimal for the treatment of deep tumors mainly due to: i) insufficient uptake of ALA; ii) nonuniform PpIX production within tumors. Here, we investigated the use of Vitamin D3 (Vit D3) as a differentiation pretreatment prior to ALA-PDT to address the above limitations. Superficial and deep tumors were generated by chemical carcinogenesis (DMBA/TPA) and by subcutaneous implantation of A431 human squamous carcinoma cells in mice, respectively. Superficial tumors were treated with topical Vit D3 (Vectical) for 3 days, topical ALA (Levulan) for 4 h, followed by noninvasive PpIX fluorimetry and tumor harvest to measure PpIX levels by confocal microscopy. For deep tumors, Vit D3 and ALA were given systemically. Tumor histology, apoptosis, cell proliferation and heme-synthetic enzymes were analyzed. Differentiation pretreatment of tumors: i) significantly (3-4 fold) enhanced PpIX accumulation in both tumor models; ii) increased the metabolic uptake of ALA as almost every cell across the tumor accumulated higher levels of PpIX than the no Vit D3 controls; iii) downregulated the expression of ferrochelatase, a key heme-synthetic enzyme, and iv) upregulated E-cadherin, a marker of differentiation. In the deep tumor model, 20-50 fold lower concentrations of Vit D3 than the maximum dose found in Vectical were found to be effective. Results indicate that a differentiation pretreatment with Vit D3 prior to ALA-PDT enhances PpIX accumulation and also may increase the tumor response to therapy. Downregulation of ferrochelatase by Vit D3 could be an underlying mechanism for this enhanced tumor response.

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Loss of epidermal PPARγ in SKH-1 mice results in absent sebaceous glands, a defect in permeability barrier function, and augmented UVB-induced apoptosis and inflammatory responses

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Peroxisome proliferator-activated receptor gamma (PPARγ) is thought to play important roles in keratinocyte and sebocyte growth and differentiation. In addition, PPARγ ligands exhibit anti-inflammatory activity in a number of tissues. Our previous studies demonstrated that UVB irradiation results in the formation of oxidized glycerophosphocholine species having PPARγ ligand activities in human epidermoid carcinoma KB cells and SZ95 sebocytes. The present studies were designed to determine the role of PPARγ in epidermal biology and photobiology using mice with specific epidermal knockout (KO) of PPARγ in the SKH-1 hairless background. We first noted that adult KO mice exhibited a nearly complete lack of sebaceous glands relative to wildtype sibling controls (WT). We also observed that the epidermis of KO mice exhibit significant defects in cutaneous permeability barrier as determined by an approximate two-fold increase in transepidermal water loss compared to WT mice. We then demonstrated that UVB-irradiation of SKH-1 mouse epidermis caused generation of PPARγ agonist species in epidermal lipid isolates. We next noted that KO mice exhibited both an augmented apoptotic and inflammatory response to UVB. Increased apoptosis was noted by an increase in caspase 3 activity 24 hrs after irradiation with 1500 J/m^2 of UVB in KO mice relative to WT mice. KO mice also exhibited an augmented UVB-induced inflammatory response as noted by significantly increased skin thickness, increased inflammatory cell infiltrate, and significantly increased myeloperoxidase (MPO) activity relative to WT mice at 24 and 72 hrs post irradiation. These findings suggest that PPARγ regulates sebaceous gland development and/or maintenance, as well as permeability barrier homeostasis and protects against UVB-irradiation induced apoptosis and inflammation.

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UVA irradiation silences IGF-1 expression in dermal fibroblasts both *in vivo,* and *in vitro*

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Aging-associated silencing of IGF-1 expression in geriatric dermis has been demonstrated to result in an inappropriate response of geriatric keratinocytes to UVB irradiation. This inappropriate UVB response has been correlated with increased susceptibility to non-melanoma skin cancer in elderly individuals. Because oxidative stress will also silence IGF-1 expression in young fibroblasts and the UVA component of sunlight can penetrate the epidermis and cause oxidative stress in the dermis, we determined whether UVA irradiation can directly silence IGF-1 expression in fibroblasts. Our results demonstrated that UVA irradiation of cultured human fibroblasts led to a dose-dependent silencing of IGF-1 expression. To determine if UVA-dependent silencing could occur in intact skin, full-thickness human foreskin explant cultures were irradiated with increasing doses of UVA and harvested at 72 hours post-irradiation. Once again, UVA was demonstrated to silence IGF-1 expression. Finally, chronically sun-exposed skin was compared with sun-protected skin from the same individuals from a similar anatomic site (pre- and post-auricular skin) was examined for changes in IGF-1 expression. IGF-1 expression was found to be silenced in chronically sun-exposed skin compared to sun-protected skin. These data support and agree with our in vitro data which demonstrates that UVA irradiation can silence IGF-1 expression in dermal fibroblasts and potentially influence the susceptibility of skin to the initiation of non-melanoma skin cancer.

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Microscopic UV challenges on skin provide new information on relationship of challenge to response

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The traditional exposure area for a phototest (a test to determine the sensitivity of skin to ultraviolet radiation) has been between 1 cm^2 and 1 in^2. There have been reports that the apparent sensitivity of the skin, expressed as erythema, decreases for areas of the order of 1 mm^2 and smaller. In this study we investigated the responses of human skin to solar simulated radiation with a beam diameter of 200 μm (0.03 mm^2). Twelve human subjects were exposed to solar simulated radiation on their dorsal upper arm or on their lower back with a series of doses in increments of 20% in order to determine the threshold dose to induce a minimal perceptible erythema response (MED). Each dose was delivered with a liquid light guide of 8 mm diameter and with quartz optical fibers of 200 mm diameter. The resulting skin responses were evaluated visually for the 8 mm diameter spot and with a video microscope for the 200 μm spots. Both types of sites were investigated with a Reflectance Confocal Microscope. The threshold dose to elicit a minimal perceptible erythema was found to be the same between the two sets of exposures. The erythema response to the microscopic beam was always diffuse with no clear boundaries for all given doses extending to several times the exposed site diameter for doses > 3MED. The pigment responses, at 5-7 days after exposure, were always confined within the irradiated sites and upon microscopic inspection showed a non-uniform distribution of pigment that was modulated by the undulation of the stratum corneum. The skin was found to return to normal appearance after the microscopic challenge within two weeks following exposure while change in appearance for the larger areas persisted for several weeks to months. This new modality of testing provides the possibility to study skin at the microscopic level with a rapid recovery following challenge.

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Nitric oxide (NO)-releasing exisulind suppresses UVB-induced cutaneous photocarcinogenesis

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NO-releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs) have promise as cancer chemopreventive agents. A number of NSAIDS have been shown to manifest chemopreventive effects against UVB induced skin photocarcinogenesis. However, the mechanisms underlying the observed inhibition remain elusive. In this study, we investigated chemopreventive effects of NO-Exisulind (5 mg/mouse) against UVB-induced (180 mJ/cm2 twice weekly for thirty weeks) skin photocarcinogenesis in SKH-1 hairless mice. Sixty SKH-1 mice were randomly divided into three groups: group 1 received vehicle, groups 2 and 3 received UVB-irradiation while group 3 animals were also topically treated with NO-Exisulind (30 min prior to UVB-irradiation). At week 30, the group pretreated with NO-Exisulind had a significantly lower incidence ($p<0.003$) and number ($p<0.007$) of tumors as compared to the UVB (alone) group. Similarly, the tumor volume ($p<0.04$) in this group was also significantly reduced. NO-Exisulind diminished the expression of proliferating cell nuclear antigen (PCNA) and cyclin D1 in UVB-induced tumors. These lesions also showed an increase in apoptosis characterized by enhanced TUNEL staining and Bax:Bcl-2 ratio. In addition, a decrease in the UVB-induced phosphorylation of extracellular signal-regulated kinase 1/2 (Erk 1/2) and p38 MAP kinases was seen in NO-exisulind-treated SCCs but not in benign lesions. These changes were accompanied by a reduction in mesenchymal markers such as fibronectin, n-cadherin, SNAI, Slug and twist and an increase in epithelial marker, e-cadherin in SCCs. Our data suggest that topical treatment with NO-Exisulind affords protection against UVB-induced carcinogenesis in SKH-1 mice.

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Proteome changes in human epidermis induced by UVB irradiation

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Long-term sun exposure, principally UVB, induces skin cancer, premature skin aging and cutaneous immunosuppression. Comprehensive analysis of UVB-induced changes in human epidermal cell protein expression may assist in discovery of UVB-induced damage biomarkers. This study determined changes in the human epidermal proteome of two healthy young volunteers at 2, 7 and 24h after UVB (40 mJ/cm2) using iTRAQ and 2D LC-MS/MS. Expression of several distinct proteins identified with >95% confidence was altered by UVB during 24 hours. Across all time points, metabolic protein expression was highly increased while that of keratins and RTN4 (NogoA) expression was decreased. In the early phase (2-7 hours after UVB), expression of several metabolic proteins increased, including antioxidants, proteins involved in motility, and glycolysis/gluconeogenesis. Expression of several proteins decreased, including several proteins involved in structural components e.g. keratins (1, 5, 10, 14), apoptotic (calpain and lamin A) and calcium binding (S100A14) proteins, the neurite growth inhibitor reticulon 4 isoform A (NogoA), and the GABA-B receptor 1 (GABABR1). In the late phase (24 hours after UVB), expression of several proteins increased, including several proteins involved in glycolysis & gluconeogenesis, methane and phenylalanine metabolism, and regulation of actin-based motility. Expression of several proteins decreased, including several cytoskeletal constituents (keratins 9, 15) and myosin, proteins RTN4-A (NogoA) and GABAR1. Confocal microscopy of naive human skin biopsies confirmed the presence of RTN4 (Nogo) and GABAR1 in epidermis and select dermal cells, validating the proteomic results. These results suggest that during the first 24h after UVB challenge, human epidermal cells alter protein expression to protect the skin from oxidative damage and invite neurite extension.

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Photoactivated Rose Bengal, a possible mutagen in Chinese hamster ovary cells

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Dermatological surgery relies on sutures and adhesives for wound closures. These methods increase the risk of foreign body reactions, infection, and scarring. Recent studies have investigated Rose Bengal (RB) as a photochemical bonding agent to replace traditional wound closure techniques. RB is known to create reactive oxygen species (ROS), such as singlet oxygen. Once photoactivated, these ROS can cause DNA mutations. We investigated the toxicity and mutagenicity of photoactivated RB on epithelial Chinese hamster ovary (Cho) cells. Cells were exposed to the following concentrations of RB: 0.1%, 0.01%, 0.001%, and 0.0001%. The cultures were irradiated for 400 seconds using a high-intensity visible wavelength lamp at 81,500 lux. Control cultures were maintained in the dark. Cell viability was assessed using trypan blue exclusion and the XTT assay. Mutagenicity was assessed using the Hprt mutation assay. Exposure to concentrations of RB greater than 0.001% resulted in complete cytotoxicity following exposure to light. After 30 minutes and 24 hours, the XTT assay showed that cells exposed to light only, the lower levels of RB without light, or the lowest level of RB plus light appeared more viable than controls without light. The Hprt gene mutation assay showed that the light activated RB at 0.0001% was likely mutagenic, with roughly a doubling of the background mutation frequency. All other concentrations resulted in low or nonexistent colony formation due to RB's cytotoxicity. RB's ability to produce ROS and its potential for mutagenicity should be taken into consideration in its clinical application. RB's innate cytotoxicty at relatively low concentrations (e.g., 0.001%) appears to be significantly below the 0.1% concentration used in clinical studies.

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The role of macrophage migration inhibitory factor (MIF) in UVB-induced melanogenesis in the skin

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It is evident that keratinocytes are a key cell type in the epidermis, which mediate the tanning response of the skin to UV radiation. Protease-activated receptor (PAR)-2 activation induces melanosome transfer through the increased phagocytosis of melanosomes by keratinocytes. Recent studies have suggested a potentially broader role for MIF in skin inflammation owing to its ability to enhance PAR-2 expression. In this study, we demonstrated that MIF (100 ng/ml) stimulated PAR-2 expression in human keratinocytes. Additionally, we have shown that MIF stimulated stem cell factor (SCF) release in keratinocytes; however, MIF had no effect on the release of endothelin-1 or prostaglandin E2 in keratinocytes. MIF has no direct effect on melanin and tyrosinase synthesis in cultured human melanocytes. Next, we examined the effect of MIF on melanogenesis using a 3-dimensional reconstituted human epidermal culture model—MelanoDerm, which is a novel commercially available cultured human epidermis containing functional melanocytes. MIF induced an increase of melanin content in the epidermis after a 9-day culture period. Moreover, we observed that melanin synthesis induced by UVB (100 mJ/cm2) stimulation was significantly down-regulated by anti-MIF antibody (30 μg/ml) treatment. Our *in vivo* study showed that the back skin of MIF transgenic mice had a higher melanin content than that of wild-type mice after 12 weeks of UVB exposure. Therefore, we concluded that MIF mediated melanogenesis mainly through the activation of PAR-2 and SCF expression in keratinocytes following exposure to UVB radiation.

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Oncogenic transformation of cutaneous melanocytes by ultraviolet radiation and Bisphenol A

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Melanoma incidence is on the rise despite increased public awareness to reduce sun exposure. We hypothesized that ultraviolet radiation (UV) co-operates with environmental chemicals to alter signaling pathways that initiate or accelerate melanomagenesis. The high incidence of melanoma associated with workers exposed to plastic dust raised the hypothesis that exposure to Bisphenol A (BPA), a chemical used in the production of many plastics, may participate in mechanisms of melanomagenesis. Normal human epidermal melanocytes (NHEM) were treated with a single dose of UVB (20mJ/cm^2), BPA (1X10^{-7}M) or UV/BPA combined. Gene expression profiling and Ingenuity Pathway Analysis showed that UV and low dose BPA act synergistically on melanocytes to perturb the expression of several key oncogenes and tumor suppressor genes. The expression of PTPRK, a tumor suppressor gene downregulated in >20% of melanoma cell lines, is significantly decreased in UV+BPA treated cells (170 fold change (FC) compared to UV (80 FC) and BPA alone (2 FC). GDF15, an oncogene involved in the transition from non-metastatic to metastatic phenotype in melanoma, is upregulated 13 fold in the combined condition compared to UV (6 FC) and BPA alone (5 FC). BRCA1, a tumor suppressor involved in DNA repair, is also downregulated in the combined condition, but not in BPA or UV treated cells. These novel observations suggest synergistic roles and additive effects of the environmental stressors UV and BPA on melanoma development and suggest new disease biomarkers and preventive/therapeutic strategies.

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Frequency of somatic mutations in GNAQ in primary, pigmented dermal melanocytic proliferations

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A recent study indicates that somatic mutations in codon 209 of GNAQ, a gene encoding the signaling protein G protein alpha subunit q, may be present in up to 80% of blue nevi. Given that blue nevi are primary dermal, pigmented melanocytic proliferations, the aim of this study was to ascertain the frequency of somatic mutations in GNAQ in various dermal melanocytic proliferations including blue nevi. Genomic DNA was isolated for genotyping per protocol using techniques including laser capture microdissection to isolate nevus cells from regular blue nevi (BN, 10, all melanotic), cellular blue nevi (CBN,9, all melanotic), metastatic melanoma (MM, 9, 4 of which were melanotic) and intradermal melanocytic nevi (IDMN, 9, 2 of which were melanotic). DNA sequencing analysis was performed on GNAQ spanning codon 209, BRAFV600E, NRAS1, NRAS2 and KRAS genes. Mutations in GNAQ were noted in 40% (4/10) of BN and 44% (4/9) of CBN but in not in any of the cases of MM or IDMN. The only mutation detected was an A/T point mutation. While no additional mutations were noted in cases of BN, one CBN exhibited a concurrent NRAS2 mutation, one a concurrent KRAS mutation and a third a mutation in KRAS alone. Given that blue nevi and its variants are inherently stable lesions with a low potential for malignant transformation, our findings suggest that, like oncogenic BRAF and RAS, the relevance of a somatic mutation in GNAQ appears to be in the initiation of melanocytic neoplasia as it alone appears to be insufficient for malignant transformation. The presence of GNAQ mutations in blue nevi and its variant and its absence in IDMN and MM indicates that activation of the q class of the G-protein alpha subunit appears to be unique to primary pigmented dermal melanocytosis. The consequences of concurrent mutations in RAS and GNAQ are unclear for now and require further study.

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The p16^{INK4A} tumor suppressor regulates cellular oxidative stress

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Mutations or deletions in the cyclin-dependent kinase inhibitor p16^{INK4A} are associated with multiple cancer types, but more commonly found in melanoma tumors and specifically associated with familial melanoma predisposition. Although p16 is thought to function as a tumor suppressor by negatively regulating the cell cycle, it remains unclear why genetic compromise of p16 predisposes to melanoma rather than other cancers. Here we describe a novel role for p16 in regulating oxidative stress in several cell types, including melanocytes. Expression of p16 was rapidly upregulated following UV-irradiation, and in response to H_2O_2-induced oxidative stress in a p38 stress-activated protein kinase-dependent manner. Knockdown of p16 using siRNA increased intracellular ROS and oxidative (8-oxoguanine) DNA damage which was further enhanced by H_2O_2 treatment. Elevated ROS were also observed in p16-depleted human keratinocytes, and in whole skin and dermal fibroblasts from p16-deficient mice. Elevated ROS and p38-signaling in p16-deficient fibroblasts were normalized by expression of exogenous p16. Finally, p16-mediated suppression of ROS could not be attributed to upregulation of antioxidant enzymes or the role of p16 in cell cycle regulation. These findings suggest a potential alternate tumor-suppressor function of p16 as an endogenous regulator of carcinogenic intracellular oxidative stress. Compared to keratinocytes and fibroblasts, we also found increased susceptibility of melanocytes to oxidative stress, which may explain why compromise of p16 predisposes to melanoma over other cancers.

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Combination of Dacarbazine and Dimethylfumarate efficiently reduces melanoma lymph node metastasis

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Dimethylfumarate (DMF) has been shown to reduce melanoma growth and metastasis in animal models. We addressed the question of whether DMF is as effective in its antitumor activity as the US Food and Drug Administration-approved alkylating agent dacarbazine (DTIC). We also tested the possibility of an improved antitumoral effect when both therapeutics were used together. Using our severe combined immunodeficiency (SCID) mouse model, in which xenografted human melanoma cells metastasize from primary skin sites to sentinel nodes, we show that these treatments, alone or in combination, reduce tumor growth at primary sites. Our main finding was that metastasis to sentinel nodes is significantly delayed only in mice treated with a combination of DTIC and DMF. Subsequent experiments were able to show that a combination of DTIC/DMF significantly reduced lymph vessel density in primary tumors as examined by real-time PCR and immunohistochemistry. In addition, DTIC/DMF treatment significantly impaired melanoma cell migration *in vitro*. *In vivo*, DTIC/DMF therapy significantly reduced mRNA expression and protein concentration of the promigratory chemokines CXCL2 and CXCL11. In addition, our data suggest that this xenotransplantation model is suitable for preclinical testing of various combinations of antimelanoma agents

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Prevention and treatment of human melanoma by EGCG encapsulated in chitosan nanoparticles

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Chemoprevention, especially through the use of bioactive food components, could be a useful approach for melanoma prevention and treatment. However, the bioavailability of effective chemopreventive agents is a major limiting factor in the success of this approach. Here, we explored whether our recently-introduced approach of nanochemoprevention (Cancer Res. 2009; 69: 1712-6) could be applied to human melanoma. We encapsulated epigallocatechin gallate (EGCG) from green tea in chitosan, a polymer suitable for oral delivery, and determined the utility of chitosan-encapsulated EGCG (nano-EGCG) in augmenting the cytotoxicity on human melanoma Mel 928 cells. Treatment of cells with nano-EGCG produced remarkable growth inhibitory effects, with an IC50 of 2 μM (versus 50 μM for non-encapsulated EGCG in PBS). Next we determined whether nano-EGCG retained its mechanistic functionality. Nano-EGCG treatment (2 and 4 μM) of Mel 928 cells resulted in (i) a shift in the Bax/Bcl-2 ratio favoring apoptosis, (ii) augmentation of PARP cleavage, (iii) marked inhibition of caspase 3 and 9, (iv) inhibition of Cdk 4 and 6, (v) induction of p16, p21 and p27, (vi) significant inhibition of cyclin D1 and D3, and (vi) dramatic increases in the levels of annexin V. When we examined EGCG release kinetics, EGCG release peaked 2 hours post-incubation, with 50% released into the intestinal fluid and 30% released into the gastric juice. Thereafter, EGCG remained at a constant level with a steady release trend. To ascertain the oral bioavailability of EGCG, blood samples were collected from mice treated with EGCG and nano-EGCG 1, 8 and 24 hours after administration, and the levels of EGCG were determined by LC/MS/MS. Nano-EGCG exhibited a significant longer half-life in the plasma of mice as compared to non-encapsulated EGCG. Translation of these data to appropriate animal model systems could pave the way to new avenues of melanoma treatment and prevention.

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The melanocyte-specific glycoprotein, Pmel17/gp100, is released by ectodomain shedding

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Pmel17/gp100 is a melanocyte-specific membrane-bound glycoprotein that has amyloid characteristics and forms fibrillar structures in melanosomes after a complex sequence of post-translational processing and trafficking events, including cleavage by a furin-like proprotein convertase (PC). Ectodomain shedding is a proteolytic mechanism by which a transmembrane protein is converted into a secreted form. A secreted form of Pmel17 (termed sPmel17) was also thought to be released due to cleavage by a PC and has not been characterized well so far. We used multidisciplinary approaches to demonstrate that sPmel17 is released by ectodomain shedding at the juxtamembrane and/or intramembrane motif and to show that this is independent of cleavage by a PC. We further show that sPmel17 consists of 2 fragments linked by disulfide bonds and that the shedding is inhibited at low temperature but not by metalloproteinase inhibitors. Moreover, treatment with a phorbol ester or a calmodulin inhibitor induces Pmel17 shedding. We also refine the reactivity of HMB50 and NKI/beteb, 2 monoclonal antibodies commonly used for melanoma detection. The fact that those antibodies require physically separated domains of Pmel17 sheds interesting light on its 3-dimensional conformation. We conclude that sPmel17 is released by regulated proteolytic ectodomain shedding.

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A mechanism of acquired resistance to B-RAF (V600E) inhibition in melanoma

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Activating B-RAF V600E kinase mutations occur in ~7% of human malignancies and ~50-70% of melanomas. Early clinical experience with a novel B-RAF (V600E)-selective inhibitor, PLX4032, demonstrated an unprecedented 70% anti-tumor response rate among patients with B-RAF (V600E)-positive melanomas, but acquired drug resistance frequently develops after initial responses. To understand the potential mechanism(s) of acquired resistance, we derived drug-resistant sub-lines from B-RAF (V600E)-positive melanoma cell lines. Acquired PLX4032 resistance did not arise from secondary mutations in B-RAF. Instead, up-regulation of a specific receptor tyrosine kinase (RTK) at the RNA, protein and tyrosine phosphorylation levels emerged as a dominant feature of acquired PLX4032 resistance among melanoma cell lines, patient-derived biopsy samples, and short-term cultures. This heightened RTK activity contributed significantly to the growth of PLX4032-resistant melanoma cells. Thus, blockade of a specific RTK is a potential therapeutic strategy against appropriated selected melanomas escaping V600EB-RAF targeting.

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An advanced approach for skin whitening /lightening regulation through the inhibition of a new intracellular target

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Skin complexion is an important attribute of beauty and is of endless concern. Today's demand for skin lightening products are on the rise to achieve fairer and lighter complexion, but they are also an excellent tool to fight age-related hyperpigmentation such as age spot. In skin pigmentation, two main physiological pathways are involved: "constitutive" and "facultative" pigmentations. Traditionally, whitening cosmetic actives targeted the "facultative" pathway only, but Unipex Innovations has developed a new strategy targeting both mechanisms of action through the inhibition of Microphtalmia-associated transcription factor (MITF). Inspired by TGF-β and MITF role in skin pigmentation, Unipex Innovations presents its innovative new encapsulated biomimetic whitening peptide. Cellular constitutive and facultative pigmentation processes were addressed by several *in vitro* molecular and cellular methodologies using murine and human melanocytes. The inhibition of the expression of MITF, TRP-1, TRP-2 and the inhibition of tyrosinase activity in presence or absence of UV radiations was demonstrated. Furthermore, significant reduction of dendricity & melanosome content was established. The reduction of melanin production was also studied in comparison with market references (vitamin C and Arbutin). The *in vivo* efficacy of the active was evaluated in a clinical study with 23 Asian volunteers by evaluating the skin color reduction under dermatologist evaluation and by measuring the lightening effect with L* parameter quantification. Data showed that the active induces significant progressive skin lightening effect on face and hyperpigmented spots after 28 and 56 days. In conclusion, targeting "constitutive" and "facultative" pigmentation pathways is a safe and effective strategy to whiten and lighten the skin complexion and represents a major advance in melanogenesis modulation.

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Histochemical and immunohistochemical study in melasma. Evidence of damage in the basal membrane

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The pathogenesis of melasma has not been clearly elucidated. We included 48 melasma patients without previous treatment. Samples were taken from lesional and non lesional (buttock) skin in 24 patients. In other 24 patients, we took biopsies of lesional and perilesional skin. Biopsies were also taken from healthy control subjects of exposed skin. Histochemical (Fontana Masson, PAS) and immunohistochemical (SCF, c-kit and anti mast cell tryptase) studies were realized. In the first group of patients, Fontana Masson stain, in melasma lesions revealed an increase of melanocytes and melanin in all epidermal layers even in the stratum corneum compared with non lesional skin; interestingly many pigment basal cells protruded into the dermis in 45% of the melasma biopsies. PAS stain used in the second group of patients showed damage on the basal membrane BM in 96% of melasma lesions vs 75% of perilesional skin. In melasma skin we could not find BM in 16% vs 4% of adjacent lesional skin. Interruptions of BM with thickness were evident in 79% vs 58% of perilesional biopsies, in 4% of perilesional biopsy only thickness was evident. In 5 control biopsies of exposed skin no evidence of damage was found in BM. The expression of SCF and anti-mast cell tryptase was increased at lesional dermis compared with perilesional dermis. A protrusion of basal cells c-kit (+) into the dermis was evident in 70% vs in 29% of adjacent lesional skin. The expression of anti-mast cell tryptase was increased at lesional dermis compared with perilesional skin. The results suggest a role of SCF, c-kit and mast cells in the pathogenesis of melasma. We were surprised by the unexpected evidence of damage to BM that could facilitate the protrusion of cells and the fall of the melanin into the dermis. The protruded basal cells might be active melanocytes allowing the constant hyperpigmentation in melasma.

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A new insight into skin whitening: The accumulation of advanced glycation end products (AGEs) in the dermis triggers melanogenesis in epidermal melanocytes

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The melanin content of the epidermis is a dominant factor that determines skin color. Increased skin pigmentation after UV-exposure is well known, and it has often been observed that the skin color in UV-unexposed skin turns yellow-dark with age. Recent studies have reported that advanced glycation end products (AGEs) give adverse physiological affects via a specific receptor, RAGE, on the cell surface of skin dermal fibroblasts. In 2005, Aoki et al reported that melanocytes exposed to substances derived from lymphocytes and mast cells initiate melanogenesis, which strongly suggests that alterations of the dermal environment by the accumulation of AGEs eventually triggers skin pigmentation in the epidermis in the absence of UV exposure. This study was designed to identify a new pathway of melanogenesis that is triggered by AGEs that accumulate in the skin dermis, in which specific mediators play a critical role in facilitating dermal-epidermal cross-talk. A comparative study using protein arrays indicated that production of MCP-1 by dermal fibroblasts was dominantly stimulated by treatment with AGEs-BSA. To examine the possibility of trans-reactivity of MCP-1 on epidermal cells, we characterized MCP-1 receptor expression in epidermal keratinocytes and melanocytes. Interestingly, the two MCP-1 receptors, CCR1 and CCR2, were specifically expressed by melanocytes but not by keratinocytes. Consequently, we examined the direct effect of MCP-1 on tyrosinase protein synthesis in melanocytes. The DOPA oxidase activity and western blotting analysis clearly demonstrated that the de novo synthesis of tyrosinase protein by melanocytes was enhanced by MCP-1. These findings indicate the existence of a new pathway of melanogenesis initiated by MCP-1, which is induced by accumulated AGEs in dermal fibroblasts.

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Melanoma growth and metastasis is dependent on the presence of vitronectin in the extracellular matrix

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The transition of radial to vertical growth phase in melanoma is accompanied by the expression of alpha-v and beta-3 (avb3) integrin. This integrin has been previously shown to be important for melanoma invasion and metastasis. Recent therapeutic strategies to inhibit this integrin have proved controversial. In the present study, we assessed the role of vitronectin, the main partner of avb3 integrin, in melanoma growth and metastasis. Age-matched wild type and vitronectin deficient (VN-/-) mice were intradermally injected with B16-F10 melanoma cells. Tumour volume was significantly increased in VN-/- animals from day 13 onwards ($p<0.001$). However, fewer mice had metastases in the VN-/- group at day 16 (4/19 VN-/- vs 11/18 WT, $p=0.02$). Similarly, when injected intravenously, VN-/- mice had significantly less metastases (48 vs 79, $p=0.004$). This was accompanied by decreased lymphangiogenesis in tumours from VN-/- mice. In animals subjected to primary tumour excision at day 10, survival was significantly increased in the VN-/- group. *In vitro*, B16 cells had dramatically reduced adhesion when cultured with VN-/- serum. This was fully corrected by addition of vitronectin but only partial correction was obtained by collagen IV. The absence of vitronectin also resulted in increased cell proliferation *in vitro* that was reduced by addition of wild-type serum containing vitronectin. In conclusion, vitronectin is a major component of the extracellular matrix for melanoma cell adhesion and their subsequent spread. Its dual effect on tumour growth and spread may explain contradictory results obtained by inhibitors of avb3 integrin.

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Seasonal variation in serum level of 5-S-cysteinyldopa in postoperative cases of malignant melanoma without metastasis

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Statistical analysis of serum 5-S-cysteinyldopa (5-S-CD) levels which were examined in summer and winter was performed in 16 cases of malignant melanoma without metastasis. During the observation, the number of cases which 5-S-CD levels exceeded the upper limit of normal value, 8 nmol/l, was nine (56.3%), and the correlation between 5-S-CD level and stage was insignificant. The incidence of increase of 5-S-CD levels beyond the upper limit was 28.8% in measurement from May to August, though it was 2.3% in that from November to February. The serum 5-S-CD level measured from May to August, 9.3±1.2 nmol/l, was significantly higher than that from November to February, 4.6±0.4 nmol/l. It is possible that serum 5-S-CD levels of malignant melanoma patients without metastasis may increase and exceed upper limit of normal value by exposure of the subjects to sunlight in summer.

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Skin melanin index obtained from digital photography and videodermoscopy correlates with dermatologist assessment of skin type

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Assessment of skin photosensitivity by Fitzpatrick Skin Type (FST) is considered a predictor for the risk of developing skin cancer. Recall and respondent bias introduce error into this subjective assessment. An objective assessment of skin pigmentation is an unmet need to predict skin response to sunlight. In the last decade, several instruments have been developed for this purpose, including digital imaging with red-green–blue (RGB) spaces. This study assesses skin type by Melanin Index (MI) obtained from RGB digital images using both a digital camera and a videodermoscope compared to FST. Both instruments are calibrated at standard illumination, light source and white balance. Bilateral images of the upper ventral arm (constitutive skin) are taken for reproducibility of results. Fifty-eight subjects (20 M, 38 F) were enrolled in the study (mean age: 47 yrs; range: 20-89), skin type I-VI (I=10;II=13;III=8;IV=10;V=10;VI=7), race (36 White, 19 African American, 3 Asian). For both instruments, no significant difference was found between MI of the left vs right arm. The digital camera MI showed separation among 3 categories of skin type: a) I, b) II, III, IV, and c) V,VI (ANOVA, $p=0.02$). However, videodermoscopy MI demonstrated a progressive gradient from skin type I through VI (ANOVA, $p<0.0001$). Both digital camera and videodermoscope MI positively correlated with dermatologist assessed skin type (Spearman coefficient, $r = 0.43$; $r=0.70$). These findings indicate that MI determined by RGB images are an objective measure of skin pigmentation. The videodermoscope is a non-invasive, easy-to-use tool that may provide objective and reproducible measurements to predict human skin response to sunlight. Given that it is difficult to achieve standardization of distance, brightness and arm position with a digital camera, it is challenging to achieve consistency under clinical conditions. The videodermoscope represents a valuable instrument for future use.

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Assessment of pigmentation response to chronic solar exposure among skin phototypes I-VI

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While Fitzpatrick skin types (FST) may be a reliable and valid approach to assess skin pigmentation in fair-skinned persons, it is problematic for dark skinned persons due to reliance on self-reported ability to "tan" and "burn", which are considered culturally-biased insofar as they reflect personal experience of sun-reactivity. The aim of this study is to quantify the extent of pigment response to chronic sun exposure in skin types I-VI by assessing skin pigmentation measured as melanin index (MI). MI was obtained from RGB (Red-Green-Blue) digital images using both a digital camera and a videodermoscope. Both instruments were calibrated at standard conditions. Images were taken of the lower dorsal forearm (chronic sun exposed skin) and upper ventral arm (sun protected skin). Fifty-eight subjects (20 M, 38 F) were enrolled in the study (mean age: 47 yrs; range: 20-89), with all skin types (I=10;II=13;III=8;IV=10;V=10;VI=7). With both the digital camera and videodermoscope, there was a statistical difference between sun exposed vs. sun protected skin and there was statistical correlation for the two instruments ($p=0.0017$). Also, the difference between sun protected vs. sun exposed has a positive correlation with FST ($p< 0.05$). All skin types are affected by chronic sun exposure; however, the higher the FST the greater the change in MI between sun protected and sun exposed skin. People with skin types IV, V and VI, who may not self-report tanning, demonstrate greater increases in MI in sun exposed skin than those with skin types I –III, who self-report tanning. By FST, skin type VI shows no noticeable pigment change after sun exposure; however, the difference in mean MI of chronically sun exposed vs sun protected skin is increased. (ANOVA, $p =.008$)This MI increase in sun-exposed skin for those with skin type VI corresponds to their self-reported experience of gradually getting darker over the summer in northern latitudes. The MI provides an objective measure of the response to sun exposure for those with darker skin tones.

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Pediatric melanoma of the head and neck: A single-institution review of 41 patients

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The goal of this study is to describe pediatric head and neck melanoma in terms of general survival trends, along with patient, histologic, and tumor characteristics. The Duke Melanoma Database and Tumor Registry Database were searched for patients with a diagnosis of melanoma occurring on the head or neck before age eighteen, with exclusion of mucosal/aerodigestive melanomas. The query identified 41 pediatric subjects with melanoma of the head or neck. All patients were Caucasian with 26 (63.4%) males and 15 (36.6%) females. The primary sites were represented as follows: ocular (1 subject, 2.4%), ear (1 subject, 2.4%), facial (15 subjects, 36.6%), and scalp/neck (24 subjects, 58.5%). The follow-up time ranged from 6.2 to 13 years with a median of 9.9 years. At the time of follow-up, there were 13 (31.7%) melanoma-associated deaths—11 males and 2 females. The anatomic distribution of primary melanoma for these 13 patients follows: 1 (7.7%) ocular, 4 (30.8%) facial; and 8 (61.5%) scalp/neck. Histologic data revealed 24 (58.5%) tumors classified as superficial spreading melanoma; 5 (12.2%) as nodular melanoma; 8 (19.5%) were not classified; and 4 (9.6%) classified as other. For those patients who experienced melanoma-related mortality, the most common pathologic subtype was superficial spreading (6/13, 46.2%), followed by nodular (3/13, 23.1%). The mean Breslow depth for patients with melanoma-related mortality was 2.4 mm, compared to 1.8 mm for those who were alive at last follow-up. Twenty-three (56%) patients received lymph node dissection. Of these 23, 17 (73.9%) were found to have nodal involvement of metastatic melanoma. In almost four decades, 41 pediatric patients, with a predominance of males and superficial spreading melanoma subtype, were treated for head or neck metastatic melanoma. Survival for patients with scalp or neck melanoma was slightly worse when compared to other sites on the head.

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Genetic variants in telomere-maintaining genes and skin cancer risk

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Telomere-related genes play an important role in maintaining the integrity of the telomeric structure that protects chromosome ends, and telomere dysfunction may lead to tumorigenesis. We evaluated the associations of 39 tag-SNPs in telomere-related genes (TERT, TRF1, TRF2, TNKS2, and POT1) with the risk of skin cancer in a case-control study of Caucasians nested within the Nurses' Health Study (NHS) among 218 melanoma cases, 285 squamous cell carcinoma (SCC) cases, 300 basal cell carcinoma (BCC) cases, and 870 controls. Of the 39 SNPs evaluated, ten showed a nominal significant association with the risk of at least one type of skin cancer. After correction for multiple testing within each gene, three SNPs in the TERT gene and one SNP in the TRF1 gene showed significant associations with the risk of melanoma. The additive odds ratio (OR) (95% confidence interval (95% CI)) of these four SNPs for the risk of melanoma was 1.43 (1.14-1.81), 1.50 (1.14-1.98), 0.73 (0.59-0.91), and 1.87 (1.19-2.91), respectively. We did not observe significant associations for SCC or BCC risk. Our study provides evidence for the contribution of genetic variants in the telomere-maintaining genes to melanoma susceptibility.

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Pigmentation and skin cancer genome-wide association studies: A review and meta-analysis

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We performed a meta-analysis of summary results from 11 genome-wide association studies (GWAS) of pigmentation, sun sensitivity, nevi, melanoma, basal cell carcinoma (BCC), and squamous cell carcinoma and five replication studies expanding upon GWAS findings. Loci associated with pigmentation, freckling, sun sensitivity, and skin cancer included MC1R (melanocortin-1 receptor), TYR (tyrosinase), ASIP (agouti signaling protein), TYRP1 (tyrosinase-related protein 1), OCA2 (oculocutaneous albinism type II), and SLC45A2 (solute carrier family 45, member 2). Loci associated with nevi and melanoma included MTAP (methylthioadenosine phosphorylase) and PLA2G6 (phospholipase A2, group VI). Loci associated with only pigmentation and/or sun sensitivity included TPCN2 (two-pore segment channel 2), KITLG (kit ligand), SLC24A4 (solute carrier family 24, member 4), HERC2 (hect domain and RCC1-like domain 2), IRF4 (interferon regulatory factor 4), and SCL24A5 (solute carrier family 24, member 5). Loci distinctly associated with BCC included PADI6 (peptidylarginine deiminase, type VI), RHOU (ras homolog gene family, member u), TERT-CLPTM1L (telomerase reverse transcriptase-CLPTM1-like protein), KLF14 kruppel-like factor 14), CDKN2A/B (cyclin-dependent kinase inhibitor 2A/B), and KRT5 (keratin 5). These findings suggest that melanoma development may occur distinctly via nevi or pigmentation pathways and that BCC can develop via pigmentation or independent of pigmentation. Only three of five skin cancer GWAS, however, adjusted for pigmentation or sun sensitivity, so the effects on skin cancer cannot be fully separated from those on pigmentation. GWAS results must be interpreted cautiously since causal variants cannot be identified. MC1R exemplifies this since the functional 'red hair color' (RHC) MC1R alleles are not on the platforms used for these GWAS, and further genotyping showed that signals on chromosome 16 were due to RHC alleles.

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Elemental bi-mineral complex inhibits tyrosinase expression and melanogenesis *in vitro*

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The bio-electrical fields of living tissues are known to affect various biological functions. A novel elemental bi-mineral complex that produces biomimetic electricity was, therefore, evaluated for its ability to modulate melanogenesis. Topical daily applications of pigmented epidermal equivalents with elemental bi-mineral complex for 7 days led to a significant decrease in melanin deposition. This was reproduced using a cosmetic formulation containing elemental bi-mineral complex, demonstrating the ability of elemental bi-mineral complex to modulate melanogenesis *in vitro*. TYR and TRP-1 promoter- luciferase reporter assays showed that elemental bi-mineral complex inhibited tyrosinase and tyrosinase related protein 1 (TRP-1) expression in mouse melanoma B 16 cells. Consistently, TYR mRNA level was also reduced upon topical treatment of pigmented epidermal equivalents with elemental bi-mineral complex. Elemental bi-mineral complex did not interfere with the phagocytic activity of keratinocytes, suggesting no effect on melanosome transfer. Topical treatment of human skin explants. Based on these data we propose the use of elemental bi-mineral complex to mitigate the appearance of epidermal hyperpigmentary lesions.

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Regulation of human skin pigmentation *in situ* by repetitive UV exposure – molecular characterization of responses to UVA and/or UVB

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Exposure to ultraviolet (UV) radiation is a major environmental factor that affects pigmentation in human skin and can eventually result in various types of UV-induced skin cancers. Effects of various wavelengths of UV on melanocytes and other types of skin cells in culture have been studied but little is known about gene expression patterns *in situ* following exposure of human skin to different types of UV (UVA and/or UVB). To test the hypothesis that different mechanisms and/or factors might be involved in physiological pigmentary responses of the skin to different types of UV, we used whole human genome microarrays and immunohistochemical analyses to characterize human skin *in situ* to examine how melanocyte-specific proteins and paracrine factors are regulated by repetitive exposure to suberythemal doses of different types of UV. Repetitive exposure of human skin to UVA and/or UVB induced different levels of increased melanin content despite the same level of visible tanning at the end of the UV exposure protocol. The microarray analyses revealed that gene expression patterns induced by UVA or UVB are distinct, UVB eliciting dramatic increases in a large number of genes involved in pigmentation as well as in other cellular functions, while UVA had little or no effect on those. Surprisingly, none of the well-known melanogenic paracrine factors showed a significant change after repetitive UVA and/or UVB exposure at a suberythemal dose. These results were also confirmed at the protein level by immunohistochemical analysis. These results allow important insights into the different mechanisms of skin tanning induced by UVA or UVB in a physiological situation, provide a rich database resource of UVA- and/or UVB-responsive genes, and identify potential new melanogenic factors involved in the UV-induced responses of human skin.

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Dietary triterpene lupeol targets melanoma phenotype exhibiting activated Wnt/β-catenin signaling

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Melanomas with aberrant activation of Wnt signaling have poor prognosis. Thus, identifying non toxic Wnt targeting agents could be a promising strategy for treating this melanoma subtype. Here, we provide evidence that lupeol, a dietary triterpene preferentially inhibits the growth and proliferation of human melanoma cells exhibiting aberrant Wnt signaling whilst sparing normal melanocytes and melanoma cells that do not exhibit constitutive Wnt activation. Lupeol treatment (40-60 μM, 48 h) to human melanoma cells Mel 928 and Mel 1241 cells (harboring constitutive Wnt signaling) inhibited growth and activated key apoptotic regulators caspase 3 7 while producing no such effects in Mel 1011 cells that do not express constitutive Wnt signaling. Lupeol treatment to Mel 928 and Mel 1241 cells was observed to decrease i) β-catenin/Tcf4 transcriptional activity, and ii) the expression level of Wnt downstream target proteins Imp1 and MITF. Further, employing immunoblotting, enzyme based assays and immunofluorescence techniques, we found that lupeol inhibited the translocation of β-catenin from the cytoplasm to the nucleus in Mel 928 and Mel 1241; no such effects were observed in Mel 1011 cells. Next, we determined whether the observed in vitro effects of lupeol could be translated in an *in vivo* model. Athymic nude mice were implanted with Mel 928 and Mel 1011 cells. When tumors grew to 200 mm^3, the tumor bearing mice were divided into 2 groups and were treated with corn oil (control) or lupeol in corn oil (40 mg/Kg) i.p. thrice a week. Tumor growth was recorded weekly until tumors in control group reached 1000 mm^3. Lupeol administration was found to result in a significant decrease in the growth of Mel 928 tumors but had no effect in Mel 1011 tumors. Our findings thus highlight the anti-cancer efficacy of lupeol against melanoma subtype exhibiting constitutive Wnt signaling both *in vitro* and *in vivo*. We suggest that lupeol could be developed as a potential therapeutic agent for this subset of human melanomas.

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Exploring the role of keratinocyte-derived inflammation in melanomagenesis

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Inflammation contributes to melanoma development, growth, and metastasis, and mediators of that inflammation originate in part from keratinocytes and stromal fibroblasts, key cellular components of the melanoma microenvironment. To elucidate the role of keratinocyte-derived inflammation in melanomagenesis, a double transgenic mouse model (HGF-PKCα) was used. K5-PKCα mice – which overexpress PKCα in basal keratinocytes and develop a strong neutrophilic cutaneous inflammatory response upon topical TPA (12-tetradecanoylphorbol-13-acetate) application – were crossed with melanoma-prone MT1-HGF mice – which overexpress HGF under a metallothionein promoter – to create HGF-PKCα mice and their respective controls. These mice are unique in investigating the role of inflammation, since TPA-mediated activation of PKCα upregulates expression of pro-inflammatory cytokines by keratinocytes, and MT1-HGF mice develop melanomas after initiation with 7,12-dimethylbenz[a]anthracene (DMBA) followed by twice-weekly TPA. Here, we show that HGF-PKCα mice treated with the above-mentioned DMBA/TPA regimen did not develop melanomas until after TPA treatment ended but developed significantly more papillomas than their HGF-WT counterparts. This suggests that: 1) PKCα-mediated and keratinocyte-driven inflammation serves to inhibit melanomagenesis in HGF-PKCα mice and 2) HGF sensitizes K5-PKCα mice to the development of squamous papillomas. To investigate this further, we are developing a novel orthotopic model for melanomagenesis, whereby melanoma cells, primary keratinocytes, and dermal fibroblasts will be grafted on athymic mice or a K5-PKCα host. Finally, HGF treatment of primary keratinocytes increases expression of inflammatory cytokines such as CXCL1, CXCL2 and GM-CSF as well as pro-angiogenic factors such as VEGF. This upregulation is potentiated in keratinocytes that overexpress PKCα or oncogenic Ras. Together, these results present evidence of keratinocyte-mediated regulation of melanoma development and suggest a facilitating role of HGF in squamous cell carcinogenesis.

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MK615, a Japanese apricot extract containing triterpenoids, induces apoptosis in malignant melanoma cells and a patient with malignant melanoma

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Since malignant melanoma (MM) is an aggressive and chemoresistant skin cancer, development of innovative and effective therapies is critically important. MK615 is an extract from the Japanese apricot Prunus mume Sieb. Et Zucc (Ume) at neutral pH; it contains natural chemical substances including triterpenoids that have anti-cancer effects in several cancers. We assessed the anti-neoplastic effects of MK615 in MM cell line SK-MEL28 and in a patient with metastatic MM. Cell proliferation was examined by MTT assay. Apoptosis was studied by a flowcytometry and the annexin V assay in vitro and the TUNEL assay in specimens from the patient. Microarray chips were used to detect molecules that showed altered expression in response to MK615 treatment. MK615 suppressed the growth and induced the apoptosis of SK-MEL28 cells. The microarray analysis revealed that the mRNA expression level of the inhibtor of DNA binding-1 (Id-1) was remarkably decreased by MK615. We used MK615 for a 64-year-old woman with advanced MM that originated on her left sole. In spite of the surgical resection, regional lymphnodes dissection, and the adjuvant chemotherapy with 3 sessions of DAC-Tam therapy followed by the IFN-β for 15 months, multiple metastatic lesions appeared on her left thigh. We started MK615 without altering her chemotherapy. Her cutaneous metastatic lesions dramatically reduced after 4 months. TUNEL assay using the biopsy specimens showed that apoptosis was induced in the metastatic lesions. Our present results indicate that MK615 exerts its anti-proliferative effects by the induction of apoptosis and the inhibition of Id-1 expression in MM cells, suggesting that MK615 could be a valuable tool for the treatment of MM.

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Heat-treated vaccinia and attenuated vaccinia viruses induce apoptosis and inflammatory responses in melanoma cells and cause tumor regression in murine B16 melanoma model

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Poxviruses are large cytoplasmic DNA viruses that hold promises as vectors for immunotherapy and as oncolytic agents. Poxviruses encode many genes that evade the host immune system. Infection of dendritic cells and macrophages with heat-treated vaccinia (incubated at 55°C for 1 h) induces type I IFN and proinflammatory cytokine/chemokine production. The vaccinia E3L virulence gene encodes a key immuno-modulatory protein that subverts several antiviral signaling pathways. The E3 protein consists of an N-terminal Z-DNA binding domain and a C-terminal dsRNA binding domain. Here we report that infection of murine and human melanoma cells with heat-treated vaccinia or ΔE3L (in which the full-length of E3 is deleted) induces caspase-3 activity, a hallmark of apoptosis. Infection of melanoma cells with ΔE3L virus induces IFN-β, IL-6, CCL4 and CCL5 production, which is inhibited in the presence of araC (an inhibitor for viral DNA replication). Infection with WT vaccinia or E3LΔ83N (in which only the N-terminal Z-DNA binding domain of E3 is deleted) has no induction effects, indicating that dsRNA produced by ΔE3L virus during intermediate and late gene transcription following DNA replication in melanoma cells is the trigger for host innate immune responses. ΔE3L infection of melanoma cells also induces the phosphorylation of IRF3, a key transcription factor for IFN-β and its related genes. *In vivo* experiment demonstrates that injection of heat-treated vaccinia or ΔE3L into transplanted poorly immunogenic murine B16 melanoma results in the regression of the tumors at the injected sites as well as the generation of systemic antitumor immunity. Our results indicate that intratumoral injections of heat-treated vaccinia or attenuated vaccinia ΔE3L cause melanoma regression through multiple mechanisms.

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Novel 5,7-unsaturated steroidal and secosteroidal products of cytochrome P450scc show antimelanoma activity

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Cytochrome P450scc metabolizes 7-dehydrocholesterol to 7-dehydropregnenolone (7DHP) and vitamins D3 and D2 to 20(OH)D3 and 20(OH)D2, respectively, with further sequential additions of hydroxyl group to vitamin D side chain. P450scc also hydroxylates 1(OH)D3 to 1,20(OH)2D3 and CYP27B1 hydroxylates 20(OH)D3 to 1,20(OH)2D3. Furthermore, 7DHP can be metabolized by steroidogenic enzymes to corresponding hydroxysteroidal 5,7-dienes that can undergo UVB-induced transformation to pregnacalciferol (pD) and lumisterol-like compounds (pL). To define the clinical significance of these processes we have biochemically, chemically or photochemically generated the above compounds and tested their effects on melanomas. They inhibited the proliferation and colony forming ability by melanoma cells in a dose-dependent manner with a potency similar or higher than that for 1,25(OH)2D3. There was a difference between the biological activities of these compounds with derivatives with shorter side chains being less potent. Interestingly, unlike 1,25(OH)2D3, 20(OH)D3 and 20(OH)D2 had no toxic (calcemic) effects. Using 20(OH)D2 as a ligand, we found that these effects were mediated by interaction with the vitamin D receptor (VDR); overexpression of the VDR amplified the inhibitory effect while its silencing abolished it. Thus, we have identified novel secosteroids that are excellent candidates for anti-melanoma therapy, with 20(OH)D3 and 20(OH)D2 deserving special attention because of their lack of toxic effects and high relative potency.

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Expression of vitamin D receptor (VDR) decreases during progression of melanocytic and melanoma lesions

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Calcitriol (1,25-dihydroxy-vitamin D3) have broad range of biological activity, including immune and neuroendocrine activities, and tumorostatic and anticarcinogenic properties, affecting proliferation, differentiation and apoptosis and protecting DNA against oxidative damage. In recent years there has been growing interest in the role of vitamin D and its analogs in biology of various human tumors and their use in anti-cancer therapy. Vitamin D3 acts through a specific nuclear receptor (VDR), found in cells of normal and cancer tissues of the body. However, there is shortage of information on the changes in the expression pattern of VDR during progression of pigmented lesions. Using immunohistochemistry we analyzed expression of VDR in 140 samples obtained form 82 patients including 25 benign nevi, 70 primary cutaneous melanomas, 35 metastases, 5 re-excisions and 5 normal skin biopsies. The strongest VDR expression was observed in normal skin that significantly decreased in melanocytic proliferations with following order of expression: normal skin> melanocytic nevi>melanomas>metastases. The VDR expression in skin surrounding nevi and melanoma was also significantly reduced as compared to normal skin. Tumor-infiltrating and lymph node lymphocytes retained high levels of VDR. There was remarkable decrease of VDR expression in nuclei of melanoma cells at vertical growth phase versus radial growth phase, and metastatic melanomas showed the lowest cytoplasmic VDR staining. In addition, VDR expression was inversely correlated to melanin content. In conclusion, we propose that progression of melanocytic lesions can be linked to reduction of VDR expression and that melanogenesis can attenuate the VDR expression. Thus, the findings should have important clinical implications for diagnosis or adjuvant therapy with active forms of vitamin D or their analogs.

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A CD147-targeting siRNA enhances the cell adhesion of human malignant melanoma cells by phosphorylating focal adhesion kinase

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We previously demonstrated that CD147/basigin, a transmembrane glycoprotein belonging to the immunoglobulin superfamily, is highly expressed on the surface of malignant tumor cells including malignant melanoma (MM) and plays a critical role in invasiveness and metastasis of MM. Metastasis is an orchestrated process consisting of multiple steps including adhesion, invasion, and migration. In this study, we investigated whether CD147/basigin is involved in adhesion of MM using the human MM cell line, A375, that highly expresses CD147/basigin. To elucidate the roles of CD147/basigin, siRNA targeting CD147 was transfected to deplete its expression. Since colocalization of CD147/basigin with integrin, a cell surface adhesion molecule, was reported previously, expression and localization of integrin was examined. Expression and phosphorylation of focal adhesion kinase (FAK), a down-stream kinase of integrin, were examined by Western blot analysis. Localization of integrin was observed by a confocal microscopy. Adhesiveness of the cells to matrices was evaluated by adhesion assay utilizing matrix-coated plates. Silencing of CD147/basigin in A375 cells by a siRNA induced the phosphorylation of FAK at Y397. Distribution of integrin was spread and actin showed distinct arrangement, resulting in the morphological alteration of the cells to a dendritic appearance. Silencing of CD147/basigin in A375 cells by a siRNA clearly enhanced the adhesiveness of A375 cells to collagen I and IV. These results suggest that CD147/basigin is implicated in the adhesion of MM cells to the extracellular matrices.

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Photographic survey of scalp nevi in children

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The purpose of this protocol is to establish the typical clinical and dermoscopic patterns of scalp nevi in children younger than 18 years old. Pediatric scalp nevi may represent a source of anxiety for practitioners and parents as the clinical and dermoscopic features of typical nevi have yet to be defined. Gathering more information on these common pigmented lesions will further our understanding of signature scalp nevi and help optimize clinical care and management. We obtained clinical and dermoscopic images of 18 scalp nevi in 8 healthy Caucasian children, 5 blonds and 3 brunettes. The main clinical patterns included: eclipse (4/18), cockade (3/18), solid brown (7/18), and solid pink (4/18) nevi. On dermoscopy eclipse nevi demonstrated a light brown, homogenous center with a darker, reticulated peripheral ring. Cockade nevi, with a target-like appearance, demonstrated a darker, central globular pattern, lighter homogenous inner ring, and a peripheral darker reticular ring. Solid brown and pink nevi on dermoscopy showed the following predominant dermoscopic patterns: globular (5/18), reticular (2/18), homogenous (1/18), complex (mixed pattern) (2/18), and fibrillar (1/18). All scalp nevi had a unifying feature—perifollicular hypopigmentation, which caused the appearance of scalloped, irregular borders if occurring on the periphery, or variation in pigmentation, if occurring within the nevi. Also, a predominant vascular pattern was discerned on dermoscopy—dotted vessels (10/18)—8 nevi did not have a visible vascular pattern. All nevi were organized and benign in appearance. The predominant clinical patterns include solid-colored, eclipse, and cockade nevi. On dermoscopy, perifollicular hypopigmentation is a hallmark feature. Dermoscopy is a valuable, non-invasive tool in the evaluation of cutaneous melanocytic lesions in children—it increases the accuracy of clinical diagnosis of benign and malignant skin lesions, thus decreasing the number of unnecessary excisions. Recruitment of more subjects is needed to validate these results.

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Prognostic significance of BRMS1 expression in human melanoma

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BRMS1 (Breast Cancer Metastasis Suppressor) has been reported to suppress metastasis without significantly affecting tumorigenicity in breast cancer and ovarian cancer. To investigate the role of BRMS1 in human melanoma progression and prognosis, we used tissue microarray (TMA) to examine BRMS1 expression by immunohistochemistry in melanocytic lesions at different stages. Our data showed that BRMS1 expression is significantly decreased in metastatic melanoma compared with primary melanoma or dysplastic nevi ($P = 0.021$ and 0.001, respectively, $\chi 2$ test). There is no significant difference for the expression of BRMS1 between dysplastic nevi and primary melanoma ($P = 0.057$, $\chi 2$ test). Reduced BRMS1 staining is significantly correlated with AJCC stages ($P = 0.011$, $\chi 2$ test), but not associated with tumor thickness, tumor ulceration and other clinicopathological parameters. Furthermore, BRMS1 expression is significantly correlated with disease-specific 5-year survival of melanoma patients ($P = 0.007$, log-rank test). Multivariate Cox regression analysis also revealed that BRMS1 staining is an independent prognostic factor for melanoma patients (relative risk = 0.51; confidence interval = 0.29 to 0.91; $P = 0.022$). Moreover, our in vitro studies showed that BRMS1 inhibited the growth and tube formation of endothelial cells by suppressing IL-6 expression. In addition, our in vivo studies confirmed that BRMS1 inhibited blood vessel formation by matrigel plug assay. Taken together, BRMS1 expression is decreased in metastatic melanomas and it inhibits angiogenesis in melanoma. BRMS1 may be used as an important prognostic marker and potential therapeutic target for melanoma.

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Nucleotide excision repair (NER) gene family play an important role in the control of non-pigmented hair fiber growth

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The regulation of melanogenesis and the growth and pigmentation of hair fibers are affected by numerous intrinsic factors including general metabolism and nutritional status, hair-cycle dependent changes, body location, racial and gender differences, hormone-responsiveness, genetic defects and age-associated changes. The hair follicle bulb (HB) is the only site of pigment production for the hair shaft and melanogenically active melanocytes are located in the upper hair matrix (UH). We conducted a microarray study to discover gene expression patterns unique to non- pigmented hair follicle (HF) that may be implicated in the lack of melanogenesis in gray hair. Pigmented and non-pigmented HFs (n=10-20 per group) collected from the same individuals (n=6) were microdissected and transected into the lower one third and hair bulb and upper, non-bulbar, hair matrix and sheaths including the bulge region. Microarray data was verified with qPCR and immunohistochemistry. In comparison to pigmented upper hair matrix and HBs, several nucleotide excision repair (NER) family genes exhibited statistically significantly lower expression both in non- pigmented upper hair matrix and non- pigmented HB. These genes were identified as ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, XPA, NTPBP, HCNP, DDB2 and POLH. Immunohistochemistry showed consistent results. Our results suggest that losing NER gene function may be consistent with DNA damage accumulation in melanocytes and a lack of melanin production in gray hair follicles. These results offer a new insight into the molecular changes that occur in non- pigmented HF and may also provide novel information with regard to the importance of melanogenesis.

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Characterization of melanocytes in Becker's nevus

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Becker's nevus (BN) is an uncommon cutaneous hyperpigmentation that develops during adolescence and occurs primarily in young men. It is characterized by the presence of a light or dark brown patch with hypertrichosis. Histopathological examination shows increase in the number of melanocytes, mild acanthosis and regular elongation of the rete ridges. It may represent a distressing cosmetic handicap and a challenging issue regarding treatments like lasers. The difference of melanocytes of BN (BM) from the normal epidermal melanocytes (EM) was examined using culture of melanocytes, RT-PCR and Western blot. The comparison of cultured BM between untreated group and Alexandrite laser-treated and UVB-treated (0.5, 0.7, 0.9, 1.1J) groups was explored with morphological analysis and immunocytofluorescence(ICF). We observed that cultured BM was proliferated more than EM. In addition, there was no difference in RT-PCR and Western blot of bcl2, c-kit and melanocortin 1 receptor (MC1R) between BM and EM. We also discovered there were laser-resistant cells in cultured BM. Increase in expression of c-kit and decrease in expression of mel5, microophthalmia-associated transcription factor (MITF), and NKI-beteb were found in laser-treated group compared with untreated group using ICF. Decrease in expression of MC1R was observed in UVB-treated group compared with untreated group using ICF. On the basis of this current study, we suggest that there may be difference of BM from EM and resistance of BM to laser treatment, which lead to frequent recurrence of Becker nevus after laser therapy.

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"Pilot survey study – comparison of diagnostic and biopsy/referral sensitivity to Melanoma between dermatologists and MelaFind®"

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Melanoma (MM) is one of the most dangerous entities encountered by dermatologists and the diagnosis is often elusive. In this cross-sectional reader study, 39 dermatologists were compared to MelaFind in analysis of 23 melanomas and 24 benign pigmented lesions. MelaFind is a new instrument designed to use machine vision for non-invasive early detection of MM; the device is programmed to recommend biopsy, not to diagnose MM. Raters consisted of dermatologists who had expressed interest in the MelaFind device previously. MelaFind was applied to the lesion during an acquisition study conducted by Electro-Optical Sciences, Inc, for the FDA which preceded this reader study. Prior to the biopsy of the lesion, a close-up, distance, and dermatoscopic image were taken, which raters viewed along with a case history. By comparing to the histology, we assessed biopsy/referral recommendation sensitivity and specificity (sens/spec) for both MelaFind and raters. Diagnostic sens/spec were evaluated for raters. In addition, the reason for biopsying lesions found to be MMs was assessed by asking raters if biopsy was performed due to concern for: 1. MM; 2. A melanocytic lesion other than MM with aggressive biological behavior; 3. Non-MM skin cancer; or 4. Not concerned, but the patient expressed concern. RESULTS: The biopsy sens for raters and MelaFind were 87.2 and 95.6 respectively while spec were 29.0 and 8.3 respectively. The diagnostic sens/spec for MM among the dermatologists were 53.2 and 70.5 respectively. The responses given for biopsy of lesions proven to be MMs were: 49.8%, 36.1%, 6.1%, and 7.9% for responses 1, 2, 3, and 4 above, respectively. In this study, MelaFind appears to be more sensitive, but less specific than dermatologists in recommending biopsy of histologically proven MMs. Only 49.8% of the time were the lesions biopsied because the dermatologist believed it to be MM. These findings suggest that MelaFind would be useful for dermatologists. A larger reader study to confirm these results is underway.

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Fluorescence *in situ* hybridization for distinguishing malignant blue nevi from atypical cellular blue nevi

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Malignant blue nevus is a term employed to describe melanomas resembling, arising within, or in close proximity to blue nevi. Malignant blue nevi may have many overlapping histologic features with atypical cellular blue nevi and definitive distinction of these two entities by standard histopathology may in some cases be impossible. In this study we used fluorescence *in situ* hybridization (FISH) targeted against 6p25 (RREB1), 6q23 (MYB), 11q13 (CCND1), and the centromere of chromosome 6 (Cep6) to distinguish between atypical cellular blue nevi and malignant blue nevi. We identified 5 cases of malignant blue nevi and 10 cases of atypical cellular blue nevi. The FISH assay performed with 100% sensitivity and 100% specificity within this small cohort of cases. All five cases of malignant blue nevi showed clonal aberrations within chromosome 6. Three of 5 cases met the 6p25/Cep6 criteria, all 5 met the 6p25 gain criteria and 3 of 5 met the 6q23/Cep6 loss criteria. None of the cases met criteria for gains in 11q13. None of the 10 atypical cellular blue nevi showed significant chromosomal copy number changes in the targeted loci or met any criteria for melanoma. A combined analysis of the clinical and histologic changes in addition to FISH analysis targeting chromosome 6 and 11 with the aforementioned probes may significantly contribute to the ability of pathologists to discriminate between malignant blue nevi and benign atypical cellular blue nevi.

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Disruption of N-glycan processing by N-Acetyl Glucosamine (NAG) and its depigmenting effects *in vivo* models

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Tyrosinase, an important enzyme for melanin production, is synthesized and glycosylated in the endoplasmic reticulum (ER) and Golgi. The enzyme is subsequently transported to melanosome where it participates in melanogenesis. N-acetyl glucosamine (NAG) inhibits the glycosylation of tyrosinase accounting for its pigment-reducing abilities. Previous studies have shown that NAG effectively reduces the appearance of hyperpigmented spots and the production of melanin in skin equivalent cultures. In this study, we demonstrated that NAG decreases the pigmentation in human melanoma cell, brown guinea pig and human skin. NAG inhibits the α-glucosidase activity with dose-dependent manner and disrupts tyrosinase glycosylation in HM3KO melanoma cell, showing similar results with well-known glycosylation inhibitor, deoxynojirimycin (DNJ). The color of cell pellet and melanin contents were also reduced by various concentrations of NAG and the tyrosinase activity was slightly inhibited in HM3KO melanoma cell. The amount of MITF, transcription factor implicated in tyrosinase gene expression, was reduced in both mRNA and protein level. While the protein level of tyrosinase was reduced by NAG treatment in HM3KO melanoma cell. To investigate the *in vivo* effects of NAG, we applied topically the NAG solution (5% in propylene glycol:ethanol:water = 5:3:2) twice daily for 4 weeks in the dorsal skin of brown guinea pigs and for 8 weeks in the forearm of humans tanned by UV irradiation. As expected, the lightening effects were observed in the skin of both brown guinea pigs and humans in terms of deltaL value and histological analysis.

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Melanocyte dendrite-derived globules secreted into the culture medium are possible transporters of melanosomes in the melanosome transfer mechanism

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A thorough understanding of melanosome transfer from melanocytes to keratinocytes is crucial for the treatment of pigmentary disorders. Three possible mechanisms of melanosome transfer have been suggested, that is, (i) pinching off of melanocyte dendrite containing melanosomes by keratinocyte, (ii) direct inoculation of melanosomes into keratinocyte via melanocyte-keratinocyte membrane fusion through nanotubes and/or (iii) melanosome release out of melanocytes and uptake by keratinocytes via phagocytosis. The pathway for melanosome transfer, however, has not been fully clarified. Here we report a possible mechanism for melanosome transfer in cultured normal human melanocytes and keratinocytes. Numerous globular bodies containing multiple melanosomes generated from the extending tip of melanocyte dendrites were observed to be released into the culture medium. When those globules were collected and added to the culture medium of normal human keratinocytes, they were subsequently phagocytosed by keratinocytes. Electron microscopic observation revealed that the incorporated melanosomes into keratinocytes were enclosed by a bilayer membrane and located mostly in the perinuclear area as a melano-phagolysosome. Our present results clearly indicate a possible mechanism of melanosome transfer, i.e. melanosomes are packed in globular bodies before budding off from melanocyte dendrites, released into the extracellular space, and then phagocytosed by keratinocytes, at least *in vitro*.

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1-(2,4-dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylpheny)propane inhibits melanin synthesis by multiple mechanisms

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Acquired hyperpigmentation, whether due to acute or chronic sun exposure, or from cutaneous inflammation, is a cosmetic concern worldwide. At present, there is an intense search for more effective, safe topical materials to treat uneven skin color. A. Nesterov et al., 2008, recently described 1-(2,4-dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylpheny)propane (DP) as a tyrosinase inhibitor, but detail of its mechanism remain unclear. DP (IC_{50}= 50 nM) was 200 times more potent than kojic acid (KA)(IC_{50}= 10000 nM) in inhibiting DOPA oxidase activity of mushroom tyrosinase. In contrast, DP was significantly less effective at inhibiting tyrosinase from normal human melanocytes (NHEM) extract, whereas KA (IC_{50}= 100 μM) strongly inhibits human tyrosinase. DP, however, decreased melanin content in cultured NHEM after incubation with an IC_{50} value of approximately 10 μM for 7days. This resulted in an IC_{50} for DP against human tyrosinase activity that was at least 40 times higher than the IC_{50} against melanin synthesis in NHEM. DP was also highly effective against melanization in reconstructed skin and in clinical studies. To determine the reason for this discrepancy, we examined DP potency as an antioxidant and the effect of DP on NHEM mRNA and protein for enzymes involved in melanin synthesis, including TYR, DCT and MITF. As a DPPH radical scavenger, DP was comparable to ascorbate and Trolox. At non-cytotoxic levels DP did not inhibit tyrosinase mRNA transcription in NHEM. However, western-blotting revealed that treatment of NHEM with 15 μM DP decreased tyrosinase protein by approximately 50%. In conclusion, we show that DP acts through multiple mechanisms of action to reduce hyperpigmentation; a) direct inhibition of tyrosinase activity, b) reduction of oxidative stress, and c) alteration of post-translational modification and/or acceleration of tyrosinase degradation.

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Examining the impact of potent skin lighteners on melanocytes *in vitro*

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Melanocytes grown *in vitro* were treated with one of three well established skin lightening agents, 1) hydroquinone, 2) kojic acid and 3) niacinamide. The cytotoxicity of each of these skin lightening agents on the melanocytes was establish and the highest, non-lethal dosage of each ingredient on the cells was determined using the MTT assay. Melanocytes were then grown and treated with these skin lightening agents at these highest non-lethal doses for 24 hours. The cells were analyzed using human DNA gene microarrays employing the full range of genes (over 19k) expressed on these chips. Three interesting mechanisms of melanin control are commonly thought to be: inhibition of tyrosinase (TYR), inhibition of cytochrome oxidase 1 (COX1), and inhibition of melanocortin 1 receptor (MC1R) expression. It was found in these studies that each of these key target genes appears to be actually upregulated by these potent skin lighteners in the 24 hour timeframe examined in this study. Protein assays were conducted using each of these ingredients on melanocytes looking at these three key proteins and also at melanin expression. It was found that hydroquinone and kojic acid did demonstrate significant melanin reductions after 48 and 96 hours of treatment, but niacinamide did not in the time frame of these *in vitro* studies. Examination of the ingredients for their tyrosinase protein expression indicated that each ingredient upregulated tyrosinase at the doses examined within the timeframe of these studies. All three ingredients also increase COX1 and MC1R protein expression in melanocytes in a statistically significant fashion. This data suggests that there may be common, unexpected pathways towards skin lightening that are shared by at least these three popular skin lightening ingredients and these pathways may be more complex than anticipated.

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Seroreactivity to a mimotope of the high molecular weight-melanoma associated antigen (HMW-MAA) in patients with melanoma

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Mimotopes isolated from phage display peptide libraries by panning with tumor-associated antigen (TAA)-specific mAbs are being evaluated as immunogens to implement active specific immunotherapy in cancer patients. Although TAA-specific mAb are commonly used to isolate mimotopes, no information is available regarding the seroreactivity to the epitope that is defined by a TAA mimotope. To address this question, we have used the mimotope 225D9.2+, which was obtained by panning of a phage display library with the murine monoclonal antibody (mAb) 225.28S, a mAb which recognizes the high molecular weight melanoma-associated antigen (HMW-MAA). We established an ELISA assay and measured the levels of IgG against this mimotope in the sera of 80 melanoma patients and 40 age-and sex-match controls. Patients were stratified into four risk groups based on their stage of disease according to the American Joint Committee on Cancer (AJCC). Serum levels of IgG against the 225D9.2+ peptide were significantly higher in melanoma patients than in controls. Sixteen percent of patients showing elevated levels regardless of the stage of disease. Forty-five percent of stage I patients and 20 % of stage II patients had elevated levels of IgG against the 225D9.2+ peptide. By contrast, all patients with stage III and IV were consistently below the positive cut-off. These results suggest a spontaneous humoral immune response against the epitope on the HMW-MAA that is defined by the mAb 225.28S. Antibody recognition of a TAA mimotope by patients' sera could be used for evaluating the immunogenicity of this mimotope, and furthermore this serological test could be used for monitoring melanoma mimotope vaccine treated patients.

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Human Numb is required for proper mitotic entry and progression in melanoma cells

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Understanding melanoma genetics is crucial for its management. Proper regulation of the cell cycle, particularly DNA replication and cell division, are crucial for correct cell ploidy and genomic integrity. We recently demonstrated that the mitotic regulator Polo-like kinase 1 (Plk1) was overexpressed in clinical melanoma and a targeted depletion of Plk1 through lentiviral shRNA or a small-molecule inhibitor causes mitotic catastrophe and induction of apoptosis in human melanoma cells (J Invest Dermatol 129: 2843-53, 2009). Here, we studied the connection between Plk1 and Numb, an evolutionary conserved protein that plays critical roles in cell fate determination, in human melanoma cells. Using a double thymidine arrest and release protocol, we evaluated the expression of Numb during the cell cycle in A375 and HS294T human melanoma cells. Numb expression was found to be maintained at a low level in asynchronous melanoma cells. However, as cells entered S phase Numb levels were found to increase, peak during mitosis and then rapidly decline at mitotic exit when the cells reentered G1 phase. This cycling of Numb mirrored the mitotic specific expression of both Plk1 and Cyclin B1, however Numb showed an expression pattern with an increase beginning earlier in S phase. Plk1 inhibition and nocodazole-mediated G2/M mitotic arrest was also found to be accompanied with an increase in Numb expression. Surprisingly, lentiviral shRNA mediated knockdown of Numb resulted in reduced Plk1 protein levels, a G2/M cell cycle arrest and a significant reduction in cell growth in both melanoma cell lines. This suggested that Numb is a positive regulator of Plk1 protein stability. Indeed, we found that loss of Numb contributes to reduced Plk1 protein half-life. Overall, our data suggested that i) Numb is a regulator of proper cell cycle progression and its expression is required for proper mitosis, and ii) Numb may possess a tumor suppressor function though the regulation of Plk1 protein stability in melanoma cells.

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Targeting altered glucose metabolism in melanoma

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PET-scan imaging using radiolabeled deoxyglucose of metastases reveals increased glucose uptake and aerobic glycolysis as a cancer cell phenotype. To characterize glucose metabolism in human melanoma, 10 cell lines were examined for glucose uptake, consumption, and lactate production. Compared to adult melanocytes, all melanoma lines displayed a higher level of aerobic glycolysis (increased glucose uptake, usage, and lactate production). Immunoblots detecting mediators of glucose metabolism revealed at least 2 groups. One group displayed prominent hexokinase II (HK-II) levels correlating in many lines with B-raf mutation; another group displayed elevated glucose transporter1 (GLUT1) linked to increased p-AKT levels. To target altered glucose metabolism in melanoma, 3-bromopyruvate (3-BrPA), an agent inhibiting both HK-II and mitochondrial function, was added (dose range: 0 to 200uM; 24 hrs). Amongst treated lines, 6 of 10 displayed varying sensitivity. To elucidate cell death mechanisms, two sensitive and resistant lines were studied. 3-BrPA (100uM) treatment triggered depletion of intracellular ATP level in sensitive, but minimal ATP reduction in resistant, lines. 3-BrPA (200uM) triggered prominent (>50%) cell death in sensitive lines (Annexin-V-PI staining / FACS). However, cell cycle analysis revealed absence of sub G0 DNA content pointing towards necrosis. This cytotoxicity appeared to be caspase independent (no effect by pan caspase inhibitor-z-VAD). 3-BrPA induced cytotoxicity accompanied by lysosomal changes (increased levels of LAMP1, LAMP2, cathepsin D) and inhibition of mTOR signaling assessed by reduction of p-p70s6K; p-4-EBP-1 levels. Pretreatment with ROS scavenger N-acetylcysteine almost completely blocked killing by 3-BrPA implicating ROS production. Thus, melanoma cells exhibited uniform phenotypes of altered glucose metabolism but responses to metabolic stress were heterogeneous. 3-BrPA, as a single treatment overcomes cell death resistance of some melanoma lines by inducing necrosis resulting from ATP depletion, altered ROS, increased lysosomal enzymes, and inhibition of mTOR signaling pathways.

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Influence of the association of different tanning boosters on human skin melanin content

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We were interested in investigating the associative synergy of different melanin enhancers that have different mechanisms of action. Protease-activated receptor-2 (PAR-2) is known to be involved in phagocytosis of melanosomes by keratinocytes (HK). Our previous studies have shown that HK express a significant increase in PAR-2 when treated with compound (IV08.006) designed specifically for this purpose. Our studies on fluorescent microsphere ingestion by HK, after PAR-2 induction, showed that HK ingested significantly more microspheres after PAR-2 induction. As the PAR-2 pathway is also known to affect the keratinocyte cytoskeleton, we performed actin staining, which revealed that PAR2-induced HK developed more protrusions. Studies on *ex vivo* human skin induced with 1% and 3% of the compound for 24h, 48h, and 72h, followed by F&M staining, revealed the greatest increase in melanin after 48h of treatment with both concentrations, and melanin migration to upper keratinocyte layers was higher. Furthermore, we determined the impact of the association of two melanin boosters with PAR-2 induction. One of these molecules helps specific amino acids transport across the cellular membrane and is implicated in gluthatione synthesis; the other is designed to be a modulator of the α-MSH receptor, a melanocyte stimulating hormone that stimulates melanogenesis. Each of these molecules promoted increased melanin content in *ex vivo* human skin when used at different concentrations. Interestingly, when associated with PAR-2 induction, melanin content in the epidermis was higher than with each product alone. Moreover, melanin increase in the upper layers of the epidermis was remarkable, demonstrating an increase of melanin uptake by HK. These studies confirm the interest of associating such melanin inducers with PAR-2 induction in order to enhance melanin synthesis and its spread throughout the epidermis for tanning and skin tone homogenization purposes.

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Protease-activated receptors-1 and -2 activate protein kinase D_1 (PKD_1) signaling in human melanoma cells

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Recent data indicate an important role of proteinases and proteinase-activated receptors (PARs) in tumorigenesis. Although a role of PARs has been described in various skin tumors including melanoma, the underlying cellular mechanisms are not understood. Recent studies suggest PAR_1 to be a regulator of melanoma cell growth and metastasis by affecting angiogenic and invasive factors. Moreover, changes in the expression patterns of PAR_1 and PAR_2 correlate with skin cancer progression, and PAR_1 as well as its ligands thrombin and MMP-1 are overexpressed in melanoma. Therefore, the aim of this study was to elucidate the role of putative PAR_1 and PAR_2-mediated signal transduction pathways during melanoma progression. Activation of both PAR_1 and PAR_2 led to rapid phosphorylation of protein kinase D_1 (PKD_1) which is known to be involved in cell migration, integrin regulation and intracellular vesicle transport. Both PAR_1 and PAR_2 activate the PKD_1 pathway in various cultured melanoma cells. Downregulation of PKD_1 by siRNA resulted in diminished proliferation, migration and secretion of the proangiogenic chemokine interleukin-8 (IL-8) in melanoma cells. In conclusion, our results show that PAR_1 and PAR_2 are involved in melanoma cell proliferation, migration and secretion of IL-8 via activation of PKD_1. Inactivation of the PKD_1 pathway may be beneficial for the inhibition of PAR-induced tumor proliferation and migration.

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Expression of activating transcription factor 3 (ATF3) in human melanoma

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Activating transcription factor 3 (ATF3) is an adaptive response gene that can be induced by a wide variety of stimuli. ATF3 has been implicated in the cellular response to ultraviolet radiation and the regulation of other important cellular processes such as proliferation and apoptosis. The role of ATF3 in human malignancies depends on the cellular context and both tumor suppressor functions and oncogenic functions for ATF3 have been reported. To help define the role of ATF3 in human melanoma, we used immunohistochemistry to examine the pattern of ATF3 protein expression in human melanomas. We found that ATF3 expression in melanoma is dichotomous. While nevi and most melanomas expressed low to no detectable levels of ATF3 (ATF3-low), some showed strong ATF3 nuclear staining (ATF3-high). Using A375 cells as a model system for ATF3-low tumors, we found that ATF3 can be induced by interferon-γ, cisplatin, dacarbazine and sorafenib to varying degrees. In addition, we found that ATF3 siRNA protects A375 cells from cisplatin-induced cell death suggesting that in A375 cells, ATF3 induction is pro-apoptotic. ATF3 will likely be induced in response to immune-based, targeted and non-targeted therapies. Given that ATF3 can promote either cell survival or apoptosis, it will be important to define the expression pattern of ATF3 in human melanoma and determine how ATF3 expression influences the cellular response to anti-tumor agents.

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Polo-like kinase 1 expression is regulated through MAPK signaling pathway and is a potential therapeutic target in human melanoma

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Disruption of the cell cycle regulation has been implicated in the development and progression of malignant melanoma. By using cDNA microarray technique and pathway enrichment analysis we could identify cell cycle pathway and its member polo-like kinase 1 (Plk-1, a mitotic serine/threonine kinase) to be significantly overexpressed in primary melanomas and melanoma metastases. This finding could be confirmed using real-time RT-PCR analysis on an independent set of specimens. In *in vitro* analysis of 8 human melanoma cell lines we observed the peak expression of the Plk-1 to be at the G2/M phase of the cell cycle. Transfection of human melanoma cell lines with two independent Plk-1 siRNAs led to reduction of Plk-1 mRNA/protein, significant decrease in cell proliferation, induction of mitotic catastrophe, and apoptotic cell death. Analysis of apoptosis revealed to be caspase 3/8 dependent, TP53-independent and mediated through cleavage of Bid and decrease in Bcl-2 followed by the release of mitochondrial cytochrome c at the later time points. Comparative genomic hybridization (CGH) and SNP arrays showed no genetic alteration in locus 16p12.1 expressing Plk-1 in our samples. We presumed MAPK signaling pathway to induce Plk-1 expression in primary and metastatic melanomas as this pathway was also significantly activated. Inhibition of this pathway using the MEK inhibitor P89059 or sorafenib resulted in decreased expression of Plk-1 in a dose- and time-dependent manner, cell death and apoptosis. This study shows that in human melanoma: Plk-1 expression is dynamically regulated during the cell cycle, knock down of Plk-1 can lead to inhibition of cell proliferation/survival and induction of apoptosis and, Plk-1 expression to be regulated among others through MAPK signaling pathway. We conclude that Plk-1 could be a potentially attractive target in melanoma therapy.

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***In vivo* photoacoustic microscopy of human skin**

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Photoacoustic microscopy (PAM) is an emerging biomedical imaging modality that measures light absorption through detection of laser-induced ultrasound waves. This unique imaging technique is non-invasive, utilizes non-ionizing light, and provides optical contrast with an unparalleled combination of resolution and penetration as compared to conventional optical microscopy modalities. PAM efficiently detects both oxy- and deoxyhemoglobin as well as melanin without the aid of exogenous contrast agents; thus it is an attractive imaging technique for use in skin imaging. Recently, PAM was applied to imaging the cutaneous microvasculature of normal skin and pigmented lesions in human subjects. *In vivo*, three-dimensional images of cutaneous microvascular networks were collected from different locations on the body and from several volunteers with diverse levels of skin pigmentation. Various vascular and structural elements within the skin have been identified in the photoacoustic images, and several distinct differences between acral and non-acral skin have been observed. Additionally, a nevus was photoacoustically imaged, subsequently biopsied, and pathology was compared to the photoacoustic data. From the PAM images, the lateral dimensions, depth and thickness of the nevus were determined and compared to the dimensions from histology. After accounting for tissue distortion following excision, tissue fixation and processing, we determined that data obtained by histology and photoacoustic imaging were consistent. These findings demonstrate the potential for PAM to have a clinical impact in the diagnosis and assessment of vascular disorders, pigmented lesions and possibly other cutaneous diseases.

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CYLD inhibits melanoma growth and metastasis

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CYLD is initially identified as a tumor suppressor due to its genetic linkage to cylindroma and other skin appendage tumors. Its role has since been extended into many other types of cancer, including melanoma. However, the molecular mechanisms of CYLD in tumorigenesis are still unclear. In this study, we found that CYLD was expressed in normal melanocytes and absent in a majority of melanomas and melanoma cell lines examined. Restoring CYLD expression in A2058 and skmel28 melanoma cells by retroviral transduction significantly reduced cell growth rate, soft agar colony formation and cell migration *in vitro*, as well as tumor growth kinetics when cells were injected subcutaneously into immune-deficient SCID mice. Moreover, A2058 cells expressing LacZ control or a catalytically deficient CYLD mutant developed multiple metastatic lesions in 2 of 7 and 2 of 6 mice examined, respectively. In contrast, cells transduced to express the WT CYLD did not metastasize in all of the 8 mice examined so far. At the molecular level, CYLD expression led to a decrease of cyclin D1 and N-cadherin and an increase of p53 and E-cadherin. These findings indicate that CYLD inhibits melanoma tumor growth and metastasis in a manner that is dependent on its catalytic function and through effects on multiple molecular targets. We are currently investigating the mechanisms mediating CYLD downregulation in melanoma and the mechanisms of CYLD regulation of downstream molecular targets.

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N-Nicotinoyl dopamine inhibits skin pigmentation by reducing melanosome transfer

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Transfer of melanosome synthesized within melanocytes to neighboring keratinocytes, results in a constant supply of melanin to the epidermis, and this process determines skin pigmentation. Niacinamide is well known to be an effective skin lightening compound that works by inhibiting melanosome transfer. Recently, we synthesized a novel niacinamide derivative, N-Nicotinoyl dopamine (NDD), with prominent antioxidant activity. In this study, we have investigated whether NDD has an inhibitory effect on pigmentation process in co-culture model of human melanocyte and keratinocyte, and in reconstituted three-dimensional human epidermal model. CFDA (CarboxyFluorescein DiAcetate)-labeled melanocytes were subsequently co-cultured with keratinocyte of TRITC (Tetramethyl Rhodamine IsoThioCyanate)-labeled cytokeratin, and the transfer of melanosome assessed visually by confocal scanning laser microscopy and quantitatively by flow cytometry. The dosages of NDD not affecting cell viability produced an inhibitory effect on melanosome transfer in a dose-dependence manner while NDD had not inhibitory effect of tyrosinase and melanin synthesis in B16F10 mouse melanoma cells. In a reconstructed skin model (MelanoDerm™) topical application of 0.05 and 0.1 % NDD resulted in significant skin lightening and decrease of melanin production without effects on cell viability, melanocyte morphology and overall tissue histology. Furthermore, NDD (0.1 %) was more effective than niacinamide (2 %) on skin lightening. Also, there was no skin irritation when all of the creams (0.05, 0.1 and 0.2 % NDD) were applied in 24-hour patch-test on twenty volunteers. These results suggest that NDD may be useful for skin-whitening agent and it might be due to its down-regulation of melanosome transfer.

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β-catenin regulates melanocyte dendricity through the modulation of PKCζ and PKCδ

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The Wnt/β-catenin signaling pathway is involved in the melanocyte differentiation and melanoma development. However, the effect of β-catenin for dendrite formation has not been clearly elucidated yet in normal human epidermal melanocytes (NHEM). To investigate the effect of β-catenin, we transduced NHEM with recombinant adenovirus expressing β-catenin. Forced expression of β-catenin led to the dramatic morphological changes of NHEM, including the reduction of dendrite length and enlargement of cell body. Concomitantly, the protein levels for dendrite formation-related molecules, such as Rac1 and Cdc42, were markedly decreased. In addition, phosphorylation of p38 MAPK was significantly reduced by β-catenin, potentiating its inhibitory role for dendrite formation. Interestingly, overexpression of β-catenin led to the increase of PKCζ level, while PKCδ was decreased by β-catenin, suggesting that those PKCs were β-catenin-downstream modulators in NHMC. When PKCζ was overexpressed, dendrites were shortened, with the reduced protein levels for Rac1 and Cdc42. In contrast, PKCδ overexpression led to the elongation of dendrites, with the increased levels for Rac1 and Cdc42. These results suggest that β-catenin plays an inhibitory role for dendrite formation through the modulation of PKCζ and PKCδ.

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Requirement of NAD(P)H:quinine oxidoreductase-1 (NQO1) for the development of melanocytes in zebrafish

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NAD(P)H:quinine oxidoreductase-1 (NQO1) is a ubiquitous flavoenzyme that catalyzes the two-electron reduction of quinones to hydroquinones using NAD(P)H as an electron donor. In a previous study, we showed that NQO1 has a potential for enhancing pigmentation in primary cultured human melanocytes and melanoma cell lines. Recognizing its important role in pigmentation, we further investigated the potential role of NQO1 in the development of melanocytes using zebrafish model. Whole mount in situ hybridization showed that zNQO1 was expressed in the epithelium and yolk at 10-14 hours post-fertilization (hpf), and in the anterior head and yolk extension at 24 hpf. To suppress the zNQO1 expression, we microinjected antisense morpholino oligonucleotide. Interestingly, it was obvious that the pigmentation was dramatically decreased at 30 hpf by suppression of zNQO1 expression. Concomitantly, the expression of pigmentation-related genes including MITF, TYR and TYRP1 was also markedly decreased by morpholino microinjection. These results suggest that zNQO1 plays a pivotal role in the lineage specification of melanocytes during zebrafish development.

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Diagnostic detection of micrometastasis to sentinel lymph nodes by real-time PCR analysis in malignant melanoma

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Since the death from malignant melanoma (MM) is due to metastases, the most critical question is whether the MM is localized or already spread to the draining lymph nodes (LNs) or distant organs. Sentinel LN (SLN) status is an important prognostic factor for patients with cutaneous MM. We used a real-time PCR technique to detect the expression of gp-100, MART-1, and tyrosinase in primary cutaneous MM. First, to ascertain the accuracy of this technique, we investigated the correlation between the metastatic lymphatic mapping and the mRNA level of each dissected LNs in a patient with the advanced stage of primary vaginal MM. Forty six LNs were removed from the patients' inguinal, juxta-vaginal, and para-aortic regions and subjected to real-time PCR analysis for the three genes. Nine histologically metastatic LNs had high mRNA levels for the three genes, and 10 of 32 histologically negative LNs also demonstrated high levels of mRNAs. The positive LNs were located in vicinity of the primary tumor. We denominated these 10 lymph nodes as genetically positive LNs, 3 of which showed histological micrometastases after re-examination of serially sectioned specimens. Second, in an analysis of 41 SLNs of 28 patients with primary cutaneous MM, the expression levels of gp-100, MART-1, and tyrosinase were significantly correlated with each other (R^2, 0.5271-0.8592, $P < 0.0001$). All the 9 histologically positive LNs showed positive PCR at high expression levels. Among 32 histologically negative LNs, 10 LNs exhibited high expression levels of the three genes. According to the number of the positive genes, the LNs could be divided into +++, ++, and +, and 47% of +++ LNs were also histologically positive, whereas none of ++ and + showed histological positivity. Our study validated the accuracy of real-time PCR and suggests that this technique can be useful for lymphatic mapping.

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***In vivo* regeneration of the follicular pigmentary unit with melanocyte progenitors from adult skin**

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Mammalian coat pigmentation is maintained by melanocyte stem cells (MSCs), which generate a large number of differentiated melanocytes during each hair follicle cycle. Pigment produced by differentiated melanocytes in the base of the growing (anagen) hair follicle is transferred to keratinocytes that condense as they undergo terminal differentiation to form the elongating, pigmented hair shaft. Previous studies have defined a quiescent population of MSCs in the bulge/lower permanent portion (LPP) region, or niche, of the hair follicle where quiescent follicular epithelial stem cells reside. However, the stages leading to the generation of a large number of fully differentiated progeny from substantially fewer quiescent bulge/LPP MSCs have not been defined. We have used a doxycycline-inducible, bitransgenic mouse system to localize and isolate quiescent, follicular melanocyte progenitors *in vivo*. We show that a distinct set of melanocyte progenitor cells, located in the secondary hair germ (SHG) region of the follicle apart from the bulge/LPP, possesses the ability to regenerate the pigmentary unit during hair follicle anagen. Using direct transfer of quiescent adult melanocyte progenitors into the recipient skin from amelanocytic mice, we demonstrate that SHG melanocyte progenitors have greater regenerative potential than cells derived from the MSC niche in the bulge/LPP. De novo follicular pigmentation was durable, maintained for over one year. In resting (telogen) hair follicles, quiescent, "label-retaining" SHG melanocyte progenitors are similar to the total population of melanocytic SHG cells, constituting 20% of the cells in the SHG at this hair follicle stage. These results show that a substantial reservoir of melanocyte progenitors with stem cell potential reside in the SHG of murine hair follicles, implicating a group of cells apart from the bulge/LPP niche as intermediates in hair shaft repigmentation.

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Reflectance confocal microscopy features of cutaneous metastatic melanoma

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While the reflectance confocal microscopic (RCM) features of primary cutaneous melanoma have been characterized, the dermoscopic and RCM features of cutaneous melanoma metastases have not. Clinical, dermoscopic, and RCM images were obtained from 9 consecutive metastatic lesions which were then excised and submitted for pathologic review. Based upon clinical observations and histologic review, the 9 lesions were divided into 2 groups: superficial (5/9) and deep (4/9), using a cutoff depth of approximately 0.5mm. Dermoscopy of the 5 superficial lesions revealed either disorganized globular (3) or homogenous with atypical vasculature patterns (2). RCM revealed features similar to primary melanoma: keratinocyte disarray, multiple pagetoid dendritic cells, cerebriform nests, and severely distorted architecture. Dermoscopy of 4 deep lesions demonstrated a homogenous pink-white background with atypical vasculature. All lesions demonstrated an absence of typical dermal papillary rings, corresponding to epidermal atrophy. In addition, all lesions demonstrated small empty holes within the superficial dermis, in a singular or paired fashion, which may correspond to superficial vessels. For all deep lesions, the depth of resolution for RCM was surpassed by the depth of the dermal metastatic nodule. In conclusion, metastatic melanoma can be divided into 2 distinct categories based on the depth of the lesion. Superficial melanoma metastases are accessible by RCM imaging and demonstrate features similar to those of primary cutaneous melanoma. Deep melanoma metastases, with tumor depth greater than 0.5mm, cannot be directly visualized with RCM, and the imaged features may represent site-specific epidermal change, as all deep lesions were imaged on the leg. Further investigation is needed to fully characterize and validate the RCM and dermoscopic features of superficial metastatic melanoma to determine if these techniques can differentiate primary melanoma from in-transit disease.

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Melanogenic differentiation program control by TGF-beta signaling and the GLI2 transcription factor in melanoma cells

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Melanoma is one of the most common causes of cancer and cancer deaths in the young populations. This cancer is highly metastatic and today, no efficient treatment is currently available and its prognosis is extremely poor. The melanocyte-specific transcription factor M-MITF is involved in numerous aspects of melanoblast lineage biology including differentiation, survival, and migration. It also plays complex roles at all stages of melanoma progression and metastasis. In this study, we demonstrate that in human melanoma cell lines, expression of M-MITF and its gene targets involved in melanogenesis, TYR, TRP-1, DCT/TRP-2, is repressed by exogenous TGF-beta and by stable overexpression of a constitutively active mutant form of TGF-beta receptor type I (TbetaR1). Both induce a strong reduction in M-MITF and TYR promoter activity, as determined in transient cell transfection assays, accompanied with decreased melanin production. Inversely, the TbetaRI inhibitor SB431542 increases M-MITF and M-MITF target gene expression. We show that expression of M-MITF in human melanoma cell lines is highly variable and inversely correlated with that of GLI2, a Hedgehog mediator identified as a TGF-beta gene target, whose expression in melanoma cells is associated with loss of e-cadherin expression and increased propensity to metastasize. Overexpression of a constitutively active mutant form of GLI2 in highly pigmented melanoma cells reduced the expression of M-MITF and that of its transcriptional targets, accompanied with decreased melanin production and increased invasiveness in Matrigel™. Together, our data suggest that TGF-beta-driven GLI2 expression in melanoma cells downregulates the melanogenic differentiation program in melanoma cells while favoring migration and invasiveness.

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Role of p16INK4a mutations in melanocytic nevi induced by 7,12-dimethylbenz(a)anthracene (DMBA)

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p16INK4a, the gene that encodes cyclin-dependent kinase inhibitor 2A, is a critical locus for tumor suppression. p16INK4a inhibits the activity of the cyclin D1-cyclin-dependent kinase 4 (CDK4) complex, which drives cell cycle progression by phosphorylating the retinoblastoma (RB) protein. p16INK4a drives G1 phase cell cycle arrest, blocking the RB protein phosphorylation. Inactivating mutations in the p16INK4a gene are associated with a greatly increased risk of melanoma, as well as atypical nevus syndrome, characterized by a high number of dysplastic nevi, increased numbers of banal melanocytic nevi. We investigated the effects of an inactivating p16INK4a mutation on the growth and morphology of nevi in mice. For this purpose, p16INK4a-/- mutant mice on a C3H/HeN background were subjected to a single topical application of 100 µl of a 0.1% solution of 7,12-dimethylbenz[a]anthracene (DMBA) followed by biweekly topical application of 100 µl of a 40 nmol solution of the phorbol ester 12-O-tetradecanoylphorbol-13 acetate (TPA). They were compared to wild-type C3H/HeN mice. Both strains began developing nevi after a latency period of seven weeks. The nevi of the p16INKa-/- mice demonstrated a consistently larger size throughout the 15 weeks of the study, and comprised a larger area in total (mean 27.04 mm2, standard deviation 15.18 mm2) than those of their wild-type counterparts (mean 5.85 mm2, standard deviation 3.73 mm2; $p = 0.0007$). A greater number of nevi was observed in the p16INKa-/- group (mean 10.17, standard deviation 8.68) than in the wild-type group (mean 4.5, standard deviation 3.47), but the difference did not achieve statistical significance ($p = 0.08$). Histopathologic appearance was consistent with dermal melanocytic nevi; in no case did the nevi develop into invasive melanomas. These results provide evidence that functional p16INK4a inhibits the growth of melanocytic nevi in mice.

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Association of high c-SKI/SnoN expression and eficient TGF-β/SMAD signaling in human melanoma cells

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SKI and SnoN proteins have been shown to inhibit TGF-β/SMAD signaling, acting both as transcriptional co-repressors in the cell nucleus, and as sequestrators of SMAD proteins in the cytoplasm. TGF-β, on the other hand, induces rapid, proteasome-mediated, degradation of both proteins. How elevated SKI and SnoN protein levels often co-exist with active autocrine TGF-β signaling in cancer cells is yet to be understood. In this study, we found elevated SKI and SnoN protein levels in a large panel of melanoma cell lines, as compared to normal melanocytes. There was however no correlation between SKI protein content and the capacity of melanoma cells to invade Matrigel™, and to form subcutaneous tumors or metastasize to bone after intracardiac inoculation into nude mice. Overexpression of SMAD7, which inhibits TGF-β signaling and delays melanoma cell tumorigenicity and inhibits their metastatic propensity, did not significantly alter SKI levels. TGF-β induced a rapid and dose-dependent degradation of SKI protein, associated with solid, dose-dependent, SMAD3/4 specific transcriptional response and induction of the pro-metastatic target genes IL-11 and PTHrP, partially prevented by pharmacologic blockade of proteasome activity. Stable SKI knockdown in 1205Lu melanoma cells did not alter their invasive capacity or transcriptional responses to TGF-β. SKI knockdown did not reveal any growth inhibitory activity of TGF-β and did not allow p21 expression in response to TGF-β. Thus, while SKI somewhat attenuates basal autocrine TGF-β signaling in melanoma cells, it does not efficiently interfere with the protumorigenic and pro-metastatic activities of TGF-β, unless stabilized by proteasome blockade.

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Expressin of Neuropeptide Y in melanocytic tumors

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Introduction. Neuropeptide tyrosin (NPY) is widely expressed in the CNS and peripheral nervous systems and has been shown to have a role in numerous physiologic processes. Additionally, NPY receptors are expressed in specific cancers like breast and different sarcomas. Considering than other neuropeptides, particularly α-MSH, seem to play a role in the pathogenesis of melanoma, this study assess the expression of NPY in cutaneous melanomas and melanocytic nevi, and correlate it with α-MSH expression and several prognostic factors for melanoma. Material and methods. We performed an observational study about the immunohistochemical expression of NPY and α-MSH (Bachem UK.LTD) in samples of cutaneous melanomas diagnosed in the last 5 years in San Jorge Hospital, Huesca (Spain). Different types of melanocytic nevi were also analyzed. Results. A total of 175 pigmented lesions were studied: 49 primary cutaneous melanomas, 15 melanoma metastases and 110 melanocytic nevi. Immunostaining with NPY and a-MSH was significantly higher in melanomas than in melanocytic nevi ($p < 0.001$), although Sutton nevi showed significant expression of NPY. 83 % of nodular melanomas and 78 % of superficial spreading melanomas were positive for NPY. Nodular melanoma showed the highest percentage of positive cells for NPY (mean 59.2%, CI 95% [38.8 - 79.5]) followed by superficial spreading melanoma (57.8% [33.5-82.2]). α-MSH expression was significantly higher in superficial spreading melanoma (mean of cells 80.5% [63.3-97.7]) than in nodular melanoma (41.1% [21.9-60.4]). A positive and significant correlation of 59 % was found between the immunostaining of both neuropeptides. Significant association was found between expression of NPY and presentation of metastasis (T-Student test $p=0.03$) No significant association was observed with age, gender, location of the lesions, Breslow index, Clark level and mitotic index. Conclusion. Our study demonstrates that NPY is significantly expressed in melanomas and could be a predictor factor for development of metastasis.

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Thyrotropin-releasing hormone (TRH) is a novel is a novel pigmentary hormone *in situ* and *in vitro*

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While thyrotropin-releasing hormone (TRH) is the major regulator of the hypothalamic-pituitary-thyroid (HPT)-axis, this tripeptide is also found in peripheral tissues. Large amounts of TRH are produced in amphibian skin, where it is thought to stimulate melanophores indirectly via the release of alpha-MSH from the pituitary gland. Recently, we have reported that the epithelium of normal human scalp hair follicles (HFs) expresses TRH and TRH-receptor (TRHR) and that exogenous TRH stimulates hair growth in microdissected, organ-cultured HFs. Using a novel frog skin organ culture system, we show that TRH can directly stimulate pigmentation of xenopus skin in the abence of a pituitary gland. Moreover, we show that TRH also stimulates human HF pigment production in organ culture (up-regulation of melanin synthesis, gp100 and tyrosinase expression, and of tyrosinase activity). However, HF melanocytes *in situ* lack prominent TRHR immunoreactivity. Therefore, indirect modes of TRH action on HF pigmentation are conceivable. In cultured melanocytes from the outer root sheath of human HFs, TRH significantly increased melanosome formation, dendricity, tyrosinase activity, and melanocyte proliferation *in vitro*. melanocytes were identified in the TRH treated groups, than in the vehicle control. Taken together, our findings indicate that TRH exerts complex stimulatory effects on both frog and human HF melanocytes *in situ* and *in vitro* and that the pigmentation-stimulatory properties of this tripeptide neurohormone represent an important, novel, and evolutionarily highly conserved regulatory principle in melanocyte biology.

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MDM2 antagonists increase pigmentation and induce epidermal thinning in human reconstructed skin

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Stabilization of the tumor suppressor p53 induces hyperpigmentation of the skin without ultraviolet (UV) exposure in mouse models. Nutlins are a novel class of specific MDM2 antagonists that restore the function of p53 in tumors expressing wild-type p53, such as melanomas. Nutlins show low toxicity in normal cells but their long term effect on human skin is unknown. Since skin cells continuously proliferate under physiological conditions, we evaluated the effects of nutlin-3a in human reconstructed skin models (melanoderms) and in human primary melanocytes. Treatment with nutlin-3a over 9 days increased melanin content in the epidermis compared to untreated controls (69.7% vs. 41.9%, respectively). Histochemical analysis revealed morphological changes in keratinocytes in the viable epidermis (spindle-shape appearance) consistent with a mature phenotype that resulted in significant thinning of that layer compared to controls (40.70 ± 2.35 vs. 21.74 ± 1.00 µm; $p=0.0017$). However, there was no effect on the thickness of the stratum corneum (44.42 ± 3.0 vs. 40.75 ± 0.48; $p=0.17$). Collectively, these results show that nutlin-3a induces differentiation of both skin cell types disrupting the regulation of the melanocyte-keratinocyte unit. To further characterize these effects, we treated primary light pigmented melanocytes with 5 µM or 10 µM nutlin-3a for 48 hr or 24 hr, respectively. As expected, nutlin-3a increased the protein expression of p53 and p53-target genes (p21 and MDM2). Surprisingly, nutlin-3a reduced the protein expression of Microphthalmia transcription factor (MITF), but increased expression of tyrosinase and tyrosinase-related protein-1. These results suggest that MDM2 antagonists affect human skin cells by inducing their accelerated differentiation, which may affect basic skin functions such as wound healing or homeostasis.

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Clinical valuation of a dioic acid and rumex occidentalis complex-based formulation in a dark-skinned population

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The aim of this work was to investigate the effects of 1,18-octadecen-9-dioic acid (dioic acid) & Rumex occidentalis complex for its skin lightening action in a dark-skinned population. As part of a 12-week placebo-controlled clinical study, the skin lightening effect of a product containing 1% of dioic acid, 2% of a Rumex occidentalis botanical extract and SPF 15 was assessed on 70 Indian female volunteers. Change in skin colour was monitored by: (A) Chromameter® measurement (L*, a*, b*) and Individual Typology Angle (ITA) calculation, (B) Grading of standardised photographs by a dermatologist and by naïve graders. Colorimetric measurements on volunteers' cheeks showed a significant increase of L* compared to baseline after 4 (+1.02, $p<0.0001$), 8 (+1.01, $p<0.0001$) and 12 weeks (+1.50, $p<0.0001$) and of ITA after 4 (+2.93, $p<0.0001$), 8 (+2.83, $p<0.0001$) and 12 weeks (+4.18, $p<0.0001$) of test product application. For both L* and ITA°, changes were significantly different than the SPF 15 placebo at week 4 ($p<0.05$) and week 12 ($p<0.05$). These results were confirmed by the dermatological and naïve grading where a significant lighter skin colour was obtained after 4, 8 and 12 week of application of the test product ($p<0.001$). Its effect was significantly better than the placebo at week 4 ($p<0.05$) according to the dermatologist; at week 4 ($p<0.05$) and week 12 ($p<0.05$) according to the naïve graders. This overall skin lightening action was beyond the one induced by the SPF 15 as shown by the placebo and was achieved without inducing any side effects. These findings show dioic acid and Rumex occidentalis complex delivers a significant skin lightening effect on dark-skinned population.

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Prophylactic potential of bleaching agents for familial melanoma

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Selective removal of melanocytes may serve as a prophylactic treatment for patients predisposed to melanoma development, including familial melanoma patients and those at risk for tumor recurrence. Recently demonstrated selective cytotoxicity of topically applied phenolic compounds towards melanocytes may prevent malignant transformation and elicit immune reactivity. Here, cytotoxicity of phenolic agents mono benzyl ether of hydroquinone (MBEH) and 4-tertiary butyl phenol (4-TBP) towards melanoma cell lines was assessed by MTT assays. To assess immune activation, bleaching agents were applied to organotypically cultured human skin, and to denuded skin of C57BL/6mice or k14-SCF mice maintaining epidermal melanocytes. Upon topical application, MBEH treatment induced significant depigmentation of treated skin in both k14-SCF (17%) and C57/BL6 (64%) mice. In MBEH treated mice subcutaneously challenged with B16 melanoma cells, skin tumor volume was reduced by 90% among treated mice in comparison with control. Surprisingly, 3 out of 4 human melanoma cells lines and mouse B16 cells were markedly insensitive to 0-500 μM 4-TBP and MBEH in vitro, maintaining > 60% viability in response to 500uM of 4-TBP and MBEH after 24 hrs, suggesting that anti-tumor effects were not due to direct cytotoxicity. Three-fold increased emigration of dendritic cell subsets from human skin was observed following application of MBEH by immunohistology, parallelled by 2 fold increased infiltration of CD3+ T cells ($p<0.05$) in k14-SCF mice. These results suggest immune activation may be involved in elimination of melanocytes by MBEH. Thus topical exposure to MBEH carries potential for anti-melanoma treatment.

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The BH3-mimetic ABT-737 induces strong synergistic killing of melanoma cells when combined with the alkylating agent temozolomide

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Metastatic melanoma has poor prognosis and is refractory to most conventional chemotherapies. The alkylating agent temozolomide (TMZ) is commonly used in treating melanoma but has a disappointing response rate. Agents that can act cooperatively with TMZ and improve its efficacy are thus highly sought after. One possible agent is the BH3 mimetic ABT-737, which can induce apoptosis by targeting pro-survival Bcl-2 family members. Yet, research has shown that many cell lines are resistant to ABT-737 via the upregulation of the pro-survival protein Mcl-1 and/or the down-regulation of the pro-apoptotic protein Noxa, which can inhibit Mcl-1. We found that a combination of TMZ and ABT-737 induced strong synergistic apoptosis in multiple human melanoma cell lines, while inducing little or no apoptosis in normal melanocyte lines. However, sensitivity to the drug combinations was variable, and two melanoma cell lines were completely resistant. Further study revealed that resistance was strongly correlated with the expression of MGMT and PARP, two proteins involved in TMZ resistance by known mechanisms. Inhibition of these proteins via O6-benzylguanine and ABT-888, respectively, reduced or eliminated resistance to the drug combination. Furthermore, these two inhibitors worked together synergistically to enhance sensitivity. Immunoblot analysis and experiments with knockdown cell lines suggested that TMZ and ABT-737 synergy is mediated through upregulated Noxa. Our results show that TMZ and ABT-737 are a promising drug combination for clinical studies, particularly when combined with pretreatment by MGMT and PARP inhibitors.

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Stable isoform of the Smad1 protein inhibits melanoma cell proliferation and migration

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Bone morphogenetic protein (BMP) signaling possesses a potent anti-tumor activity in the skin. Despite the significant progress achieved in delineating the functional significance of the BMP signaling in epithelial skin cancers, very little is known about the role of the BMP pathway in melanoma. To access a role of the BMP-Smad1 signaling in the melanoma growth and metastasis, we examined the effect of Smad1 protein on the proliferation and migration of melanoma cell lines. We show here that malignant human melanoma cells express low levels of active isoform of Smad1 protein and that duration of its active signaling is reduced compared to normal melanocytes. Decreased Smad1 activity in melanoma cells is caused, at least in part, by its proteosomal degradation mediated by the ERK-mediated phosphorylation of the linker region of the Smad1 protein. In fact, most of the primary and metastatic melanoma cell lines have constitutively active MAPK/ERK as a result of the gain-of-function mutations of the BRAF or NRAS oncogenes. Using lentiviral vectors, we created the viral particles expressing Smad1 protein containing the mutated MAPK/ERK binding sites. Infection of melanoma cell lines with those lentiviruses showed significant increase of the extent of BMP-dependent activation of a Smad1 transcriptional reporter construct. Melanoma cell lines expressing constitutively active Smad1 protein containing mutated MAPK/ERK binding sites showed significant inhibition of cell proliferation and the migration ability compared to non-infected cells. Smad1-mediated growth inhibition in melanoma cells correlated with upregulation of the p16INK4a and p21Cip1 expression on mRNA and protein levels. Our results demonstrate that MAPK-mediated Smad1 degradation promotes melanoma cell survival and suggest that further exploration of the mechanisms involved in stabilization of the Smad1 protein may serve as a new treatment strategy for melanoma.

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Characterizing regression in melanomas: A population-based study

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Spontaneous regression is considered as partial or complete resolution of a tumor in the absence of any treatment. The true incidence and prognostic impact of regression is unknown. The purpose of this study is to examine the epidemiology of melanomas demonstrating regression, which is reported to occur, at least partially, in a significant number of cases. Using data from NCI's SEER (Surveillance, Epidemiology, and End Results) Program, we analyzed cases of "malignant melanoma, regressing" occurring on the skin between 1973 and 2006, as specified by International Classification for Diseases in Oncology 3 (ICDO3). The demographics, tumor characteristics and relative survival of these were compared with cases of "malignant melanoma" of skin sites. Comparisons used chi-square and Wilcoxon tests for categorical variables and t-test for continuous variables. Relative survival was compared using Z-test. A total of 1,126 cases of regressing melanoma and 85,808 of malignant melanoma were identified. For regressing melanoma cases, 65.8% were male, 73.0% were diagnosed at age 50 or higher, and 94.7% occurred in whites. The average Breslow's thickness was 2.44mm. The majority (52.7%) occurred on the skin of the trunk. Relative 5-year survival of regressing melanomas was 95.5% (sem= 1.5%). For melanomas that were not classified as regressing, 55.9% were male, which was significantly lower than regressing melanomas ($p<0.001$), and fewer melanomas without regression occurred at 50 or higher (60.6%). The average Breslow's thickness was significantly higher at 3.43mm ($p=0.044$). Relative 5-year survival of regressing melanomas was significantly higher than for non-regressing melanoma cases, whose relative survival was 86% (sem 0.2%) ($p<0.001$). For cases of regression in melanomas, assessment of prognosis is difficult as original tumor thickness cannot be accurately assessed. Examination of these cases gives important clues to the clinical significance of regression.

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Blocking laminin alpha 5 expression inhibits melanoma cell proliferation, migration and invasion

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Laminin-511 (composed of α5, β1 and γ1 chains) is a major extracellular matrix component of basement membrane of epithelia and blood vessels. Our previous studies indicate that laminin α5 chain play important roles in tumor cell invasion and progression. The objective of the study was to explore the role of laminin α5 in melanoma growth and invasion. Using lentivirus-mediated RNA silencing strategy (lenti-shRNA), we blocked the expression of laminin α5 in melanoma cells by transfection of human melanoma cells with lenti-Lα5 shRNA construct (with lenti-LUC shRNA as control). The gene expressions were examined by reverse transcription and polymerase chain reaction analysis. The effects of laminin α5 gene knockdown on melanoma cell growth, migration and invasion were studied using cell proliferation, chamber migration and metrigel invasion assays. The results demonstrated that blocking laminin α5 expression significantly inhibited melanoma cell proliferation. Compared with control cells, total viable cells were decreased 34.49% and 26.28% on day 3 and 5 respectively (both $p<0.01$). Also, blocking laminin α5 expression markedly inhibited the abilities of melanoma cell migration and invasion. The rate of cell migration through the membrane was significantly decreased at 24 hours (75% down, $p<0.001$). The rate of cell invasion through the metrigel was markedly reduced at 24 hours (79% down, $p<0.001$). In addition, the study showed that the down-regulation of laminin α5 correlated with the disregulated expression of matrix metalloproteinase family proteins. The mRNA expression levels of MMP-2, MMP-9 and membrane-type 2 (MT2) MMP were markedly down (44%, 42% and 12% respectively) while the expression of tissue inhibitor of metalloproteinase (TIMP)-2 was significantly up (16%). These findings demonstrate the critical roles of laminin α5 in the cellular functions of human melanoma and strongly suggest the involvement of MMPs/TIMP proteins.

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MAGE-C2 expression affects phosphorylation of Kap1, DNA repair and p53 activity

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Melanoma associated antigens (MAGE)/cancer testes antigens (CT antigens) are preeminent owing to their exclusive expression in normal germ line tissues (testis, ovary etc.) and cancer cells. DNA damage occurs at high frequency in cells and can be a result of ionizing radiation, reactive oxygen species, mutagenic chemicals, ultraviolet radiation etc. The DNA damage response of cells incites a variety of signal transduction pathways and proteins involved in DNA repair, arrest of cell cycle progression and changes in chromatin structure at the site of DNA damage. The Krüppel-associated box zinc finger protein (KRAB-ZFP) silencing complex mediates repression through an association with KRAB-ZFP–associated protein 1 (KAP1), a molecular scaffold protein. KAP1 coordinates the deacetylation and methylation of histones, resulting in heritable gene silencing through the formation of heterochromatin. Phosphorylation of KAP1 at ser824 results in relaxation of chromatin at the site of DNA damage allowing for repair. Kap1 phosphorylation is mediated by ATM kinase and is considered critical in response to DNA damage. In this study, we show that down-regulation of MAGE-C2 in the A375 melanoma cell line results in apoptosis, increased p53 activity and altered expression of p53 downstream targets. MTT and TUNEL assay showed that knock down of MAGE-C2 enhances the effects of DNA damage, resulting in decreased cellular proliferation and cell death, respectively. MAGE-C2 knockdown decreases DNA damage induced phosphorylation of Kap1 at ser824 in melanoma cells and over-expression of MAGE-C2 in 293T cells results in phosphorylation of Kap1 at ser824 even in absence of DNA damage. Since phosphorylation of Kap1 at ser824 facilitates relaxation of heterochromatin, loss of gene repression, DNA repair and cellular proliferation, our results suggest that MAGE-C2 can regulate the cellular DNA repair machinery by affecting phosphorylation of Kap-1.

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Regulatory T lymphocyte infiltrate is inversely related to breslow depth in melanoma

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CD4+CD25+ regulatory T cells (Treg) represent a functionally distinct lineage of T lymphocytes that are crucial for the maintenance of immune tolerance. In the absence of regulatory T cells, severe autoimmunity develops. Tumors express antigens allowing for immune recognition and activation of anti-tumor immune mechanisms. Treg can suppress the immune system's response to neoplasms allowing for tumor progression. Melanoma has been noted to have a lymphocytic infiltrate. Immune mediated treatments for melanoma have had mixed success. Treg have been implicated as a possible etiology for the lack of efficacy in immune based therapies. We proposed that Treg were important in initiating peripheral tolerance to tumor antigens in early, thin melanomas thereby allowing them to progress. We hypothesized that thinner melanomas would have a greater percentage of regulatory T cells in their lymphocytic infiltrate as compared to melanomas with a greater breslow depth. We examined benign nevi, dysplastic nevi, melanomas with a breslow depth <1 mm and melanomas with a breslow depth greater than 1 mm for the presence of regulatory T lymphocytes. Immunofluorescence microscopy was used to detect T cell markers CD25 and CD4, the Treg transcription factor FOXP3, and the melanocyte marker Mart 1. Two blind observers examined the slides for the immunohistochemical profile of the infiltrate. Our data revealed that nevi had very few inflammatory cells present in the surrounding stroma. In contrast, melanomas <1 mm in breslow depth had a brisk inflammatory infiltrate with an average of 20% of the infiltrate being Treg. Melanomas greater than 1 mm had a Treg infiltrate significantly different than their thinner counterparts. In melanomas with a breslow depth greater than 1 mm the Treg population was 10% of the total CD4+ population. Thus, there are an increased number of Treg in thin melanomas possibly allowing for tumor progression despite immune surveillance.

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An issue of safe inferior surgical margin in subungual melanoma: Is the distance from nail matrix to phalangeal bone enough to eradicate?

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Subungual melanoma (SM) has traditionally been managed by radical digital amputation, "functional" surgery or even the wide local excision (WLE) and grafting. If excisions of the subungual soft tissue were enough to eradicate melanoma, there would be no problem in performing WLE for SM. However, the amount of the subungual tissue, i.e. the distance from the nail matrix to the phalangeal bone has not yet been studied. The purpose of this study is to measure the distance, on an aspect of inferior surgical margins, in order to elucidate the safety of WLE for SM. Nine male and six female cadavers, whose ages ranged from 41 to 74 years (avg. 57.1±10.7), were included in this study. Each of them offered examples of the 1st and 5th fingers and toes. The shortest distance between the lowermost base of nail matrix and phalangeal bony surface was measured. The mean distances were 0.90mm (1st finger), 0.72mm (5th finger), 0.87mm (1st toe) and 1.09mm (5th toe), respectively. So, total average of all digits was 0.90mm. Considering 5mm of lateral surgical margin is recommended even in melanoma *in situ*, approximately 1mm, the measured distance does not seem sufficient for eradication. Therefore, we propose that a more aggressive treatment, such as functional surgery or radical amputation instead of WLE with grafting, may be regarded as a better option for treatment of SM until a consensus of the safety of inferior surgical margins is established from further randomized and well-controlled studies.

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GLO1 overexpression in human malignant melanoma antagonizes glycolytic methylglyoxal stress

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Glyoxalase I [lactoylglutathione lyase (EC 4.4.1.5) encoded by GLO1] is a ubiquitous cellular defense enzyme involved in the detoxification of methylglyoxal, a reactive 1,2-dicarbonyl compound and cytotoxic byproduct of glycolysis. Apart from exerting general cytotoxic effects associated with protein crosslinking and mutagenic adduction of DNA bases, methylglyoxal-induced posttranslational adduction of selected target proteins is rapidly emerging as a novel mechanism of transcriptional control and cancer cell survival signaling. Based on the hypothesis that GLO1 upregulation may play a functional role in glycolytic adaptations of cancer cells, we examined GLO1 expression status in human melanoma tissue. Quantitative RT-PCR analysis of a cDNA tissue array containing human melanoma tissues (stages III and IV) and healthy controls revealed pronounced upregulation of GLO1 expression at the mRNA level. Immunohistochemical analysis of a melanoma tissue microarray confirmed upregulation of glyoxalase 1 protein levels in malignant melanoma tissue versus healthy human skin. Consistent with an essential role of GLO1 in melanoma cell defense against methylglyoxal cytotoxicity, siRNA interference targeting GLO1-expression (siGLO1) sensitized A375 and G361 human metastatic melanoma cells towards the antiproliferative, apoptogenic, and oxidative stress-inducing activity of exogenous methylglyoxal and impaired cellular proliferation under hypoxic conditions. Taken together, our data suggest that overexpression of GLO1 in metastatic melanoma may reflect a cellular response to elevated methylglyoxal stress associated with glycolytic adaptations in advanced stages of the disease.

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Automated computer analysis of pagetoid spread and irregularity at the basal layer in confocal images of superficial spreading melanoma vs junctional nevi

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We aimed to extract quantitative morphological diagnostic information from noninvasive 3-dimensional confocal images of 10 melanomas and junctional lentiginous nevi. This study tested computer-automated in vivo noninvasive identification of two criteria for melanoma: 1. presence of pagetoid melanocytes (PM) in the epidermis, and 2. irregularity in the depth position of the melanocytic cells (DMC) at the dermal-epidermal junction. Melanocytic lesions were imaged with resolution and contrast comparable to standard histopathology. A confocal reflectance microscope acquired 3D images at a clinically suspicious region of each lesion in about 1 minute over a 500um x 500um (en face) field of view to a depth of 200 um, in 5 melanomas and 5 junctional lentigious nevi. Melanocytic lesions presented a high optical reflectivity, which enabled detection of isolated PM against the less reflective surrounding epidermis, and enabled characterization of the smoothness of the DMC, which we quantified by calculating the mean axial gradient between laterally adjacent points. The cellular atypia at the basal layer caused by melanoma gave the DMC surface a high mean spatial gradient (2.99 ± 0.32) in the MM data and a lower ($p = 0.00001$) mean spatial gradient (1.23 ± 0.32) in the junctional lentiginous nevi. By analyzing local fluctuations in the superficial pigmented surface we were able to automatically identify the coordinates of pagetoid melanocytes in all the melanomas and none of the nevi. We conclude that two diagnostic trates can be automatically assessed with ease. The melanoma sites presented an irregular DMC due to focal melanocytic proliferation, while the nevi presented a smooth DMC. The melanoma sites presented PM, while the nevi did not. The feasibility to rapidly screen melanocytic sites for two criteria for melanoma was demonstrated.

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Investigation of melanosome-based mechanisms underlying melanoma treatment resistance.

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We have previously demonstrated that genes regulating melanosome formation in primary murine melanocytes and human and murine melanoma cells influence multi-drug resistance. Here we have initiated studies to assess what, if any, melanosome-based mechanisms contribute to that resistance. We utilized a series of immortalized murine cell lines, each carrying a mutation in a gene regulating melanosome function (*Oca2, Slc7a11, Tyr, Hps5*) and tested each cell line for increased susceptibility to cisplatin, correlating loss of specific melanosome functions with changes in drug sensitivity. In an alternative and complementary approach, we used shRNA gene silencing to stably deplete the human melanoma cell line MNT1 of molecules regulating protein trafficking to the melanosome (VPS33A, pallidin, cappuccino) and tested cisplatin sensitivity. Drug sensitivity increased with loss of function of the tyrosinase enzyme and HPS5 protein, decreased with loss of function of the SLC7A11 transporter and remained unchanged with loss of function of the P protein encoded by *Oca2*. It was found that depletion of VPS33A caused increased drug sensitivity. We conclude that melanin synthesis by tyrosinase as well as HPS5 and VPS33A function (both implicated in melanosomal protein trafficking) are necessary for maintenance of drug resistance. We also conclude that the transporter SLC7A11 influences drug response. Melanosomal pH as regulated by the P protein does not influence drug response. In summary, selected melanosome functions contribute to melanoma drug response.

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Paving the way for personalized approaches to melanoma prevention

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In the United States, the incidence of Cutaneous Malignant Melanoma (CMM) has increased faster than that of any other type of cancer in the last 50 years. In addition to the human tragedy of CMM, the economic consequence in terms of loss of years and income per death places CMM second only to adult leukemia. Furthermore, the stark difference in outcome for patients with early- and advanced-stage disease highlights the impact that effective strategies for early detection and disease prevention may have on reducing the burden attributable to CMM. Melanocytic nevi are an important indicator for risk of melanoma in later life, and can be an intermediate for CMM formation. We have established the Colorado Kids Sun Care Program (CKSP) to examine the sun exposure profiles and genetic factors that predispose to nevus formation. Over 900 CKSP study subjects have been followed from ages 6 to 10 with annual skin exams collecting data on nevus counts, sun exposure, phenotype and behavioral variables. We collected DNA samples and are genotyping SNPs in all presently known Caucasian pigmentation and melanoma risk genes in order to test for susceptibility to nevus formation. As an example, we report a new association between the major blue/brown eye color OCA2/HERC2 rs12913832 SNP, UV exposure and nevus counts. In particular, individuals who are homozygous for the blue eye color SNP are particularly sensitive to modification of nevus counts by UV exposure. Furthermore, this modification is most obvious in the presence of intermittent vs chronic UV exposure behaviors. Our ongoing analysis thus identified key pigmentation and melanoma risk genes that interact with different UV exposure profiles to give maximal nevus counts in children. We anticipate that these data may be used to select individuals with nevus and melanoma susceptibility genotypes who may in turn be better targeted for primary prevention and secondary screening.