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A differential response between normal and psoriatic keratinocytes' N-methyl-D-aspartate receptor subtype 2C following tumor necrosis factor alpha exposure

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After wounding, psoriatic skin heals significantly faster than normal skin. Expression of the N-methyl-D-aspartate receptor (NMDAR) subtype, 2C (NR2C), is reduced in psoriatic skin in vivo and this receptor is induced in normal keratinocytes by tumor necrosis factor alpha (TNFα) in vitro. NMDARs can regulate Ca++ influx in keratinocytes, and levels of intracellular Ca++ regulates these cells' migration and proliferation. To determine whether differential expression of NR2C occurs in psoriatic and normal keratinocytes after TNFα exposure, we performed qPCR on cell cultures (normal, psoriatic involved and psoriatic uninvolved), after TNFα treatment. Keratinocytes obtained from normal and psoriatic skin were grown to confluence in supplemented keratinocyte growth medium (60 μM Ca++). The cells were then incubated in medium without the additives but with 150 μM Ca++ for 2 days. Representative cultures were incubated with TNFα for 3 days, and RNA extracted; the NR2C mRNA was determined by qPCR. TNFα significantly induced NR2C mRNA but only in normal keratinocytes. Immunocytochemistry showing TNFα induction of NR2C protein in only normal keratinocytes confirmed these results. In addition, to determine whether an induction of NR2C was associated with an effect of TNFα on epiboly, we cultured all three cell types (normal, involved and uninvolved), with or without induction of NR2C and then scratched the representative monolayers with a pipette tip to create a "wound." TNFα reduced cell migration into the denuded zone only in normal keratinocyte monolayers that expressed NR2C. Thus, NR2C expression may play an important role with TNFα on regulating normal keratinocyte migration. Differential expression of NR2C and its interplay with TNFα may explain, in part, the increase in the rate of wound healing in psoriasis.

Enhanced cellular internalization of FGF1/CPPC fusion protein promoted its radioprotective effect in the hair follicles

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Signals for FGF1 functions come from not only activated FGF receptors (FGFR) but also internalized FGF1. Although FGF11 subfamily members (FGF11-14) have structural similarity with FGF1, FGF11-14 possess unique C-terminal polypeptides. Novel cell-penetrating peptide domains (CPPC11-14) were identified from the C-terminal region of FGF11-14 proteins, which could readily deliver FGFs into cells independently of FGFRs. In this study, we created FGF1/CPPC fusion proteins (FGF1/CPPC11-14) following the alignment of FGF12 C-terminal region and evaluated the protective activity of FGF1/CPPC11-14 against radiation damage. FGF1/CPPC11-14 fusion proteins were internalized into the rat intestinal epithelial cell line IEC6 more efficiently than FGF1. However, the mitogenic activity of FGF1/CPPC12 through FGFR1c was less than that of FGF1. In contrast, FGF1/CPPC11-14 significantly reduced radiation-induced apoptosis in IEC6 cells in the presence of heparin, and the administration of FGF1/CPPC11-14 to BALB/c mice without heparin reduced the induction of apoptosis in hair bulbs 24 h after irradiation. In addition, pre-treatment with FGF1/CPPC11-14 maintained K15 positive stem cells in the bulge after irradiation, although K15 positive stem cells decreased in anagen hair follicles after irradiation. These findings indicate that FGF1/CPPC fusion proteins protect the hair follicles against radiation-induced injury and suggest that cellular internalization of FGF1/CPPC11-14 is involved in their activities independently of FGFRs.

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Dysregulated iRHOM2/ADAM17 in Tylosis with oesophageal cancer affects Ephrin- and EGF-family-mediated keratinocyte adhesion and migration

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We have linked mutations in the inactive rhomboid protein iRhom2 with the autosomal dominant disorder Tylosis with Oesophageal Cancer (TOC). Symptoms include palmoplantar keratoderma and a greatly increased risk of oesophageal cancer. Previously, we showed increased activation of Epithelial Growth Factor (EGF) signalling in TOC keratinocyte cell lines, which migrate significantly faster than controls during scratch wound assays, particularly in the absence of exogenous EGF. This led us to investigate the role of ADAM17 (TACE), whose maturation is regulated by iRhom2. Western blotting of ADAM17 in TOC keratinocytes demonstrated increased processing of ADAM17, shown by increased mature ADAM17 compared to controls and elevated levels of the ADAM17 pro-domain. Immunocytochemistry showed increased ADAM17 at the plasma membrane in TOC cells. After stimulation with PMA or LPS, keratinocytes and PBMCs from TOC patients showed increased production of ADAM17 substrates, including amphiregulin and pro-inflammatory cytokines TNFα and IL-8 ($p<0.05$). This was abrogated by siRNA knockdown of ADAM17. Amphiregulin was also upregulated in TOC cells after scratch wounding ($p<0.001$). A protein array comparing phospho-receptor tyrosine kinases in control and TOC keratinocytes showed reduced phosphorylation of the EphA4 receptor. Western blotting showed increased levels of total EphA4 and EphA2 in TOC cells compared to controls. EphA2 is targeted by EGF signalling. The Eph/Ephrin pathway is important in adhesion and migration and is dysregulated in many cancers. Dispase assays suggested increased adhesion in TOC cells consistent with dysregulation of this pathway. These data indicate that TOC-associated mutations in iRhom2 lead to dysregulation of ADAM17 maturation, affecting EGFR and Ephrin signalling and the inflammatory cytokine response. Thus the TOC phenotype may be due to altered wound healing as a result of dysregulated adhesion and migration.

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Sustained β-catenin activity leads to dermal fibrosis by promoting expression of various pro-fibrotic mediators

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The purpose of this study is to determine the mechanism of pro-fibrotic β-catenin activity in dermal fibroblasts. We have examined fibrotic human tissue by immunohistochemistry and have analyzed novel mouse models with tissue-specific manipulation of β-catenin. β-catenin, the central transducer of canonical Wnt signaling, is a powerful regulator of gene transcription and a key determinant of dermal fibroblast identity. Fibroblasts are the primary cellular effectors of pathological fibrosis, in which excess extracellular matrix leads to sclerosis and organ dysfunction. First, we found that β-catenin is active in an increased percentage of fibroblasts in fibrotic skin associated with systemic sclerosis, localized scleroderma, keloids, and nephrogenic systemic fibrosis. Second, constitutively active β-catenin in mouse dermal fibroblasts is sufficient to cause spontaneous, progressive skin fibrosis with 2.7-fold increased collagen ($p=0.004$), thickened collagen fibrils, and increased fibrillin. Third, expression of known pro-fibrotic effectors is strongly dependent upon β-catenin. In the presence of β-catenin, expression of Tgfb1 is elevated 26.5-fold ($p<0.001$) and expression of genes encoding members of integrin (adhesion) and matrix signaling networks and components of the Wnt signaling pathway is increased >3.5-fold ($p<0.002$). Interestingly, expression of potent transcriptional repressors of lineage selection and cell differentiation (Prdm1/Blimp1, Twist2, Runx1/3) also strongly depends upon β-catenin activity (>7-fold increase, $p<0.001$). Our results support a model in which β-catenin activity can robustly promote a fibrosis phenotype not only by positive regulation of growth factors, adhesion proteins, and matrix proteins, but also by negative regulation of other genes via interactions with transcriptional repressors. Elucidating expected and novel effects of pro-fibrotic β-catenin activity is critical for understanding the pathogenesis of fibrosis and identifying therapeutic targets for treatment of fibrotic skin diseases.

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HOXD10 is a major mediator of VEGF-C-induced lymphangiogenesis

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Activation of the lymphatic vasculature promotes cancer metastasis and modulates inflammatory reactions, whereas impairment of lymphatic function results in lymphedema. The major lymphangiogenic factor, vascular endothelial growth factor-C (VEGF-C), mainly acts via activation of VEGF receptor-3 (VEGFR-3). Recently, VEGF-C and its receptor have become the targets for new therapeutic strategies that have entered the clinic. However, little is known about signaling downstream of VEGFR-3 activation. We aimed to identify transcriptional networks that mediate the VEGF-C effects on lymphatic endothelium via VEGFR-3, utilizing a mutant form of the VEGF-C protein, named VEGF-C156S, which specifically activates VEGFR-3 but not VEGFR-2. We treated human lymphatic endothelial cells (LEC) with VEGF-C156S and obtained mRNA at 15 time points (0min to 8h), followed by cap analysis of gene expression (CAGE) sequencing. We found that 84 transcription factors (TFs) were differentially expressed over the time course. Many of these TFs are known early response genes, with expression peaks between 30 and 80 min after VEGF-C addition. To identify TFs specifically involved in the LEC response to VEGF-C, we analyzed TF binding sites in the differentially activated promoters using oPOSSUM3, together with gene set enrichment analyses using published results of different cell types after growth factor treatment. These analyses revealed HOXD10 to be activated in LECs after VEGF-C stimulation. HOXD10 was found to be LECs specific, as compared to blood vessel ECs, and to be a target gene of the lymphatic TF Prox1. Adenoviral gain-of-function and loss-of-function studies revealed that the TFs ATF3, FOSB and NR4A1, that were also induced by VEGF-C, are under transcriptional control by HOXD10, and that HOXD10 mediates the VEGF-C-induced upregulation of NR4A1. HOXD10 modulated LEC migration and sprouting. Together, these results reveal an unanticipated role of HOXD10 in the control of lymphangiogenesis.

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Ethnic differences in dermal extracellular matrix

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Although the majority of the world's population is comprised of people with highly pigmented skin (~85%; WHO, 2004) few studies have attempted to characterise skin composition according to ethnic origin. The aim of the current study was to perform a robust and novel survey of epidermal morphometrics and dermal extracellular matrix (ECM) composition in photoprotected human skin from three ethnic groups. Biopsies were obtained from buttock skin of young, healthy volunteers ($n = 7$ per group): Caucasian (18-29 years), Far East Asian (19-30 years) and African (18-28 years). Morphometric analysis identified increased epidermal thickness ($P < 0.001$), rete ridge height ($P < 0.01$) and rete ridge interdigitation ($P < 0.05$) in individuals of African descent compared to both Caucasian and Far East Asian subjects. Variations in epidermal morphometrics were accompanied by compositional differences in the dermal ECM as assessed by immunohistochemical methods. Elastic fibre network components, that confer the ability to passively recoil, were differentially deposited in African skin with significantly increased fibulin-5 ($P < 0.001$) and fibrillin-rich microfibrils ($P < 0.001$) and less elastin ($P < 0.042$) immunostaining than Caucasian and Far East Asian skin. Furthermore, immunoreactivity for the fibrillar collagens type I and III, which resist tensile forces, was also significantly increased in skin of African origin ($P < 0.025$ and $P < 0.026$, respectively). This study demonstrates that skin from diverse ethnic origins exhibits fundamental differences in both epidermal morphometrics and dermal ECM composition. As a consequence, further research into non-Caucasian skin types is required to help inform future management of disease and ageing in individuals from diverse ethnic groups.

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The spontaneous development of dermal fibrosis in mice with double heterozygous deficiency of *Klf5* and *Fli1*

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Systemic sclerosis (SSc) is a multisystem autoimmune vascular disease characterized by fibrosis of skin and certain internal organs. Although its pathogenesis still remains unknown, the deficiency of transcription factor Fli1 has been shown to be potentially involved in fibrotic process of SSc. Fli1 knockdown in normal fibroblasts recapitulates many features of those cells stimulated with TGF-β1 and SSc fibroblasts. However, *Fli1*+/- mice exhibit normal dermal tissue, suggesting that other factors are required to develop dermal fibrosis in SSc. We herein identified transcription factor KLF5 as a candidate inducing spontaneous dermal fibrosis in murine models acting together with Fli1. In SSc fibroblasts, similar to Fli1, KLF5 expression was suppressed at the transcriptional level by an epigenetic mechanism. In normal fibroblasts, KLF5 knockdown resulted in the increase of CTGF, while Fli1 knockdown upregulated type I collage expression. Notably, double knockdown of KLF5 and Fli1 resulted in a robust increase of CTGF expression. Furthermore, KLF5 bound to the CTGF promoter at -112 to -105 bp, which was closely located to the Fli1 binding site (-117 to -114 bp). KLF5 directly interacted with Fli1, and these transcription factors synergistically repressed the CTGF expression at the transcriptional level. Of note, mice with double heterozygous knockout of *Klf5* and *Fli1*, but not single heterozygote mice, developed spontaneous dermal fibrosis. Consistent with in vitro data, *Col1a2* and *Ctgf* mRNA levels were markedly elevated ($p<0.05$) in the skin of these mice. Given that both TGF-β and CTGF are required for persistent tissue fibrosis, fibroblast activation due to Fli1 deficiency, which mimics TGF-β-dependent activation, and CTGF upregulation caused by a synergistic effect of KLF5 and Fli1 deficiency interact and eventually develop spontaneous dermal fibrosis in double heterozygote mice. Thus, we herein established a new murine model of SSc and the present data provide us with a new clue to better understand the mechanism of tissue fibrosis in SSc.

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Anti-aging properties of the association of "Retinaldehyde, Pretocopheryl glucopyranosyl, Glycylglycine oleamide"

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Skin is subject to an unpreventable intrinsic ageing process and influenced by exogenous factors. Protein damage mediated by oxidation, protein adducts formation with advanced glycated end products and with products of lipid peroxidation, have been implicated during skin aging. The aim of this study was to evaluate the properties of a combination of molecules on skin aging: Retinaldehyde (RAL, a retinoid), Pretocopheryl glucopyranosyl (Pretoco, an anti-oxydant) and Glycylglycine oleamide (OGG, an inhibitor of glycation). We have showed on senescent fibroblasts (Hayflick model), that RAL, Pretoco and OGG increased "trypsin-like" and "caspase-like" proteasomal activity up to +31%. Moreover, RAL increased type I collagen synthesis by +21%, and decreased MMP1 synthesis by -29%. Using Affymetrix micro-arrays, we have also showed that topical application of RAL (1%) on human skin explants induced an increase of methionine sulfoxide reductases A (MSRA) mRNA level (+131%), and an inhibition of MMP-1, -3 and -9. MSRA are enzymes that reverse the effects of oxidative damage by reducing methionine sulfoxide and recovering protein function. Furthermore, we have also showed that Pretoco is a strong inhibitor of H202-induced lipid peroxydation on fibroblasts. Finally, we have evaluated the anti-glycation properties of OGG on human skin explants. We have showed that OGG is able to prevent the inhibitory effect of methyl glyoxal (a strong glycation inducer) on fibrillin-1 expression. In conclusion, our results demonstrated the anti-aging properties of an association of Retinaldehyde, Pretocopheryl glucopyranosyl and Glycylglycine oleamide. Indeed, RAL is able to increase proteasomal activity and MSRA that reverse the effects of oxidative damage. RAL is also able to increase type I collagen and decrease MMP1 synthesis. OGG is a powerfull inhibitor of glycation and Pretoco is a strong anti-oxydant. By acting on different mechanisms involved in skin aging, these molecules could be able to reduce extrinsic aging on skin.

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Age-dependent increase of caveolae and caveolae-mediated endocytosis contributes to fibroblast dysfunction in elderly skin

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Age-dependent skin fibroblast dysfunction, such as reduced production of type I procollagen, contributes to thinning and fragility of skin in the elderly. Mechanisms underlying decline of fibroblast function are not fully understood. Caveolae are endocytic plasma membrane invaginations that function in cell signaling and endocytosis. Caveolin-1 is the predominant protein found in caveolae. We have investigated age-associated alterations of caveolae number, composition and function in human dermal fibroblasts. Electron microscopy revealed that caveolae, visualized by immunogold stained caveolin-1, are the predominant membrane pits in fibroblasts in human dermis. The abundance of caveolae in fibroblasts in aged skin (80+ years old) was significantly increased 1.8-fold ($N=5$, $P<0.05$), compared to young skin (21-30 years old). Caveolae-mediated endocytosis was 1.5-fold ($N=6$, $P<0.01$) greater in fibroblasts in aged skin compared to young skin, as demonstrated by quantitation of cholera toxin B-subunit internalization, a selective caveolae cargo, assessed by flow cytometry and scanning confocal fluorescence microscopy. In addition, caveolin-1 protein was increased 1.8-fold ($N=9$, $P<0.05$), measured by immunostaining, and 2.5-fold ($N=6$, $P<0.05$), measured by Western blot, in fibroblasts in aged skin, compared to young skin. Primary adult human skin fibroblasts have abundant caveolae (13±0.91 per fibroblast per microscopic field, $N=30$) and actively internalize cholera toxin-B. Reduction of caveolin-1 level, using specific siRNA, enhanced expression of type I collagen mRNA (2.2-fold, $N=4$, $P<0.05$) and protein (1.8-fold, $N=3$, $P<0.05$). The above data indicate that caveolae and its endocytic activity are enhanced in fibroblasts in aged human skin. Increased caveolae likely contributes to age-dependent fibroblast dysfunction, such as reduced collagen production. Our study presents caveolae and caveolin-1 as potential therapeutic targets for mitigating age-dependent decline of fibroblast functions.

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Orai1-dependant Ca2+ signals control epidermal proliferation

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Cytosolic Ca2+ signals are performed by Ca2+ releases from the endoplasmic reticulum and Ca2+ influx from the extracellular medium. Releases rely on the refilling of the intracellular Ca2+ stores by the Ca2+ influx "Store Operated Ca2+ Entry" (SOCE) via the Ca2+ channel Orai1. Here, we show that SOCE controls epidermal proliferation. We first find that SOCE amplitude and epidermal proliferation are dramatically decreased in human atrophic skin. Second we show that SOCE inhibition, with BTP2 or in vivo silencing of Orai1, decreased epidermal thickness and proliferation in mice. Furthermore, the pro-proliferative effect of heparin-binding epidermal growth factor relies on Ca2+ signals promoted by SOCE in keratinocytes. Importantly, keratinocytes proliferation correlated linearly with SOCE amplitude in vitro and Orai1 expression in vivo. Finally, we show that the topical application of the SOCE activators (cyclopyazonic acid and benzohydroquinone) induced epidermal hyperplasia and revert steroid-induced skin atrophy in mice.

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Comparative study of various growth factors and cytokines on type I collagen and hyaluronan production in human dermal fibroblasts

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Dermal fibrobast is a primary cell type responsible for synthesis and remodeling of extracellular matrix in human skin. Type I collagen and hyaluronan are main components that have roles in skin fibrosis, wound healing, tissue remodeling as well as skin aging. Several studies have reported cytokine-dependent changes of collagen expression or hyaluronan production; however, the cytokines' effect was controversial in human dermal fibroblasts. In order to clarify the role of various growth factors, cytokines or chemokines in dermal connective tissue, we assessed the effects of 33 human recombinants on the production of interstitial type I collagen and hyaluronan from dermal fibroblasts. PDGF-AA, PDGF-BB, EGF, TGF-β1, MCP-1, IP-10, IL-1α, IL-1β, and IL-15 were effective on both type I collagen and hyaluronan production in human dermal fibroblasts, as compared with no stimulated control. On the other hand, IL-10 and IFN-α caused a significant decrease in type I collagen production, and IL-8 and GM-CSF caused a slight decrease in hyaluronan production compared with no cytokine-treated control. PDGF-AA is the most potent stimulator of type I collagen (2.5-fold increase) and hyaluronan production (9.0-fold increase) in this study. Interestingly, some chemokines, such as MCP-1 (CCL2), RANTES (CCL5), eotaxin-2 (CCL24), IP-10 (CXCL10), or fractalkine (CX3CL1) significantly induced the type I collagen or hyaluronan production. In this regard, our results suggest the roles of various growth factors and cytokines on the regulation of two main extracellular matrix components in human dermal skin which probably function as key factors in skin remodeling and skin aging.

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Disassembly of desmosomes by an E-cadherin function-blocking antibody

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Desmosomes and adherens junctions are two types of intercellular junctions that mediate keratinocyte cell cohesion. Previous studies have shown that desmosome assembly requires classical cadherin–mediated adhesion. While blockage or ablation of E-cadherin delays the assembly of desmosomes, blockage or ablation of both E- and P-cadherin prevents desmosome formation. In this study we investigate whether classical cadherin is required for maintenance of desmosome stability. Human keratinocytes were cultured in low calcium medium to confluence and then switched to high calcium medium to induce the assembly of intercellular junctions. Following 16-18 hours calcium switch, cells were treated overnight with a commercially available E-cadherin neutralizing monoclonal antibody. Immunofluorescence staining of treated cells reveals loss of cell border localization of components of the adherens junctions as well as the desmosomes. Phalloidin staining shows disruption and aggregation of the actin cytoskeletal network. Western blot analysis reveals cleavages of E-cadherin and a reduced level of desmosomal cadherins. Interestingly, stabilization of the actin cytoskeleton by jasplakinolide is able to block the effect of the E-cadherin antibody on desmosome stability. We also evaluated the effect of the E-cadherin antibody on desmosome assembly by adding the antibody to cultured cells at the time of calcium switch. The results show that the E-cadherin antibody is more effective in disrupting the already formed desmosomes than impairing de novo formation of the desmosomes. Taken together, these data suggest that classical cadherin is not only required for desmosome formation, but also for desmosome maintenance. The mechanism of desmosome disassembly by the E-cadherin blocking antibody is likely through perturbing the integrity of the actin cytoskeleton.

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Surface plasmon resonance biosensor detects the altered patterns of signals in the fibroblasts expressing oncogenic H-Ras

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Surface plasmon resonance (SPR) biosensor detects intracellular signaling events as a change of the angle of resonance (AR). We previously reported that the activation of epidermal growth factor receptor (EGFR) on keratinocytes causes a unique triphasic change of AR. We also reported that the activation of EGFR of non-tumorigenic cells (e.g. HaCaT cells) showed a full triphasic change of AR, whereas five out of the six carcinoma cell lines showed mono- or biphasic changes of AR, indicating that SPR biosensor could be applied to the functional diagnosis of cancers. In this study, we hypothesized that SPR biosensor could distinguish between non-tumorigenic cells and tumorigenic cells by the pattern of AR change. To test this hypothesis, we genetically introduced oncogenic human H-Ras (H-Ras-G12V) into the non-tumorigenic fibroblasts (MEF/3T3 tet-off fibroblasts) and studied the effect of the oncogenic transformation on the pattern of SPR signals induced by EGF. The overexpression of wild type human H-Ras (H-Ras-WT) and H-Ras-G12V both induced the similar morphological transformation of cells. SPR analysis revealed that the mock-transfected fibroblasts showed a full triphasic change of AR in response to EGF stimulation. In contrast, H-Ras-G12V-transformed fibroblasts showed a monophasic change of AR, whereas H-Ras-WT-transformed fibroblasts showed a bi-/triphasic change of AR. Moreover, Western blot analysis revealed that H-Ras-G12V transformed fibroblasts showed altered pattern of activation of Erk and Akt in response to EGF when compared with the mock-transfected fibroblasts. These results indicated that the altered pattern of AR change observed in tumorigenic, transformed cell lines could be due to the alteration of signaling pathways mediated by oncogenes.

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Essential role of collagen XVII in basement membrane formation and keratinocyte migration

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Collagen XVII (COL17) is a hemidesmosomal component in basal keratinocytes (KCs). The main role of COL17 is to maintain stable adhesion between the epidermis and dermis, and genetic dysfunction of COL17 results in the blistering disease junctional epidermolysis bullosa (JEB). Interestingly, JEB patients with homozygous c.3908 G>A (p.R1303Q) mutations in COL17A1 show distinct clinical and histological features, including photosensitivity, poikiloderma and basement membrane duplication, all of which are characteristic findings of Kindler syndrome, a subtype of EB associated with focal contact dysfunction. These findings suggest that COL17 plays unknown but pivotal roles; however, the pathomechanisms remain unknown. To address this, we isolated and investigated primary KCs with the R1303Q mutation. The R1303Q KCs showed normal adhesion and expression of focal contact was not solely affected, but their motility was significantly reduced. Notably, the R1303Q KCs show numerous cell-membrane-bound migration tracks containing COL17. In addition, deposition of laminin 332 in the extracellular matrix (ECM) was increased. These findings indicate that dissociation of the cell membrane from the ECM was hampered. In light of this, we assessed the presence of COL17 in migration tracks. Transfection of GFP-conjugated COL17 into COL17-null KCs revealed COL17 to be expressed not only in hemidesmosomes but also in migration tracks. Finally, we found C-terminal cleavage of the mutant COL17 to be resistant in the migrating KCs, which may explain why the mutant KCs show reduced motility and disorganized basement membrane. In summary, this study suggests that cleavage of COL17 is essential for correct migration and organization of basement membrane in basal keratinocytes.

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Upregulation of periostin by histamine via ERK1/2 pathway is essential for histamine-induced collagen production in dermal fibroblast

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Backgroud: We previously demonstrated that histamine may play a critical role in the synthesis of extracellular matrix in skin. However, the mechanism in it is still unclear. Periostin, a novel matricellular protein, has been reported to be involved in tissue repair and remodeling. Here, we investigated whether periostin was involved in histamine-induced collagen production. **Methods:** Dermal fibroblasts derived from wild-type (WT) or periostin-knockout (PN-/-) mice were cultured and stimulated with histamine for 12 and 48 h. Histamine-induced gene production was evaluated by real-time quantitative PCR, western blotting analysis and ELISA assay. H1, H2, H4 receptor antagonists and H1 receptor knockout mice (H1R-/-) mice were employed to identify the type of histamine receptor. To investigate the key signal transducer for transactivating periostin gene in downstream of H1 receptor, phospho-kinase assay was performed. **Results:** Histamine up-regulated collagen gene expression in WT fibroblasts at the late phase (48 hrs), but not at the early phase (12 hrs). In contrast, histamine did not affect collagen expression in PN-/- fibroblasts. Subsequent real-time PCR analysis, western blot analysis, and kinase array revealed that histamine increased expression of periostin *via* activating extracellular signal-regulated kinase (ERK) 1/2 through H1 receptor mediated pathway. Periostin induction was also inhibited by either H1 antagonist or ERK1/2 inhibitor *in vitro*, and also attenuated in H1R-/- mice. **Conclusion:** These findings revealed that histamine mediates periostin induction and collagen production through H1 receptor-mediated ERK1/2 activation pathway. Histamine might be a therapeutic target for refractory allergic diseases accompanied by tissue remodeling.

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An *in vitro* method for identifying agents with lipofilling-like activity: Parallel assessment of lipid accumulation, toxicity and gene expression

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An in vitro assay was developed to facilitate the identification and characterization of agents demonstrating lipofilling-like activity. The method involves exposing replicate cultures of primary human pre-adipocytes to various concentrations of test agents during cell differentiation. The cultures are then divided into groups for analysis of lipid accumulation using AdipoRed, cell viability using Cell Titer Blue, and collection of RNA for gene expression analysis. Agents for which enhanced lipid accumulation is observed relative to vehicle controls without adverse effects on cell viability are flagged for gene expression analysis to characterize the metabolic pathways affected. The effect of three materials purported to enhance lipid accumulation in adipocytes was evaluated and compared to a positive control represented by a PPAR-gamma agonist. Two of the test agents were found to promote lipid accumulation in differentiating pre-adipocytes as evidenced by AdipoRed signals that were approximately 3.5x and 8x of the corresponding vehicle controls, respectively, with no obvious toxicity. In contrast, the third agent failed to enhance lipid accumulation relative to the corresponding vehicle control. Data obtained from a subsequent test of the method involving the same 3 agents along with 12 additional test agents revealed significant inter-assay variability that is likely related to the use of different batches of primary human pre-adipocytes in each assay run. Despite the need for further assay optimization, the method described here represents an important first step towards developing a reliable in vitro method for identifying and characterizing compounds with lipofilling-like activity for further study.

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The dynein light chain Tctex-1 is a novel interacting protein of desmoglein 1 that regulates epidermal morphogenesis

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Desmoglein-1 (Dsg1) is a desmosomal cadherin that is first expressed as keratinocytes transit out of the basal layer and becomes concentrated in the upper cell layers of the epidermis. Dsg1 is not only required for epidermal integrity, but also coordinates EGFR/MAPK signaling events to promote differentiation, and supports cellular shape changes during stratification. However, how Dsg1 regulates epidermal morphology is not yet understood. Using a modified yeast two-hybrid screen, we identified the dynein light chain, Tctex-1, as a novel binding partner of Dsg1 that interacts with the last 140 amino acids of the cytoplasmic desmoglein unique region. The interaction was confirmed by co-immunoprecipitation of endogenous Tctex-1 with Dsg1-Flag and Dsg1-GST. Dsg1-Tctex-1 association was also observed in the apical cell layers of human foreskin using a proximity ligation assay. Tctex-1 was originally characterized as a cargo adaptor for dynein motor transport, but recent data suggest its additional role in coupling microtubule and actin dynamics. Hence, we hypothesized that Dsg1-Tctex-1 interactions can orchestrate cytoskeleton organization events in keratinocytes during epidermal stratification. Supporting this idea, knockdown of Tctex-1 or Dsg1 led to reorganization of the actin cytoskeleton and alterations of cell shape in both submerged and 3-dimensional organotypic epidermis cultures (rafts). Additionally, silencing Tctex-1 or overexpression of a Dsg1 mutant lacking the domains involved in Tctex-interaction induced defective tissue architecture, including the appearance of basal-like rounded cells in the superficial granular layers of the rafts. Interestingly, knockdown of Tctex-1 did not affect the expression of early differentiation markers, thus raising the possibility that Dsg1-Tctex-1 binding may govern a distinct morphogenesis pathway independent of Dsg1-mediated differentiation.

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Low-coherent quantitative phase microscope reveals a novel therapeutic target of H1 antihistamines

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Vascular hyperpermeability plays a crucial role in promoting common skin diseases such as urticaria. However, it remains unclear how endothelial cell adhesion is altered during the development of disease. To address the exact mechanism, we investigated the biological change of endothelial cell adhesion using a novel low-coherent quantitative phase microscope that measures the three-dimensional kinetics of cell surface on a nanometer scale. Cultured dermal human microvascular endothelial cells (EC) were initially stimulated with histamine and subjected to the microscopic analysis up to thirty minutes. This real-time quantitative assessment revealed that EC increase their height by two differential time points. The first peak was found at very beginning with a marked change of cell body, corresponding to the remodeling of cytoskeleton without impairment of cell adhesion. Thereafter, the second peak of increase in cell height happened with the loss of EC cell adhesion in response to histamine. Therefore, we next investigated the biochemical cleavage of VE-cadherin, a key cell adhesion molecule that is specifically expressed by cultured EC. Western blot analysis from cell lysates and conditioned media revealed that VE-cadherin undergoes ectodomain shedding in response to histamine, leading to the disruption of EC junctions and the induction of vascular hyperpermeability. Furthermore, siRNA technique showed that ADAM10 or ADAM17 contributes to the ectodomain shedding of VE-cadherin in response to histamine. Importantly, H1 antihistamines such as levocetirizine efficiently inhibited the ectodomain shedding of VE-cadherin and maintained the cell adhesion even in the presence of histamine. These results indicate, for the first time, that the biochemical cleavage of VE-cadherin plays an essential role in the pathogenesis of urticaria, and that the impairment of endothelial cell adhesion may be a new therapeutic target for the prevention of disease that is associated with vascular hyperpermeability.

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Cyclic epithelial fibroblast growth factor 18 signaling regulates telogen phase of hair cycle

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Cyclic growth of hair follicles through growth (anagen), regression (catagen), and resting (telogen) phases is closely correlated with stem cell activation and quiescence. Although the signaling molecules involved in the anagen and anagen-catagen transition have been extensively studied, the signaling that controls telogen has not been fully understood. We show that fibroblast growth factor (Fgf)-18 is expressed in a hair stem cell niche throughout telogen, and that it regulates the hair cycle through the nongrowth phases. When Fgf18 gene is conditionally knocked out in keratin 5-positive epithelial cells in mice, telogen becomes very short, giving rise to a strikingly rapid succession of hair cycles. In wild-type mice, hair follicle growth during anagen is strongly suppressed by local delivery of FGF18 protein. Formation of hair follicle-like structures in vitro is also suppressed by FGF18. Our results demonstrate that epithelial FGF18 signaling and its reduction in the milieu of hair stem cells are crucial for the maintenance of resting and growth phase, respectively.

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Topical delivery of stem cell derived growth factors by microneedle fractional radiofrequency activates fibroblasts in aged human skin *in vivo*

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The use of growth factors in skin rejuvenation is emerging as a novel anti-aging treatment. However, there have been no report whether stem cell derived growth factors could reverse cellular senescence of fibroblasts, which have vital roles for maintaining dermal extracellular matrix. In previous study, we demonstrated that microneedle fractional radiofrequency (RF) enhanced the topical delivery of growth factors with high molecular weight. Here, we report that age-related alterations of fibroblasts are reversed by topical delivery of stem cell derived growth factors by fractional RF. In this study, 10 healthy volunteers (70-80 years old) were included for split-face comparative study. One side of each subject's face was treated with fractional RF alone, and the other side was treated with fractional RF and stem cell derived growth factors. Punch biopsies (4mm) were obtained at baseline and after 4 weeks. We performed immunostaining for type 1 procollagen, HSP 47 and Ki67. We also performed fibroblast cultures and detected SA-β-gal activity, αSMA, TGF-β expression and functional capacities. As a result, fractional RF and stem cell derived growth factors significantly increased the expression of type 1 procollagen, HSP 47 and Ki67.($p<0.05$) Fibroblast cultures 4 weeks after treatment showed decreased percentages of SA-β-gal activity positive cells than fibroblast cultures at baseline. ($p<0.05$) Fibroblast cultures 4 weeks after treatment also showed higher expression of TGF-β and higher myofibroblast differentiation under TGF-β activation than fibroblast cultures at baseline. In conclusion, topical delivery of stem cell derived growth factors by fractional RF restored age-related changes of fibroblasts. Thus, this treatment can effectively applied to improve skin aging or wound healing.

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The impaired response of dermal fibroblasts to anti-fibrotic effect of adiponectin may contribute to the development of skin sclerosis in patients with systemic sclerosis

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Systemic sclerosis (SSc) is a multisystem autoimmune disease characterized by initial vascular injuries and resultant fibrosis of skin and certain internal organs. Although the pathogenesis of SSc still remains unknown, adipocytokines, including adiponectin, have been potentially implicated in the developmental process of fibrosis, vasculopathy, and/or aberrant immune responses in this disease. Importantly, serum adiponectin levels and adiponectin mRNA levels in the lesional skin are markedly decreased in the early stage of diffuse cutaneous SSc, suggesting the possible contribution of adiponectin to the fibrotic process of this disease subtype. We herein investigated the role of adiponectin in dermal fibrotic process of SSc using skin samples from SSc and healthy control subjects and Adipo$^{-/-}$ mice applied with bleomycin-induced dermal fibrosis model. In normal fibroblasts, adiponectin suppressed the TGF-β1-induced expression of COL1A2 mRNA in a dose-dependent manner, while failed to affect the basal COL1A2 mRNA levels in SSc fibroblasts. Of note, mRNA levels of AdipoRI and AdipoRII genes were significantly lower in SSc fibroblasts than in normal fibroblasts ($p<0.01$, respectively). In a series of studies using animal models, bleomycin-induced thickening of dorsal skin was 1.5 times thicker in Adipo$^{-/-}$ mice than in wild type mice ($p<0.01$). In addition, the Col1a1 and Col1a2 mRNA levels and the number of myofibroblasts in lesional skin were significantly increased in Adipo$^{-/-}$ mice compared with wild type mice. Taken together, these results suggest that anti-fibrotic effect of adiponectin regulates the fibrotic process *in vivo* and the impaired response to adiponectin of SSc fibroblasts due to the decreased expression of its receptors potentially contributes to the development of skin sclerosis in this disease.

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Role of endothelin-1/endothelin receptor signaling in fibrosis and calcification in nephrogenic systemic fibrosis

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Nephrogenic systemic fibrosis (NSF) is an acquired disorder characterized by systemic fibrosis and abnormal calcification in patients with severe renal dysfunction. Growing evidence supports the hypothesis that gadolinium (Gd)-containing contrast agents used for magnetic resonance imaging trigger the development of NSF. However, the causative role of Gd and the mechanism of Gd-induced fibrosis and calcification in NSF are unknown. Recently, it has been known that endothelin-1 (ET-1)/ET receptor (ETR) signaling regulates fibrosis and calcification, therefore, we hypothesized that ET-1/ETR signaling plays a role in Gd-induced fibrosis and calcification in NSF. First, we demonstrated that Gd enhanced proliferation and osteogenic differentiation of human adipose tissue derived mesenchymal stem cells (hMSCs) in vitro. Next, we examined the expression of ET-1 and ETR-A in hMSCs using proliferation or calcification assay. ET-1 and ETR-A expression in hMSCs treated with Gd were elevated. To examine the role of ET-1/ETR signaling in Gd-induced proliferation and calcification of hMSCs, we assessed the effect of ET-1/ETR signaling inhibitor, bosentan, on the proliferation or osteogenic differentiation of hMSCs. Bosentan inhibited Gd-induced proliferation and osteogenic differentiation of hMSCs. In addition, bosentan inhibited Gd-induced phosphorylation of ERK and Akt in hMSCs. Finally, we examined plasma ET-1 level in the patient of NSF treated in our department. Plasma ET-1 level of the patient was significantly higher than that of normal and systemic sclerosis patients. In immunofluorescence staining, the expression of ETR-A in dermal fibrosis and calcification lesions in NSF was increased. We conclude that Gd induces proliferation and osteogenic differentiation of hMSCs via enhancement of ET-1/ETR signaling. Our results contribute to understand the pathogenesis of NSF, and suggest that bosentan can be a choice of treatments on fibrosis and abnormal calcification in NSF.

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Notch signaling attenuates epidermal growth factor receptor activation by induction of receptor protein tyrosine phosphatase-kappa in primary human keratinocytes

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Receptor protein tyrosine phosphatase-kappa (RPTP-κ) specifically and directly dephosphorylates epidermal growth factor receptor (EGFR), thereby down-regulating EGFR signaling and function. Expression of RPTP-κ in primary human keratinocytes (KC) is increased by KC-KC contact. Notch is a cell surface receptor that is activated by binding of ligand that is displayed on the surface of adjacent cells. Once activated, Notch is proteolytically processed by γ-secretase and translocates to the nucleus, where it regulates transcription of target genes. We hypothesized that Notch pathway mediates KC contact-induced RPTP-κ expression and consequent suppression of EGFR function. KC contact up-regulated both Notch target gene Hes-1 (3-fold, $n=6$, $p<0.05$) and RPTP-κ (3.5-fold, $n=3$, $p<0.05$). Suppression of canonical Notch signaling pathway by γ-secretase inhibitors substantially inhibited cell contact-dependent increase of Hes-1 mRNA (80%, $n=3$, $p<0.05$) and RPTP-κ (90%, $n=3$, $p<0.05$) expression. Furthermore, treatment of primary human KC with Notch ligand Jagged-1 peptide induced Hes-1 (2-fold, $n=3$, $p<0.05$) and RPTP-κ (5-fold, $n=3$, $p<0.05$) mRNA. Transforming growth factor-β (TGF-β) pathway induces RPTP-κ in KC and cross-talks with Notch signaling pathway via physical interaction between Notch intracellular domain and Smad3. Suppression of Notch signaling by either lenti-viral shRNA-mediated knockdown of Notch1 or by γ-secretase inhibitors significantly reduced TGF-β-induced RPTP-κ mRNA expression. In contrast, induction of RPTP-κ mRNA by TGF-β in primary human KC was further enhanced by Jagged-1 peptide. The above data provide a mechanism for inhibition of EGFR signaling by KC contact. Notch can function as either an oncogene or a tumor suppressor depending on the cellular context. In KC, Notch may act as a tumor suppressor, via up-regulation of RPTP-κ to inhibit EGFR activity.

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The cell-cell adhesion protein Desmoglein 1 and the histone deacetylase inhibitor Trichostatin A aid in recovery of epidermal differentiation after ultraviolet light exposure

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One function of the epidermis is to protect against damaging environmental agents such as ultraviolet (UV) light. While myriad studies have examined DNA damage/repair in UV-exposed keratinocytes, almost nothing is known about the molecular mechanisms governing repair of the UV-damaged epidermal structure. This study aimed to understand the impact of different UV dosages and wavelengths on epidermal differentiation and to uncover novel molecular mechanisms governing structural repair of UV-damaged skin. UVB (290-320 nm wavelengths) exposure reduced the expression of differentiation-associated proteins Desmoglein 1 (Dsg1), Desmocollin 1 (Dsc1), and Keratins 1 and 10 (K1/10) in a dose-dependent manner in normal human epidermal keratinocytes (NHEKs) while UVA (320-400 nm wavelengths) exposure did not. The impacts of UVB exposure on expression of these proteins were more pronounced when cells were UV-exposed prior to, rather than after, induction of differentiation by increasing calcium concentration. Since the desmosomal cadherin protein Dsg1 was shown to help regulate epidermal differentiation in addition to its role in cell-cell adhesion, it was hypothesized that Dsg1 may help restore differentiation and proper epidermal architecture after UV damage. Compared to controls, knocking down Dsg1 via shRNA resulted in abnormal epidermal architecture days after organotypic skin models were UVB-exposed and a further reduction of Dsc1, K1, and K10 expression in either UVA- or UVB-exposed NHEKs. Induction of differentiation was delayed in both UVB-exposed NHEKs and in NHEKs depleted of Dsg1. Over-expression of Dsg1 or treatment of cells with Trichostatin A, a drug shown to increase cadherin expression, resulted in rapid recovery of Dsg1, Dsc1, K1, and K10 expression in UV-exposed NHEKS. This work has clinical implications for reversing photoaging, for skin cancer prevention, and for improving phototherapy for diseases such as psoriasis and eczema.

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Down-regulation of type I collagen expression in silibinin-treated human skin fibroblasts through blocking smad2/3-dependent signaling pathway: Its potential therapeutic use in the chemoprevention of keloid

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Inhibition of the Smad2/3 pathway is the key step involved in the down-regulation of type I collagen synthesis, eventually preventing keloid formation in the tissue. In this study, we investigated the effect of silibinin on the proliferation, expressions of type I collagen, matrix metalloproteinase-1, and Smad2/3 of human skin fibroblasts (HSF) and keloid fibroblasts. Our data showed that the proliferation rates of fibroblasts were not markedly decreased in a dose and time-dependent manner of silibinin. Even though silibinin did not exert any cytotoxic effect on HSF and keloid fibroblasts, the expression of type I collagen was markedly decreased in a dose and time dependent manner in silibinin-treated HSF and keloid fibroblasts. Consistent with this finding, decreased promoter activity of type I collagen was observed in HSF and keloid fibroblasts by silibinin treatment. MMP-1 and MMP-2 expression levels were increased in silibinin-treated both cells. Moreover, silibinin-induced down-regulation of type I collagen was associated with the inhibition of Smad2/3 activation in TGF-beta1-treated HSF. We further demonstrated that silibinin attenuated the translocation of Smad2/3 into the nucleus in TGF-beta1-treated HSF or keloid fibroblasts. Taken together, our data indicated that silibinin has the potential to protect the fibrotic change of skin through inducing the down-regulation of type I collagen expression, which is partly mediated by inhibition of the smad2/3-dependent signaling pathway in fibroblast cells.

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NFAT and NFkB activation and keratinocyte migration post-wounding is dependent on extracellular calcium concentration and SOCE

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Cutaneous wound healing is a major clinical problem resulting in significant burden to patients' quality of life and health care resources. Injury to skin requires immediate repair to avoid chronic perturbation of the external barrier, infection and hypertrophic scarring. Intracellular calcium ($[Ca2+]_i$) signalling and activation of transcription factors NFAT/NFκB have been shown to regulate cell proliferation and differentiation. However, the role of these transcription factors in regulating keratinocyte migration and wound healing remains unclear. To address this, dual-luciferase reporter assays were used to investigate NFAT/NFκB activation 24 h post-scratch wounding and keratinocyte migration was assessed following 2D scratch wounding by live-cell imaging every hour for 48 h using a Biostation. When the endoplasmic reticulum calcium store is depleted, calcium influx across the plasma membrane is activated resulting in a process known as store-operated calcium entry (SOCE). To examine the potential role of SOCE in wound closure, keratinocytes were treated with the SOCE inhibitor GSK-7975A. Wounding in 1.2mM $[Ca2+]_o$ resulted in significant increased NFAT and NFκB activation compared to 0.06mM $[Ca2+]_o$ ($p<0.001$), suggesting higher calcium external environment is necessary for activation of NFAT/NFκB transcription factors. Cell migration analysis demonstrated wound closure rate was also dependent on $[Ca2+]_o$. Thus, scratch-wounds made in 1.2mM $[Ca2+]_o$ closed significantly faster than those in 0.06mM $[Ca2+]_o$. At 24 h post-wounding in 1.2mM $[Ca2+]_o$ the wound area had decreased by 75.7% compared to 32.8% when wounding in 0.06mM $[Ca2+]_o$ ($p<0.001$). Inhibiting SOCE had no effect on the rate of wound closure but the co-ordinated pattern of wound closure was disrupted by SOCE inhibition. These data highlight the important role of calcium signalling in regulating NFAT/NFκB activation post-wounding and in promoting keratinocyte migration resulting in co-ordinated wound closure.

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Functional role for HSP27 in pemphigus acantholysis

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We first identified a role for p38 in the mechanism by which acantholysis proceeds in the human autoimmune blistering disease pemphigus vulgaris. In this initial biochemical screen for alterations in intracellular protein phosphorylation stimulated by incubation of cultured keratinocytes with purified PV IgG, we observed increased phosphorylation of the small heat shock protein HSP27. P38 phosphorylates MK2 which in turn phosphorylates HSP27. Subsequent studies in vitro and in vivo demonstrated that p38 inhibition blocked HSP27 phosphorylation, dsg3 endocytosis, actin reorganization, keratin retraction and blistering. Although these studies demonstrated a clear functional role for p38 in the mechanism of acantholysis, we have yet to demonstrate a role for the downstream component HSP27. This study was undertaken to explore the role of PV IgG mediated HSP27 phosphorylation in the mechanism of acantholysis. Cultured normal human keratinocytes were incubated with PV IgG in the presence and absence of MK2 inhibitor III and assayed for HSP27 phosphorylation, dsg3 endocytosis, and loss of adhesion. MK2 inhibitor III blocked PV IgG mediated (i) HSP27, but not p38, phosphorylation, (ii) dsg3 depletion, and (iii) loss of adhesion as measured by the dispase assay. These observations suggest a functional role for HSP27 phosphorylation in the mechanism by which acantholysis proceeds in pemphigus vulgaris.

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The N terminal domain of BPAG1e and the regulation of keratinocyte migration

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In stationary keratinocytes the plakin molecule BPAG1e (BP230) is a component of the cytoplasmic plaque of the hemidesmosome. BPAG1e binds the cytoplasmic tails of β4 integrin and BPAG2 and tethers keratin bundles to the cell surface. In contrast, in motile cells, BPAG1e localizes to the base of the leading lamellipodium. There it functions as an important mediator of signal transduction. It supports the activation of the small GTPase Rac and the actin severing protein cofilin which both regulate processive migration via their effects on lamellipodial dynamics. Indeed, keratinocytes exhibiting a loss in expression of BPAG1e do not move in a processive fashion, a motility defect which is rescued by forced expression of either constitutively active Rac or cofilin. To delineate further the domains of BPAG1e involved in determining skin cell motile behavior, we expressed either the N- or the C- terminal domains (residues 1-665 and 1811-2649, respectively) of BPAG1e in wild type and BPAG1e knockdown human keratinocytes. The N- but not the C-terminal domain of BPAG1e localizes with β4 integrin. Moreover, expression of the N-terminal domain induces a reduction in lamellipodial stability and directed motility of wild type cells whereas the C-terminal domain has no impact on cell motility or lamellipodial dynamics. Neither the N- nor the C-terminal domain rescues the motility defects of BPAG1e knockdown cells. Rather, expression of the N-terminal domain exacerbates the aberrant migration of the knockdown cells by reducing lamellipodial stability. Taken together these data indicate the importance of the N-terminus of BPAG1e in mediating BPAG1e-cell surface protein interactions which appear necessary for C- and/or rod domain-mediated signaling.

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The effects of UVB radiation on elastic fiber components in mice

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Ultraviolet radiation induces profound changes in the architecture of the elastic fibers in the dermis. Elastic fibers comprises two distinct components: an amorphous core of cross-linked tropoelastin surrounded by a peripheral mantle of microfibrils which are mainly composed of fibrillin-1 and -2. In the past 10 years, it has been proved that fibulin-5 and latent TGF-β binding proteins (LTBP) −4 play an important role in the formation of elastic fibers. However, the influence of ultraviolet radiation on the molecule is not well understood, despite its important contributions to elastogenesis. Therefore, we examined the effects of ultraviolet B (UVB) irradiation on elastic fibers and elastic fiber components in mice. Female HR-1 hairless mice (8 weeks of age) were exposed to UVB radiation three times a week, with a total amount of UVB of 3 J/cm^2. Normal elastic fibers in the dermis decreased, and the fiber-shaped structures disappeared and fused together. Immunofluorecent staining revealed that the immunoreactivities for elastin and LTBP-4 were reduced in the dermis. RT-PCR showed decreased expression of elastin (RT-PCR: 0.381 fold $P \leqq 0.005$) and LTBP-4 (RT-PCR:0.355 fold $P \leqq 0.005$), increased expression of fibulin-5 (RT-PCR: 1.26 fold $P \leqq 0.05$), and no change in fibrillin-1 expression after UVB radiation. These results suggest that LTBP-4 contributes to the reduced generation of elastic fibers by UVB radiation.

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Norepinephrine-induced IL-6 regulates fibrosis in systemic sclerosis

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Raynaud phenomenon is commonly observed in patients of systemic sclerosis (SSc) and characterized by episodic vasospasm and ischemia of extremities in response to cold or emotional stress. It has been recogized that cold- or stress-induced norepinephrine (NE) stimulates adrenoreceptor (AR) on pericytes or vascular smooth muscle cells, resulting in vasoconstriction. However, the roles of NE in fibrosis of SSc are not well understood. The aim of this study was to elucidate the role of NE in fibrosis in SSc. IL-6 is elevated in the serum of patients with early SSc, and is correlated with the extent of skin fibrosis. At first, we examined the effect of NE on the production of IL-6 in normal and SSc fibroblasts. Protein and mRNA levels of IL-6 expression in normal and SSc fibroblasts was increased by NE in a dose-dependent manner by ELISA and real-time PCR. In addition, NE-induced IL-6 production in SSc fibroblasts was significantly higher than that in normal fibroblasts. There is no change in ARβ expression in normal and SSc fibroblasts treated with or without NE. To confirm whether NE induced IL-6 production via ARβ on fibroblasts, we assessed the effect of ARβ blocker, proplanolol, on NE-induced IL-6 production. Proplanolol inhibited NE-induced IL-6 production in normal and SSc fibroblasts. In addition, proplanolol inhibited NE-induced phosphorylation of ERK in normal and SSc fibroblasts. Next, we assessed the effect of the treatment with NE and endothelin-1 (ET-1), which are supposed to contribute to the pathogenesis of fibrosis in SSc. Treatment with a combination of both NE and ET-1 resulted in a synergistic increase in production of IL-6 in SSc fibroblasts. Finally, we identified that NE enhanced proliferation of SSc fibroblasts compared with that of normal fibroblasts. We conclude that NE induces IL-6 production, phosphorylation of ERK and proliferation of fibroblasts in SSc via ARβ. Our results indicate that avoidance of cold exposure or emotional stress may attribute to the suppression of fibrosis as well as Raynaud phenomenon in SSc.

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New insights in the biochemical properties and possible function of the human OATP5A1 transporter

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Organic anion transporting polypeptides (OATP/SLCO) have been identified to mediate the uptake of a broad range of mainly amphipathic molecules, thereby playing a critical role in the bioavailability of drugs. The human member OATP5A1 was found to be expressed in the epithelium of many cancerous and non-cancerous tissues throughout the body but protein characterization and function have not yet been determined. Therefore, the gene expression of this transporter was analyzed in different human cell types and OATP5A1 was biochemically characterized using genetically modified Hela cells and Xenopus laevis oocytes. Functional studies should help to reveal the biological role of OATP5A1. Data showed that the SLCO5A1 expression is increased in melanoma cell lines and correlates with the differentiation status of primary macrophages and dendritic cells. A core- and complex- N-glycosylated protein monomer could be localized in the cytosol and on the cell membrane, respectively. Inducible expression of OATP5A1 in Hela cells led to an inhibitory effect on the cell proliferation. Gene expression profiling with these cells identified genes that are mainly involved in system- and anatomical structure development and cell adhesion. Surprisingly, known substrates of OATPs such as oestrone-3-sulfate, prostaglandin E2 and arachidonic acid were not transported by OATP5A1. A single nucleotide polymorphism of amino acid 33 (L→F) revealed no differences regarding OATP5A1 protein expression and function. In conclusion, we provide evidence that OATP5A1 is involved in biological processes that require the reorganization of the cell shape, such as differentiation and migration. However, further studies are needed to elucidate the substrate specificity and specific function of this novel transmembrane protein.

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Amphiregulin increases sebaceous gland size and tail epidermal thickness but not basal or imiquimod-induced skin inflammation

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Multiple EGFR ligands are overexpressed in psoriasis, and antibody blockade of human amphiregulin (hAREG) improves psoriasis in skin xenografts (AJP 166:1009). To further examine the role of AREG in inflammatory epidermal hyperplasia, we overexpressed full-length hAREG in FVB/N mice using a bovine K5 promoter. Two founder lines were generated, both of which produced viable TG offspring at Mendelian ratios and expressed high levels of hAREG mRNA and protein. Both lines yielded undersized TG offspring during the neonatal period, with delayed development of the first hair pelage that progressed to tousled, greasy fur as animals aged. Using ImageJ for quantification of H&E-stained sections, both K5-hAREG lines manifested a marked increase in sebaceous gland size (0.0047 ± 0.0001 vs 0.0024 ± 0.0001 mm2/gland, mean ± SEM, n = 8 TG, 4 WT, p = 2 E-39), with no increase in gland density. Similar phenotypes were seen in mice expressing the closely related EGFR ligand epigen under control of the CMV promoter (CMV-hEPGN mice, JID 130:623). K5-hAREG mice showed no clinical or microscopic signs of inflammation, leading us to ask whether hAREG might modulate the inflammatory response to topical imiquimod (IMQ), following established protocols (JI 182:5836). Like CMV-hEPGN mice, K5-hAREG mice had thicker tail epidermis, which was similar in VEH and IMQ mice (VEH 1.55-fold, 54.8±1.1 vs. 35.3±1.1 um, p=8.2E-24; IMQ 1.29-fold, 44.5±0.7 vs. 34.6±0.8 um, p=1.3E-14, n = 4-5). While IMQ markedly increased skin inflammation and epidermal thickness of back skin in both WT and AREG mice (thickness up 2.2-fold and 1.7-fold, p<5E-27 and p<5E-60 respectively), there was no significant difference between them. Finally, IMQ did not increase skin inflammation or epidermal thickness in tail skin, possibly due to decreased drug delivery. These results suggest demonstrate similar effects of hAREG and hEPGN in altering sebaceous gland and tail skin development, but provide little support for the pro-inflammatory role of hAREG reported in other TG models.

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Caveolin-1 has an inverse correlation with collagen I in the skin: Caveolin-1 inhibitors can be novel anti-aging agents in the skin

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Aged skin is characterized by decreased thickness due to loss of dermal collagen (COL). Besides the structural role, caveolins play a gate-keeping role to modulate various signaling molecules localized to the membrane component. Concerning COL metabolism, a few studies have reported the role of caveolin-1 (Cav-1) in the skin or dermal fibroblasts. This study was aimed to evaluate the role of Cav-1 in relation with COL expression in the skin. For studies, age-dependent changes in mRNA and protein levels of Cav-1 and COL I were measured. Also, we tested whether methyl-β-cyclodextrin (MβCD) as a Cav-1 inhibit could modulate COL I level in human dermal fibroblasts (HDFs) or hairless mice. In results, there was an inverse correlation between COL I and Cav-1 levels in the chronologically aged skin of humans and mice in vivo and senescent HDFs in vitro, which was further confirmed by Cav-1 siRNA transfection and knock-out experiments. Treatment of HDFs with MβCD dose-dependently up-regulated COL I mRNA and protein, as it up-regulated mRNA levels of transforming growth factor (TGF)-β1, TGF-β receptor I (TβRI), and Smad2, indicating MβCD-mediated activation of TGF-β/TβRI/Smad2 pathway. In hairless mice, repetitive intra-dermal injection of 2.5% MβCD induced down-regulated Cav-1 and up-regulated COL I levels of mRNA and protein, leading to skin thickening. There were no adverse reactions in the skin and major internal organs in MβCD-injected mice. In conclusion, Cav-1 negatively modulates COL I expression in the skin and that intra-dermal injection of MβCD results in anti-aging effect via its anti-Cav-1 activity. Cav-1 inhibitors can be developed as novel anti-aging agents through their COL-stimulating activity in the skin.

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ANGPTL4 is a paracrine factor in tuberous sclerosis tumorigenesis with a potential role in lymphangiogenesis

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Skin hamartomas in tuberous sclerosis complex (TSC) contain fibroblasts with hyperactivation of mTOR signaling due to loss of TSC2 expression. To identify paracrine factors important in TSC tumorigenesis, we used transcriptomic analysis of cells incubated with or without the mTOR inhibitor rapamycin (20 nM) and under normoxic or hypoxic (1% O2) conditions, using TSC2-null fibroblasts from angiofibromas and paired TSC fibroblasts from normal-appearing skin from 4 patients. Unbiased hierarchical clustering identified three genes with angiogenic functions whose expression levels were greater in tumor than normal fibroblasts, increased by hypoxia, and negatively regulated by rapamycin: *VEGFA*, *GPI*, and *ANGPTL4*. Angiopoetin-like 4 was selected for further study since it is a novel factor implicated in tumorigenesis and angiogenesis in human cancer. *ANGPTL4* mRNA and protein were measured using real-time PCR and ELISA, respectively, in paired samples of skin tumor fibroblasts (angiofibroma, periungual fibroma, and forehead plaque) and patient normal fibroblasts from 18 subjects with TSC. *ANGPTL4* mRNA content in tumor fibroblasts was 4.2-fold that in patient normal fibroblasts (p<0.01), and ANGPTL4 protein released into the media by tumor fibroblasts over 24 hours was 2.1-fold more than patient normal fibroblasts (p<0.01). Levels of ANGPTL4 released by TSC2-null angiofibroma fibroblasts into media over 24 hours were increased by hypoxia and decreased by rapamycin (p<0.05). Since we had previously shown that TSC tumors contain more lymphatic vessels than normal skin, we tested the effect of recombinant ANGPTL4 on the proliferation of human dermal lymphatic endothelial cells (HDLECs) using BrdU incorporation, and observed that ANGPTL4 stimulated HDLEC proliferation (p<0.05). These studies identify ANGPTL4 as a paracrine factor in TSC tumorigenesis with a previously unrecognized role in lymphangiogenesis.

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Stabilization of integrin-linked kinase by the Hsp90-CHIP axis regulates cellular force generation, migration and the fibrotic response

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The extracellular matrix (ECM) provides structural support for tissues as well as actively guides tissue morphogenesis and homeostasis through regulating cell adhesion, migration, and growth factor activity. Therefore, tight regulation of ECM remodeling is a prerequisite for tissue function. Excessive and progressive accumulation of ECM results in tissue fibrosis leading to scarring and destruction of normal tissue architecture and function. Integrin-linked kinase (ILK) is an adaptor protein required to establish and maintain the connection between integrins and the actin cytoskeleton. This linkage is essential for generating force between the ECM and the cell during migration and matrix remodeling. The mechanisms by which ILK stability and turnover are regulated are unknown. Here we report that the E3 ligase CHIP - heat shock protein 90 (Hsp90)-axis regulates ILK turnover in fibroblasts. The chaperone Hsp90 stabilizes ILK and facilitates the interaction of ILK with α-parvin. When Hsp90 activity is blocked, ILK is ubiquitinated by CHIP and degraded by the proteasome. As ILK is essential for fibroblast migration and ECM deposition, chemical inhibition of Hsp90 impairs fibroblast migration and dramatically reduces the fibrotic response to bleomycin in mice. Together, our results uncover the molecular regulation of ILK stability and identify a potential therapeutic strategy targeting fibrotic diseases.

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The interaction of plectin with intermediate filaments is modulated by MAPK-interacting kinase 2 and PKA. Insights into the molecular basis of plectin-related human diseases

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Plectin is a versatile cytolinker, tethering the intermediate filament (IF) system to specific plasma membrane sites and organelles, thereby contributing to the maintenance of cytoarchitecture and cell resilience in tissues such as skin and skeletal muscle. Plectin-IF connections must be transitory regulated during remodeling of the cell shape. We have identified serine 4642 in the COOH extremity of plectin as an in vivo phosphosite. Phosphorylation of Ser 4642 disturbs plectin localization to IFs in various cell lines, whereas in the epidermis at junctional adhesion complexes, plectin is unphosphorylated at S4642. After fractionation of transfected cells, the COOH-terminal IF-binding domain of plectin, phosphorylated at S4642, was not found in the insoluble, IF-containing fraction in contrast to their corresponding unphosphorylated recombinants. These data and yeast two-hybrid assays indicated that phosphorylation of S4642 (pS4642) inhibits the binding of plectin to various types of IFs, including cytokeratins, desmin and neurofilaments. Treatment of HeLa cells with EGF, phorbol ester, sorbitol, 8-bromo-cyclic AMP or okadaic acid enhanced pS4642 levels. We identified two different kinases critically modulating the phosphorylation of S4642 plectin, the MAPK-interacting kinase 2 (MNK2), downstream ERK1/2, and PKA. Our study provides for the first time evidence for the key role of the plectin COOH extremity in the regulation of its linkage to IFs, identifies the implicated kinases and gives insights into the molecular basis of plectin-related human diseases, as deletion of the COOH extremity by distinct pathogenic mutations in plectin gene results in severe epidermolysis bullosa, myopathy and central nervous system manifestations.

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Increased dermal collagen deposition is a feature of chronic plaque psoriasis

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Psoriasis is a common immune-mediated inflammatory skin disease. Most research and concepts on the pathogenesis of psoriasis focus on the adaptive and innate immune responses with scant regard for changes in the dermal extracellular matrix (ECM). We were interested to assess the role of the ECM in psoriasis. Subjects (n=5; 4M, age range, 47-64 yr) with established chronic plaque psoriasis were recruited and 6mm biopsies were obtained from both involved (PP) and uninvolved (PN) skin; healthy volunteers (NN; n = 5; 3M, age range, 36-54 yr) were recruited as controls. Cryosections (6μm) were used for immunohistochemical identification of type I (pCI) and type III (pCIII) pro-collagen. Immunostaining was quantified on a semi-quantitative scale (0 = absence of staining; 4 = intense staining) and the total immunopositive area was calculated (Image J). Relative COL1 gene expression was assessed by real time qPCR (n=3 per group) and normalised to the housekeeping gene GAPDH. Semi-quantitative analysis of pCI showed a significant increase in the deposition of the amino pro-peptide in PP as compared to PN and NN skin (ANOVA; p<0.05). Similarly a significant increase in pCIII was seen in both PP and PN skin when compared to NN control (ANOVA; p<0.05). Histological evaluation of pCI deposition in PP skin showed a greater depth of staining into the papillary dermis from the dermal-epidermal junction as compared to PN and NN skin (ANOVA; p<0.05); quantification of pCIII identified a diffuse, uniform staining pattern throughout the dermis of PP and PN skin as compared to NN control (ANOVA; p<0.05). The relative gene expression of COL1 was increased in PP versus PN skin (paired Student's T-test; p<0.05). This study demonstrates an increase in both the production and deposition of pCI and pCIII in the dermis of PP skin as compared to the skin of healthy volunteers. Further work is needed to elucidate the mechanism underlying these changes in the dermal ECM in psoriasis and the role this might play in the pathophysiology of the disease.

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Keratinocyte motility is regulated by ganglioside GM3-induced suppression of insulin-like growth factor-1 signaling

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We have previously shown increased ganglioside GM3 in diabetic mouse skin and that depletion of GM3 synthase, which catalyzes GM3 synthesis, dramatically improves diabetic wound healing in diet-induced obese knockout mice. We hypothesized that GM3 similarly accumulates in human diabetic skin and that insulin-like growth factor-1 receptor (IGF-1R) activation is key to the increased keratinocyte motility with GM3 depletion. Indeed, GM3S expression, as shown by qRT-PCR, was increased by 4-5-fold in human diabetic vs. control foot skin (p<.001). GM3 biochemical supplementation or GM3 accumulation by treatment with spherical nucleic acids (SNAs) to block GM3 downstream metabolism: i) suppressed normal human keratinocyte (NHEK) migration in scratch assays (p<.01); ii) reduced cell polarization (p<.001); iii) virtually eliminated IGF-1R phosphorylation (p<.001); iv) increased cofilin phosphorylation with and without growth factor stimulation ((p<.001); and v) suppressed Rac1 activity by more than 75%. In contrast, SNA-induced suppression of GM3S expression or biochemical depletion of GM3: i) accelerated NHEK migration in scratch assays (p<.01); ii) increased the percentage of NHEKs with a single lamellipodium in standard (p<.05) and hyperglycemic (increased from 2.9% to 71% of cells, p<.001) medium; iii) increased IGF-1R phosphorylation up to 9-fold; iv) reduced cofilin phosphorylation by 2.3-fold; and v) increased Rac1 activity both without (p<.001) and with (p<.05) insulin or IGF-1 stimulation. Specific biochemical inhibition of IGF-1R or addition of GM3 reversed the stimulatory effect of GM3S SNAs on NHEK migration (p<.001). These data provide strong evidence that GM3 regulates keratinocyte polarization and migration by IGF-1R signaling and suppression of Rac1. These studies further implicate GM3 depletion as a new strategy for accelerating human wound healing.

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Differential modulation of ECM components and GFR by retinoic acid and a physiologically balanced GF formulation

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Overall appearance of skin is a combination of health, ethnicity, lifestyle and age, which determine skin color, texture, firmness and smoothness. Uneven color, rough, lax and wrinkled skin are associated with aged skin. Microscopically, aged skin has an atrophic epidermis, flattened dermal-epidermal junction, decreased number of dermal fibroblasts and diminished synthesis of extracellular matrix components and growth factors (GF). Treatment with topical formulations that contain a physiologically balanced and stable GF mixture has shown to improve skin appearance. Environmental conditions such as solar UV-light exposure strongly modulate skin appearance triggering premature aging or "photoaging". Retinoids are the gold standard for the treatment of photoaged skin. The goal of this study was to compare the effect of a physiologically balance GF formulation (PB-GFF) and generic retinoic acid (RA, 0.05%) on simulated solar (SS)-UV-light treated skin. In brief, EpiDermFT™ tissues were produced in the MatTek Corporation and subjected or not to SS-UV-light radiation (200 mJ/cm2, 23 min). Immediately after UV-exposure, tissues were treated with 25 microl of PB-GFF, RA or a combination of both and allowed to recover for 12 and 24 h. Total mRNA was isolated and expression of different genes were analyzed by qPCR. The results show that all treated groups have a significant increase in COL1A1 and COL3A1 expression in comparison with untreated tissues, although the kinetics and intensity of these increases were different. ELN and elastin-related proteins (LOXL1 and MFAP2) mRNA levels were also significantly induced (24 h after SS-UV-exposure). Changes in the mRNA expression of GF-receptors (FGFR, FLT1 and EGFR) were also observed. Non-physiologically balanced GF formulations did not trigger any changes in the genes previously mentioned. These data demonstrate that both RA and PB-GFF induce synthesis of key ECM components in EpiDermFT tissues, and therefore may be beneficial for preventing skin damage.

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Characterization of the molecular mechanisms and cellular phenotypes underlying inversa type of recessive dystrophic epidermolysis bullosa

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Type VII collagen (C7), the major component of anchoring fibrils (AF), consists of a central triple helical domain (TH) flanked by non-helical domains, NC1 and NC2. Mutations in the type VII collagen gene (COL7A1) cause dystrophic epidermolysis bullosa (DEB). Inversa recessive DEB (RDEB-I) is a rare form of RDEB associated with missense mutations within the TH. The prominence lesions in the mucosa and flexural areas of the skin in RDEB-I strengthens the hypothesis that the pathophysiology of RDEB-I is temperature dependent. However, the biological consequences of specific COL7A1 mutations leading to the unique RDEB-I phenotypes remain unclear. In this study, we screened 22 patients with RDEB and found that six patients affect with RDEB-I and have missense mutations G1907D, R2069C and G2719A. These six patients exhibit normal C7 expression at their basement membrane zone assessed by immunofluorescence. However, by transmission EM, all of these patients had either significantly reduced, complete absence or abnormal AF. We isolated keratinocytes and fibroblasts from these patients, characterized their cellular phenotypes under different temperatures and showed: (1) All of the mutations caused the intracellular accumulation of C7 when cells were grown at higher temperature but displayed normal secretion in cells grown at lower temperature. (2) All the RDEB-I mutations produced mutant C7s with reduced thermal stability, as demonstrated by increased sensitivity to protease digestion and reduced ability to form trimers at higher temperature. (3) All RDEB-I keratinocytes and fibroblasts exhibited abnormal cellular phenotypes including hypermotility and reduced matrix attachment at higher temperatures that were normalized at lower temperature. We conclude the effects of RDEB-I mutations on C7 secretion, folding, stability, and cellular motility are highly temperature dependent and correlate with the observed clinical phenotype.

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Decreasing deacetylated ganglioside GM3 prevents AKT3 expression and triggers cell apoptosis in metastatic melanomas

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GM3, the simplest ganglioside, regulates cell proliferation, migration and invasion by influencing cell signaling at the membrane level. Although the N-acetylated form of GM3 (c-GM3) is expressed in most normal cells, deacetylated GM3 (d-GM3) is mainly found in metastatic melanomas. We have found that decreases in d-GM3 by knocking down either GM3 synthase (GM3S, which catalyzes GM3 formation) or histone deacetylase 3 (HDAC3, which deacetylases GM3 to d-GM3) reduce melanoma cell proliferation and/or prevent growth of melanoma xenografts in mice. However, the mechanism of d-GM3 regulated melanoma metastasis is largely unknown. Increased expression of AKT3 is known to protect cells from apoptosis in more than 70% of metastatic melanomas. We hypothesized that d-GM3 promotes melanoma metastasis by increasing AKT3 expression and promoting AKT downstream signaling, thus preventing cell apoptosis. By qRT-PCR, immunoblotting, ELISA, WST and flow cytometry, we observed that in metastatic SKMEL-28 and A375M melanoma cells, stable knockdown of GM3S with its shRNA-lentivirus significantly reduced AKT3 mRNA and protein expression. The reduction in d-GM3 also decreased phosphorylation of proline-rich Akt substrate (pPRAS40), mTOR and sensitized melanoma cells to the apoptosis-inducing agent staurosporine. Conversely, overexpression of HDAC3 significantly increased AKT3 expression in poorly invasive A375P melanoma cells, increased A375P cell proliferation and migration, and rendered A375P cells insensitive to the induction of apoptosis. These data suggest that d-GM3 plays a critical role in metastatic melanoma survival by activating the AKT-mTOR survival pathway and provide an additional rationale for development of HDAC3- or d-GM3 specific inhibitors for treating metastatic melanoma.

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Expression of Drebrin, an actin binding protein, in basal cell carcinoma, trichoblastoma and trichoepithelioma

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Drebrin is an F-actin binding protein originally identified in brains of chick embryos. Four isoforms with a molecular mass of ~120kDa, drebrins E1, E2, A and sA, are known to play important roles in the cell migration, synaptogenesis and neural plasticity. Although drebrin had been thought to be specific for neuronal cells, it has recently been reported that drebrin is also expressed in non-neuronal cells. As for the skin cells, drebrin E2 has been shown to be enriched at adhering junctions in cultured primary keratinocytes and also be highly expressed in basal cell carcinomas (BCC). BCC and two benign neoplasms, trichoblastoma and trichoepithelioma, are all considered to derive from the same origin, follicular germinative cells. Because of this, it is sometimes difficult to distinguish BCC from trichoblastoma and trichoepithelioma. In this study, we prepared a specific polyclonal antibody for drebrin, and performed immunohistochemical staining of drebrin in BCCs, trichoblastomas and trichoepitheliomas, and examined whether drebrin could be a biomarker of BCC diagnosis. In Western blotting, drebrin was detected highly and moderately in the lysates from a squamous cell carcinoma cell line, DJM-1, and normal human epidermis, respectively. In immunofluorescence analyses, drebrin was accumulated at adherens junctions in DJM-1 cells and cell-cell junctional areas of human normal epidermis. Next we examined the expression patterns of drebrin in BCC, trichoblastoma, trichoepithelioma. In BCCs, intense drebrin expression was observed especially at tumor cell boundaries. In contrast, drebrin was stained only weakly in trichoblastoma and trichoepithelioma. These results indicate that drebrin might be involved in malignant tumorgenesis. For diagnosis of BCC, drebrin may be a novel and useful marker.

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Collagen XII and XIV - new partners of COMP in the skin extracellular matrix suprastructure

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Cartilage Oligomeric Matrix Protein (COMP) is an extracellular glycoprotein abundantly expressed in cartilage. We now describe COMP as a constituent of healthy human skin with a characteristic distribution that is strongly altered in several pathological skin diseases that are accompanied by a fibroproliferative response. In healthy human skin COMP is deposited in a linear fashion in the papillary dermis immediately subjacent to the dermo-epidermal basement membrane and concentrated in anchoring plaques. Expression is strongly augmented in fibrotic skin of patients with scleroderma and keloids, in lipodermatosclerotic regions of venous ulcers and in the stroma surrounding epithelial tumor islets. In vitro and in vivo studies demonstrated that COMP is produced exclusively by fibroblasts. Interestingly, COMP deposition overlaps extensively with that of collagen XII and XIV indicating a molecular interaction. Indeed in vitro binding assays using recombinant proteins revealed high affinity binding (KD = 1 nM) of pentameric COMP to the collagenous domains of FACIT collagens XII and XIV. Interactions were confirmed by immunogold electron microscopy of healthy skin indicating that these interactions occur in vivo. We propose that in skin, COMP functions as pentavalent nucleation structure binding collagen I fibrils via FACIT collagens XII and XIV and thereby modifying the supramolecular arrangement of the dermal collagen network. Mechanisms leading to increased COMP expression in fibrotic skin conditions might involve coordinated upregulation of collagens and COMP by TGFβ1. Alterations of the dermal collagen suprastructure might result in altered biochemical and mechanical properties in affected skin areas, known to be critical for profibrotic and proliferative responses in fibroblasts. This model could provide a mechanistic explanation for the structural alterations observed in the collagen/ECM network of some fibrotic skin diseases.

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Reduced fibrotic response in COMP-deficient mice

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We previously showed that fibrosis in scleroderma, lipodermatosclerosis in chronic wounds and the stroma surrounding basal and squamous cell carcinoma are all characterized by extensive deposition of Cartilage Oligomeric Matrix Protein (COMP) into the dermal extracellular matrix. To better understand the functions of COMP in these conditions and to facilitate mechanistic studies, we analyzed skin morphogenesis, homeostasis and development of dermal fibrosis (bleomycin injection) in wild type and mice with global ablation of COMP (kindly provided by A. Oldberg, Lund). In contrast to human skin, COMP was not detected (immunohistochemistry) in healthy skin of adult wild type mice. In young mice however, deposition was strong around growing hair follicles during follicle morphogenesis. This resembled the strong signals around tumor islets and suggested potential regulation of COMP expression by mechanical forces or pressure. We developed an in vivo organ culture model and showed that COMP expression was strongly induced in wild type skin subjected to compression forces (p=0.038 compared to untreated controls). Further factors enhancing COMP expression include profibrotic mediators e.g. TGFβ and IL-13 but not IL-4. As in human fibrotic diseases, marked COMP deposition was detected in the fibrotic dermis elicited by bleomycin injection. Interestingly, fibrotic responses following bleomycin treatment were significantly attenuated in COMP-deficient mice (p≤0.001 versus wild type) and were intermediate in heterozygous deficient animals suggesting dose-dependent effects. These findings point to a positive correlation of COMP expression with extent of fibrotic alterations. These might be caused by elevated mechanical forces acting in concert with augmented TGFβ levels in the dermis.

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Resemblances between scleroderma and ageing in skin fibroblasts

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Systemic scleroderma (Ssc) is a fibrotic disease characterized by abnormal and excessive deposition of collagen and other extracellular matrix components in various tissues. Typical for this disorder is the presence of extracellular matrix producing fibroblasts in the affected organs displaying an activated phenotype. We employed stable isotope labeling by amino acids in cell culture (SILAC), a metabolic labeling strategy, which allows mass spectrometric comparison of different cell types to unravel proteomic changes between normal and affected skin fibroblasts. Using this global, hypothesis-free approach we were able to detect that proteins involved in DNA replication are down-regulated in Ssc affected fibroblasts. Follow-up experiments validated these findings and proved that the nuclear levels of these proteins are different in affected vs. control cells. It is well established that these proteins are markers of cell proliferation, thus it might be inferred that they are indirectly involved in the up-regulated process of protein expression in Ssc affected cells. In addition, as affected cells grew slower than their healthy counterparts we hypothesized that Ssc mimics an ageing phenotype and characterized ageing in skin fibroblasts by using cells taken from donors of different ages. Indeed, we observed high resemblances between Ssc and the behavior of older fibroblasts, e.g. we evaluated the extent to which autophagy is impaired in affected cells and fibroblasts from old donors and obtained strong evidence that the autophagic capacity is dramatically decreased in both cell types. Altogether, we propose as current working hypothesis that Ssc affected cells resemble those from older donors.

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MMP1, 2 and 7 but not 9 or 13 promote cutaneous SCC keratinocyte invasion and migration

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Cutaneous squamous cell carcinoma (cSCC) affects over 20,000 people in the UK each year and 5-year survival rates for metastatic cSCC are estimated at 25-50%. Immunohistochemical studies of human samples showed increased expression of matrix metalloproteinases (MMPs) in invasive cSCC compared to normal skin or non-invasive tumours suggesting a role in cancer dissemination. Our aim was to identify MMPs necessary for cSCC keratinocyte invasion and migration. We assessed the contribution of MMPs to cell motility using organotypic invasion and scratch wound migration assays in two cSCC keratinocyte populations (SCCRDEB4 and SCCT8) using two broad-spectrum and two narrow-spectrum chemical inhibitors as well as siRNA targeting MMP1, 2, 3, 7, 9 and 13. In addition, we analysed the expression of E-cadherin and vimentin, proteins involved in epithelial-to-mesenchymal transition. Broad-spectrum MMP inhibitors almost completely prevented cSCC organotypic invasion (p= 2.1E-3 and p= 3.6E-9) while narrow-spectrum inhibitors had a milder effect, confirming that MMPs are necessary for cSCCK invasion and suggesting that several MMPs may be involved (n=3 in each case). In scratch assay, MMP1, 2, 3 and 7 siRNA inhibited migration (n=3). Invasion in organotypic culture was inhibited by siRNA specific for MMP1 (p=2.5E-3), MMP2 (p=2.9E-3) and MMP7 (p=3E-2) (n=3 in each case). In both scratch and organotypic assays, knock-down of MMP9 and 13 either increased or had no effect on cell motility dependent on the cSCC population. Knock-down of MMP1, 2, 9 and 13 were associated with increased levels of E-cadherin mRNA but only MMP1 and 2 knock-down showed decreased expression of vimentin mRNA. In conclusion we demonstrate the importance of clearly identifying which MMPs should be targeted to efficiently prevent cSCC dissemination.

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Genetic and *in vitro* observations support the key role of the protease inhibitor cystatin A in basal epidermal adhesion

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Autosomal recessive exfoliative ichthyosis presents shortly after birth with a fine peeling of non-erythematous skin on the palms and soles. Electron microscopy revealed prominent intercellular oedema and aggregates of keratin filaments in the basal and suprabasal cell layers of lesional skin (Hatsell et al., 2003). We have identified loss-of-function mutations in the protease inhibitor cystatin A (CSTA) as the underlying genetic cause of this condition (Blaydon et al., 2011), and investigated further the role of CSTA in cell-cell adhesion. In vitro dispase-based, cell stretch and scratch-wound assays combined with robust siRNA knockdown have demonstrated that loss of CSTA leads to destabilised intercellular connections. All these observations suggest a possible role in maintaining cell-cell adhesion via desmosomes in the basal keratinocytes. Furthermore DSG2 was relocated in CSTA knockdown stretched cell monolayers from a membranous to a cytoplasmic compartment. As CSTA is known to inhibit cathepsins B, H and L, we have shown all three to be expressed in cultured keratinocytes by western blotting. Also, IHC of the three cathepsins in normal skin shows that whilst cathepsins B and L are expressed in the basal layers of the epidermis cathepsin H is expressed mostly at the suprabasal level. Ongoing work is investigating the activity of cathepsins B and L in supernatants from CSTA knockdown stretched and scratch-wounded keratinocyte monolayers with or without small molecule cathepsin inhibitors. These molecular and cellular data suggest a major role for CSTA in basal keratinocyte adhesion in addition to its previously suggested role in the epidermal barrier and house dust mite cysteine protease inhibition.

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The association of plectin with epidermal keratins 5 and 14 depends on several COOH terminal subdomains within plectin, the central keratins coil 1 domain, and the quaternary structure induced by K5/K14 polymerization. Molecular insights into plectin- and intermediate filament-associated diseases

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Plectin is a member of the plakin protein family, composed of cytolinkers connecting elements of the cytoskeletal system to each other and to various membrane sites, conferring to cells critical resilience to mechanical stress in various tissues, such as the epidermis. To gain further insight into the regulation of plectin-intermediate filament (IF) connection as well as to plectin and IF-related human diseases, such as epidermolysis bullosa, in which IF-plectin linkage is compromised, we have dissected the interaction of plectin with the basal epidermal keratins 5 (K5) and 14 (K14) by yeast-three hybrid, cell transfection, chemical crosslinking and in vitro binding assays using recombinant proteins encompassing different subdomains of K5 and K14 keratins and the IF-binding COOH-terminal portion of plectin. The results showed that: 1) plectin interacts much better with monomeric K14 than K5; 2) the quaternary structure induced by hetero-polymerization of K5/K14 favors the association with plectin; 3) the coil 1 domain of K14 and K5/14 contains sequences critical for their binding to plectin; 4) deletion of the COOH-extremity of plectin affects the saturation level of plectin-IFBD to polymeric K5/K14; and 5) a recombinant form of plectin encompassing the last plakin repeat domain (PRD-C) and the COOH extremity is able to interact with K5/K14. These observations indicate that the COOH-terminal portion of plectin contains more than one binding site for K5/K14. Our data unravel the complexity of the binding of plectin to K5/K14 and provide insights into the molecular basis of plectin- and IF-related human diseases, associated with pathogenic mutations affecting functionally relevant sites within these molecules.

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Stimulation of tensotrophin expression and ECM remodelling in an innervated – dermal equivalent system

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Maintenance of connective tissue homeostasis relies on a finely tuned balance between extracellular matrix (ECM) constituents production and degradation. In the dermis, fibroblasts (HDF) anabolic and catabolic activities are regulated by a complex signalling network, where Transforming Growth Factor (TGF-β) plays a pivotal role. Autocrine TGF-β stimulation of HDF may not be exclusive, considering that other cell types, such as neuronal cells, also secrete TGF-β, thus questioning for a paracrine participation in the ECM remodelling. To study the relationship between skin innervation, TGF-β production and ECM remodelling, Nerve Growth Factor (NGF)-differentiated PC12 cells (a model of sympathetic neurons) were grown in presence of dermal equivalent (DE). In this model, metabolic activity of cells result in a progressive contraction of DE, and exogenous TGF-β increases contraction. In coculture, DE contraction was improved compared to DE alone, which supports an effective participation of the cutaneous nervous network in ECM remodelling relying on its capacity to secrete tensotrophins such as TGF-β. To mimic age-associated decrease of TGF-β expression in human skin in vivo, DE were prepared in low serum condition. We found that serum deprivation slows down DE contraction, which is restored upon addition of differentiated PC12 cells. In line with a decline of NGF production in aged skin, we further modelled the effect of aging on ECM remodelling by cultivating PC12 cells with suboptimal amounts of NGF. Cocultures containing suboptimal amounts of NGF secreted less TGF-β, and contracted less efficiently. Finally, a synthetic molecule endowed with neurotrophic properties that potentiates NGF signalling, was tested in this model. This molecule could enhance neuritogenesis from NGF-deprived PC12 cells and also potentiated TGF-β secretion. Accordingly, DE contraction was enhanced in cocultures treated with this molecule. Taken together, our results suggest that preservation of an optimum cutaneous nervous network may limit skin aging by maintaining tensotrophin expression.

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Type VII collagen regulates front-to-rear polarity in primary cutaneous squamous cell carcinoma keratinocytes

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Cell polarity is essential for tissue homeostasis and has a well-established role in cancer during both early onset of tumourigenesis and malignant transformation. Polarity can be defined as either the distribution of membrane domains along the apico-basolateral axis of a cell or the planar positioning of cells within the tissue itself, referred to as planar cell polarity (PCP). In the absence of established polarity, cancer cells do not respond to growth inhibitory signals from neighbouring cells and the microenvironment and acquire a migratory and invasive phenotype that ultimately results in metastasis. Recent studies have shown that ELMO2 positively regulates apico-basolateral keratinocyte polarity by serving as a bridge between active RhoG and ILK. We studied the regulation of this protein after manipulating COL7A1, the gene encoding type VII collagen. COL7A1 is mutated in recessive dystrophic epidermolysis bullosa (RDEB), a rare inherited skin disorder that predisposes to developing aggressive cSCC. Our data shows that ELMO2 expression is positively regulated by type VII collagen in both UV induced and RDEB cSCC ($p<0.005$, n=7, 4 independent primary cultures). We show that retroviral expression of type VII collagen confers increased structural polarity in 3D-spheroids cultured from RDEB cSCC cells. To test whether type VII collagen can influence planar cell polarity, pathway specific PCR arrays were used. Preliminary mRNA expression data suggests that APC expression, a tumour suppressor gene involved in directional migration, is significantly increased ($p=0.01$, n=3) in RDEB cSCC primary keratinocytes cultured on type VII collagen containing matrix. We show FZD3, a Wnt receptor and planar cell polarity marker, is also positively regulated by type VII collagen. These data suggest that type VII collagen positively regulates apico-basolateral and PCP polarity in cSCC and can play an important role in tumour progression through modulating key drivers of migration and invasion.

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LaNt α31, via interaction with laminin 332, mediates keratinocyte attachment and motility

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Recently, we described the identification of a new family of extracellular matrix proteins, termed the LaNts, that share similarities with laminins and netrins. We generated a rabbit polyclonal antibody against one member of this novel family, LaNt α31, and demonstrated that it is found at the dermal-epidermal junction of intact skin and is deposited into the extracellular matrix of keratinocytes in culture where it decorates sites of laminin 332 deposition. Through a combination of knockdown and exogenous addition studies we have analyzed the functional roles of LaNt α31 in human keratinocytes. Cells displaying knockdown in LaNt α31 attach less rapidly and less robustly compared to scrambled siRNA treated controls. Additionally, knockdown keratinocytes display defective motility with lower migration velocities in low-density migration assays and exhibit significantly reduced scratch wound closure rates. Consistent with these data, the expression of LaNt α31 is increased in low-density culture and following introduction of a scratch wound. Moreover, exogenous addition of purified LaNt α31 to culture medium enhances the migration rates of keratinocytes at low-density and promotes the early stages of scratch wound closure. Based on its domain architecture and the protein localization, we hypothesized that LaNt α31 may interact with laminin 332. To test this, we undertook far western analyses. These analyses reveal LaNt α31 binds laminin 332 subunits and led us to assess whether LaNt α31 modulates attachment of cells to laminin 332. Keratinocytes adhere more rapidly to plates where the laminin 332 is supplemented with low concentrations of LaNt α31 than to laminin 332 alone. Together these data have identified important functional roles for a new family of extracellular matrix proteins in keratinocyte behavior. Moreover they demonstrate that cellular responses to the major structural components of the extracellular matrix is, at least in part, modulated by the action of small linker proteins.

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Epidermal EGFR signalling controls cutaneous host defense and prevents inflammation

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The Epidermal Growth Factor Receptor (EGFR) plays an important role in tissue homeostasis and cancer progression. Interestingly, patients treated with EGFR inhibitors (EGFRI) frequently develop a papulo-pustular rash, which is regarded as important surrogate marker of the patient's treatment response. Here we show that the early inflammatory infiltrate of the rash is dominated by T-cells. EGFRI induce the expression of chemokines (CCL2, CCL5, CCL27, CXCL14) in epidermal keratinocytes, while the production of antimicrobial peptides and skin barrier genes is impaired. Correspondingly, EGFRI-treated keratinocytes facilitate lymphocyte recruitment, but show a significantly reduced cytotoxic activity against Staphylococcus aureus. Mice lacking epidermal EGFR (EGFRΔep) show a similar phenotype, which is accompanied by chemokine-driven skin inflammation, decreased host defense and deficient skin barrier function as well as early lethality. Most importantly, skin toxicities were ameliorated in a Rag2-deficient background indicating that lymphocytes play a critical role. Our findings demonstrate that EGFR signaling in keratinocytes regulates key factors involved in skin inflammation, barrier function and host defense, thus providing novel insights into the mechanisms underlying EGFRI-induced skin pathologies.

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Regulation of normal epidermal homeostasis by the basement membrane

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The basement membrane of skin has an essential structural role in aiding the tight attachment of the epidermis to the underlying dermis. In addition to its structural properties the basement membrane is also an important mediator of keratinocyte homeostasis, adhesion, proliferation, migration and gene expression. In this study we performed siRNA knock-down of the basement membrane proteins collagen IV, collagen VII, BP180 and the laminin alpha3 chain in primary keratinocytes. Organotypic collagen gels generated using normal fibroblasts and siRNA transfected keratinocytes showed altered epidermal differentiation compared to non-targeting control. Blistering was observed at the basement membrane in type VII collagen and laminin alpha3 knock-down epidermal 3D cultures. We have shown in monolayer cultures that keratinocyte stem cells have increased expression of laminin alpha3 and knock-down of this basement membrane protein causes altered stem cell marker expression. We have generated a long term organotypic model using an inert scaffold to culture keratinocytes with stable knock-down at the air-liquid interface for more than 4 weeks. Using normal primary keratinocytes we can show expression of basement membrane proteins and epidermal integrin receptor expression. This will allow us to analyse beyond an epidermal regeneration cycle to observe the impact of basement membrane loss on the stem cell compartment and epidermal homeostasis.

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Micro-spectroscopic and complementary ultrastructural observations of adipocytes

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Adipocytes play several critical physiological roles including energy storage and thermal insulation. The purpose of this work is to establish an experimental baseline using infrared imaging micro-spectroscopy for studies of adipocyte composition and morphology including their lipid droplet content. This work expands upon recent studies from our laboratories utilizing this approach to study isolated corneocytes and cultured human fibroblasts. To this end, human adipocyte cells have been grown under standard conditions, harvested, and applied to infrared transparent windows to allow spatially resolved infrared imaging using Fourier transform infrared (FTIR) imaging spectroscopy and Transmission Electron Microscopy (TEM). Infrared imaging micro-spectroscopy allows differentiation of the spatial distribution of chemical species within the cells such as proteins (eg. Perilipins and Caveolins), lipids, nucleic acids, and other components. The conformational states of these molecules or molecular assemblies can be extracted from the spatially resolved individual spectra collected from specified areas of the cells, thereby providing information pertaining to molecular organization and dynamics. The FTIR images are compared directly to visible images of the same cells providing points of reference that relates specific molecular information to specific cellular domains. The imaging results are supported with observations of the same cells using Transmission Electron Microscopy (TEM). Cells have been examined in non-fixed and fixed states. For TEM, cells were also fixed with a microwave fixation protocol meant to fix cells on an ultrafast time scale (ensuring maximum integrity for the fixed cell). The current work demonstrates that FTIR imaging micro-spectroscopy of adipocytes is a powerful method to characterize the molecular structure, organization and composition of lipid droplets in cultured adipocytes without the use of chemical labels or histological stains.

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Loss of type VII collagen in cutaneous SCC increases angiogenesis

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We have previously shown that loss of ColVII in SCC, modelling recessive dystrophic epidermolysis bullosa (RDEB), increases iinvasion with increased TGF-beta signalling. In this study, we explored the role of angiogenesis, a TGF-beta driven process, in tumourigenesis of SCC cells with absent ColVII. Stable knock-down of ColVII (shCOL7) was established in SCC cell lines using lentiviral shRNA. Collagen:matrigel gels were generated using shCOL7 and control (shC) cells and normal fibroblasts and grafted onto immunodeficient nude mice for 6 weeks. Histological analysis revealed that shCOL7 cells are more invasive than control cells in vivo, accompanied by an increase in blood vessel formation and diameter quantified using Meca-32 labelling. Data from an angiogenesis proteome array showed a significant increase in several angiogenesis markers, including VEGF-A and thrombospondin-1 in shCOL7 cells. Conditioned media from shCOL7 cells increased in vitro tube formation and branching in a HUVEC angiogenesis assay. Conditioned media from RDEB SCC cells with knockdown of TGFBR2, but not TGFBR1, reduced in vitro tube formation. Twist and VEGF expression was increased in shCol7 xenografts compared to shC and in RDEB SCC tumours compared to sporadic SCC sections. Interestingly, CD31 labelling also revealed an increase in the number and diameter of blood vessels in RDEB SCC tumours. Using a zebrafish xenograft model, we demonstrate that recombinant collagen VII protein significantly reverses intratumoural vascularisation of RDEB SCC cell lines. Our data provide evidence for the first time that loss of epithelial ColVII in SCC specifically increases angiogenesis driven by TGF-beta signalling, Twist and VEGF and may contribute to a better understanding of the accelerated metastasis in RDEB SCC.

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Short-term ozone exposure promotes wound healing by stimulating keratinocyte proliferation and migration

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As a potent disinfectant and oxygen (O2) donor, O3 has been studied for therapeutic use. By a quasi-total body exposure of O3 in a thermostatically controlled cabin to a patient with chronic limb ischaemia or by topical application of ozonated tea oil to the lesional area of chronic wounds, we have observed improved oxygenation and enhanced healing. The mechanism of O3 action on skin cells during wound healing is largely unknown. We hypothesized that short-term exposure to O3 facilitates wound healing through stimulating keratinocyte proliferation and migration. Using an in vitro diabetic cell culture model, we studied the effect of O3 exposure on keratinocyte proliferation, migration and signaling pathways. By WST (water soluble tetrazolium) assay, in vitro wound scratch assay and immunoblotting, we observed that exposure to O3 for only 10 min daily for 5 days increased cell proliferation (p<.05 by day 4, and p<.01 by day 5) and enhanced cell migration (p<.05 by 12h post scratch). Furthermore, we found that brief O3 exposure increased ERK phosphorylation by 4.8-fold, increased AKT phosphorylation at Thr 308 by 2.6-fold and AKT at Ser 473 by 1.8-fold, and increased mTOR phosphorylation by 2.8-fold. Inhibition of ERK (PD98059), AKT (MK-2206 2HCl) or mTOR (rapamycin) prevented O3-mediated cell proliferation (p<.01) and migration (p<.05). We have also noted that O3 exposure regulates keratinocyte function in a time-dependent manner. Exposure to O3 for 10 min daily maximally elevated cell proliferation, migration and related signaling, whereas more prolonged exposure (20 -30 min) markedly inhibited cell proliferation, migration and related signaling. These findings suggest that long-term exposure to O3 damaged keratinocytes, while short-term exposure to O3 exerts a beneficial effect.

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Amphiregulin cytoplasmic domain regulates G2/M in human keratinocytes

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Amphiregulin (AREG), one of seven EGFR ligands, has been strongly implicated as an autocrine growth factor for keratinocytes (KC) and other epithelial cells. It is expressed as a transmembrane precursor that undergoes ectodomain cleavage to release soluble ligand. We have previously shown that AREG knockdown (KD) strongly inhibits KC proliferation and cell cycle related gene expression that cannot be reversed by addition of soluble AREG (sAREG), suggesting that the AREG cytoplasmic domain (CTD) might be involved in a distinct intracellular signaling pathway that regulates KC growth. To further explore the effects of AREG KD on KC proliferation and cell cycling, we analyzed AREG KD cells by flow cytometry. Consistent with the appearance of many bi-nucleated cells, we found a large increase in KC with ≥4n DNA content (67.4% of the total population 6 days after AREG KD) accompanied by a strong decrease in cell division (~4-fold reduction relative to control KC, as assessed by CSFE dye dilution), which could not be reversed by addition of sAREG. To assess the functional importance of the AREG CTD, we transduced AREG KD KC with "silencing-proof" lentiviral constructs encoding the AREG CTD, full-length AREG, and sAREG. As assessed by resazurin reduction (which is sensitive to cell size and cell number), cell metabolism was markedly diminished in AREG KD cells (to 16.0% of control) but was restored by expression of the CTD (53.1%) or full-length AREG (67.8%) but to lesser extent by sAREG (38.4%, n≥3 for all). Expression of AREG CTD, but not sAREG, in KD cells efficiently restored expression of FoxM1 (29.6% of control in KD cells vs. 86.5 % for CTD and 39.8% for sAREG) and several of its target genes known to be important for G2/M transition and cytokinesis (e.g., PLK1: 39.1%, 86.6% and 42.4%; AurkA: 37.2%, 91.1% and 31.9%). Taken together, these data suggest that the AREG CTD is required for autocrine proliferation, G2/M transition and cytokinesis of human KC.

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Lipid soluble components of bee venom prevents oxidation-related changes in collagen metabolism in normal human dermal fibroblasts

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Bee venom has been used for treatment of cancer, immuological diseases, pain, and recently for anti-aging cosmetics. While the biological activities of water soluble fractions or ingredients of bee venom, e.g., melitin, are relatively well studied, there are limited knowledge about the lipid soluble components. In this study, bee venom was separated in fractions, and the effects on dermal extracellular matrix metabolism were inverstigasted. Lipid soluble fractions of bee venom reduced interstitial collagenase expression in both nontreated and hydrogen peroxide-treated dermal fibroblasts. These fractions also showed protective effect on oxidation-induced decrease of procollagen I expression. In contrast, water soluble fractions displayed no significant alteration in collagen metabolism. Because abnormal shift of cellagen turnover is an important mechanism of dermal aging and wrinkle formation, a novel anti-aging material can be developed from lipid soluble components of bee venom

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Nox4 - an emerging target for the future treatment of scleroderma?

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The pathogenesis of systemic sclerosis (SSc) is incompletely understood and effective therapies are urgently needed. There is evidence that abnormal expression and activity of distinct NADPH oxidase (Nox) isoforms are crucially involved in fibrotic diseases. Here, we analyzed the expression pattern of Nox enzyme isoforms in neonatal and adult human dermal fibroblasts (HDF) and in HDF from patients with SSc. We also investigated the regulation and function of the detected Nox isoform in the context of collagen synthesis and myofibroblast activation. Normal and diseased HDF expressed mRNA transcripts for Nox4 as well as its associated adaptor proteins p22phox and Poldip2. In contrast, Nox1, Nox2/gp91phox, Nox3, Duox2, p47phox, P67phox and p40phox were undetectable in these cells. Importantly, stimulation of normal HDF with the profibrotic cytokine TGF-β1 strongly upregulated Nox4 expression at mRNA and protein level as determined by real-time RT-PCR and Western immunoblotting. Immunofluoresence analysis revealed that TGF-β1-mediated induction of Nox4 protein localizes to the cytoplasm as well as to the perinuclear space of the cells. Pharmacological inhibition of Nox enzyme activity not only suppressed TGF-β1-mediated expression of collagen type I but also induction of both α-smooth muscle actin (α-SMA) and fibronectin 1. To finally test if agents suppressing experimentally induced fibrosis likewise reduce Nox4 expression, we pretreated HDF with the rapamycin derivative everolimus, the antifibrotic agent tropisetron, and α-melanocyte stimulating hormone (α-MSH). Only α-MSH suppressed TGF-β1-mediated Nox4, α-SMA and fibronectin 1 expression supporting a modulatory role of this peptide on fibroblast activation and ECM production. In summary, our results describe a novel and unexpected function of a specific NADPH isoform, Nox4, in fibroblast biology of the skin. Our findings are also encouraging to further dissect the functional role of this NADPH isoform in experimentally induced fibrosis models of the skin in order to tailor more effective therapies against fibrotic skin diseases.