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The mitogen-activated protein kinase pathway modulates the interaction of plectin with intermediate filaments (IF) to the plasma membrane and cell organelles in tissues exposed to mechanical stress such as the epidermis and muscle. Inherited mutations in the plectin gene result in epidermolysis bullosa (EB) and/or muscular dystrophy (MD). We have identified a phospho-plectin site within the COOH extremity of plectin, which regulates its binding to epidermal keratins and as well as to muscle-specific IF protein desmin. This phosphorylation site is lost in an EB-MD patient with amniotic ﬁndings of the COOH extremity of plectin. By yeast three-hybrid assay, biochemical, cell transfection and immunofluorescence studies, plectin was found to be phosphorylated on S4642 (pS4642). Phosphorylation of S4642 affected the potential of plectin to colocalize with IFs. In both cultured keratinocytes and in skin sections, functional plectin associated with desmosomes, as IFs anchorage site, showed a reduced phosphorylation of S4642. In contrast to unphosphorylated and unphosphorylatable forms, pS4642 phosphorylated plectin forms were not associated with the interaction of plectin with F-actin, desmin, and α-actin. These findings highlight the importance of integrin-dependent cell-collagen interactions in the regulation of fibroblasts in the periodontal ligament that express collagen XVII. Nearly all cells in multicellular organisms are surrounded by an extracellular matrix (ECM) that provides structural support, organization, and orientation to cells. Extensive communication between cells and their surrounding matrix is mediated mainly by integrins, among them α2β1 and α3β1. We aimed to delineate the functions of these integrins in tissues rich in fibrillar collagen I by generating mice deficient for either of both α2β1 and α3β1, both of which confer high avidity cell binding to fibrillar collagen I. The relevance of this model was tested in murine corneal epithelium, in which we demonstrated that both integrins interact with K5/K14. This observation suggests the presence of more than one binding site on keratin filaments. The interaction of desmoplakin and plectin with keratins 5 and 14: critical aspects of cell architecture and plasticity and explain how even minor truncations of the COOH-extremity of plectin leads to devastating consequences.

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E-cadherin is required for melanocyte maintenance in the basal layer and is altered in vitiligo-affected skin.** V. Demara, 1 A. Rubald, 1 F. Luciani, 2 R. Wagner, 1 M. Cario-André, 1 E. Khédiri, 1 L. Benzekri, 1 Y. Gaither, 1 A. Tsoli 1 and L. Larue 1 Developmental Genetics of Melanocytes, Institut Curie, France. 1 Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, Switzerland and 2 Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois. E-cadherin is a transmembrane glycoprotein that mediates cell-cell adhesion through homophilic interactions. These adhesions are essential for the maintenance of an epithelial monolayer and epithelial morphology. This is because the basal layer provides a mechanical support for the epidermis, helping to maintain the integrity of the stratum basale. In addition, E-cadherin is expressed in several cell types outside the epithelium, including fibroblasts, monocytes, macrophages and lymphocytes. However, the role of E-cadherin in epithelial cells and its regulation have not been extensively studied. In this study, we investigated the role of E-cadherin in melanocytes. Firstly, we analyzed the expression of E-cadherin in melanocytes from skin biopsies of patients with vitiligo, a skin disorder characterized by the depigmentation of the skin. We used immunohistochemistry to assess the expression of E-cadherin in melanocytes. Our results showed that E-cadherin expression was significantly reduced in vitiligo-affected skin compared to normal skin. These findings suggest that E-cadherin plays a role in melanocyte maintenance and may be a potential therapeutic target for the treatment of vitiligo.

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Collagen-binding integrins α2β1 and α3β1 – new players in controlling endocrine homeostasis.** K. Bismarch, 1 A. Niehui, 1 B. Belgacem, 1 M. Schuber, 1 J. Schulz, 2 B. Brüning, 1 D. Gullberg, 1 T. Krieg, 1 and B. Eikes 1 1 Dermatology, University of Cologne, Cologne, Germany, 2 Biomembranes and Orthopaedics, German Sport University Cologne, Cologne, Germany, 3 Medical Genetics and Metabolism, University of Cologne, Cologne, Germany. The interaction of cell-surface integrins with the extracellular matrix (ECM) plays a critical role in the regulation of cell functions. These interactions are mediated by the adhesive domains of ECM proteins and enable cells to transmit mechanical signals. In the context of endocrine homeostasis, the interaction of cell-surface integrins with collagen XVII (COL17A1) has been shown to be crucial. COL17A1 is an ECM protein that is upregulated in response to mechanical stress such as the epidermis and muscle. It interacts with specific cell-surface receptors, such as integrin α2β1 and α3β1, to transmit mechanical signals to the cell interior. These integrins are known to be involved in the regulation of cell functions, such as proliferation and migration. In this study, we aimed to investigate the role of COL17A1 in the regulation of cell functions in the context of endocrine homeostasis. We found that COL17A1 expression was significantly elevated in tissues from patients with diabetes mellitus, suggesting a potential role in the regulation of endocrine homeostasis. These findings highlight the importance of COL17A1 in the regulation of cell functions and its potential role in the development of diabetes mellitus.
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The receptor tyrosine kinase Axl regulates intercellular adhesion, epithelial-mesenchymal transition and stemness in cutaneous squamous cell carcinoma

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Axl is a receptor tyrosine kinase upregulated in a variety of tumours including cutaneous squamous cell carcinoma (SCC). Axl expression correlates with the metastatic potential of some tumours, and we hypothesized that Axl might play a role in epithelial-mesenchymal transition (EMT), a crucial step in cancer progression causing disruption of cell-cell adhesion to allow invasion and metastasis. To investigate this hypothesis, we created cutaneous SCC cell lines with stable Axl knock-down using retroviral vectors. Subsequently, colony morphology, cell adhesion and related signalling pathways were studied. Cells with Axl knock-down formed tighter colonies and had altered expression of several junctional proteins with increased functional cell-cell adhesion and decreased cell-matrix adhesion. EMT markers downregulated by Axl knock-down included TWIST, SNAIL and regulation of EMT and stemness TGFBR1 and TGFBR2. Flow cytometry sorting of CD44-high enriched adherent cells revealed increased epithelial tight junctional adhesion. TJs alterations occurred without gross changes in adherens junctions or desmosomes, or loss of cell polarity. Using preparative immunoprecipitation, we determined that Axl regulates the composition and function of tight junctions (TJ) that are critical to epithelial integrity. Axl knockdown in epidermal cells and TJ function increased phospho-epac1 and decreased claudin levels. Claudin knockdown in HaCaT cells with TJ proteins showed marked changes in tight junctional adhesion as well as loss of cancer stem cell properties including sphere formation, drug resistance, reversal of EMT and tumour formation, confirming Axl as a potential target for future therapeutic intervention in cutaneous SCCs and other Axl-overexpressing cancers.

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Loss of keratinocyte type VII collagen in SCC increases TGF-beta signalling and angiogenesis

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Rheostatic dysregulation of epimorphosis pulling (RDEP), caused by mutations in the type VII collagen (C7) gene, is associated with increased risk of metastatic squamous cell carcinoma (SCC). We have previously shown that loss of ColVII in SCC increases migration/mesenchymal transition with increased TGF-beta signalling in vitro. In this study, we explored the role of angiogenesis, a process downstream of TGF-beta signalling, in tumourigenesis of SCC cells with absent ColVII. Stable knock-down of ColVII in shCOL7 was established in SCC cell lines using lentiviral shRNA. qPCR demonstrated increased TGFBR1 expression in vitro. Increased expression of alpha5beta integrin and fibronectin, major TGF-beta pathway components, was observed in shCOL7 cells. Interestingly, incubation with a TGFBR1 inhibitor reduced SCC cell invasion and fibronectin expression in shCOL7 SCC as well as in RDEB SCC cell lines. Collagen-matrigel gels were generated using shCOL7 and control shC cells and seeded onto immunodeficient nude mice for 6 weeks. shCOL7 xenografts had an increased number and tortuosity of blood vessels macroscopically compared to shC. Histological analysis revealed that shCOL7 cells display an increase in blood vessel formation and diameter quantified with the endothelial cell marker, Meca-12. Data from an angiogenesis protein array showed a significant increase in several angiogenic factors, including VEGF, in shCOL7 compared to shC. We are currently developing a mouse model, where we observed tumour formation in embryos injected with Axl-expressing, but not Axl-knockdown cells. We show that Axl knockdown increases adhesion of TGF-beta signalling in vitro. Produced collagen matrixes in vitro. Produced increased trans-epithelial electroresistance. TJ alterations occurred without gross changes in adherens junctions or desmosomes, or loss of cell polarity. Using preparative immunoprecipitation, we determined that Axl regulates the composition and function of tight junctions (TJ) that are critical to epithelial integrity. Axl knockdown in epidermal cells and TJ function increased phospho-epac1 and decreased claudin levels. Claudin knockdown in HaCaT cells with TJ proteins showed marked changes in tight junctional adhesion as well as loss of cancer stem cell properties including sphere formation, drug resistance, reversal of EMT and tumour formation, confirming Axl as a potential target for future therapeutic intervention in cutaneous SCCs and other Axl-overexpressing cancers.

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Regulation of tight junction function and composition by the claudin-associated protein EpCAM (CD326)

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EpCAM (CD326) is a transmembrane glycoprotein expressed in invasive carcinomas and some normal epithelia. Recent work from our laboratory demonstrated that EpCAM promotes motility and epithelial migration of epidermal Langerhans cells (LC), perhaps by modulating LGa-adhesion and LC-apoptosis. Germline mutations in EpCAM cause epidermal cell dysfunction and dysfunction (congenital tufting enteropathy). We studied intestinal epithelial cells to gain additional insights into EpCAM function and determined that EpCAM regulates the composition and function of tight junctions (TJs) that are critical to epithelial integrity. EpCAM accumulated on lateral interfaces of T84 and Caco-2 human colon carcinoma cells and normal intestinal epithelial cells, but did not co-localize with TJ. Knockdown of EpCAM in T84 and Caco-2 cells using shRNAs led to morphological changes and alterations in adherens and tight junctional adhesion as well as loss of cancer stem cell properties including sphere formation, drug resistance, reversal of EMT and tumour formation, confirming EpCAM as a potential target for future therapeutic intervention in cutaneous SCCs and other Axl-overexpressing cancers.

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Hailey-Hailey disease and tight junctions: Claudins 1 and 4 are 4 regulated by ATP2C1 gene encoding Ca2+/Mn2+ ATPase in cultured keratinocytes

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Claudin-1 is a transmembrane glycoprotein expressed in normal keratinocytes, whereas claudin-4 expression is lost in keratinocytes from patients with Hailey-Hailey disease (HHHD). We have recently demonstrated that ATP2C1 gene encoding Ca2+/Mn2+ ATPase is up-regulated in non-treated control keratinocyte cultures and that ATP2C1 expression is increased after calcium stimulation. We have shown that ATP2C1 is a key player in regulating cell-cell adhesion as well as loss of cancer stem cell properties including sphere formation, drug resistance, reversal of EMT and tumour formation, confirming EpCAM as a potential target for future therapeutic intervention in cutaneous SCCs and other Axl-overexpressing cancers.

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Importance of o-linked glycans in complex epithelial formation

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A hallmark of the transepithelial barrier is the tumour barrier of O-linked glycans with the expression of short cancer associated O-linked glycans. It is, however, unknown if the de novo expression of cancer associated O-linked glycans promotes tumour development. One cause for the expression of cancer associated glycans is somatic mutations in the molecular chaperone Cosmc. Cosmc encoding Ca2+/Mn2+ ATPase SPCA1 in cultured keratinocytes

Hailey–Hailey disease and tight junctions: Claudins 1 and 4 are regulated by ATP2C1 gene encoding Ca2+/Mn2+ ATPase in cultured keratinocytes

J Groh, 1 L Kobilka, 1 P Thompson, 2 and S Polakiewicz, 3 1 Departments of Dermatology and Cell Biology and Anatomy, University of Turku, Turku, Finland, 2 Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA, 3 Department of Cell Biology, University of Turku, Turku, Finland

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Disease proteomics reveals modulation of cell microenvironment in recessive dystrophic epidermolysis bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB), a genetic skin disease, was used as a model system to investigate the role of epithelial membrane proteins involved in its pathogenesis by a disease proteomics approach. A strategy using quantitative mass spectrometry (MS), combined with bioinformatics data processing, was developed to assess quantitative differences in the microenvironment of skin fibroblasts derived from normal and pathologically altered skin. A standard extracellular proteome of normal skin fibroblasts was generated and compared to that of RDEB fibroblasts. This global, unbiased approach revealed unanticipated differences in protein abundance across all examined patient samples, as well as differences observed only for specific patients. RDEB is caused by mutations in the gene encoding collagen VII, a dermal-epidermal adhesion protein. The proteomics approach showed that profiles of collagen VII was associated with a number of other changes, i.e. decrease of 3D organotypic monolayers and increase of dermal matrix proteins, TGF-beta, and metalloproteinases, but not with higher protease activity. Thus, by combining high-resolution, high-accuracy MS with a bioinformatics data processing pipeline we generated a comprehensive list of protein changes in RDEB fibroblasts, which led to the identification of potential therapeutic targets for RDEB.
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**p180 Mitogen-Activated Protein Kinase Regulates Tight Junction Protein ZO-1 in Human Epidermal Keratinocytes**

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Increasing evidence has recognized tight junctions as the lower epidermal inside-out diffusion barrier located in granular cell layers of the epidermis. The main components of epidermal tight junctions are transmembrane proteins occludin and members of claudin family, as well as cytoplasmic plaque protein zonula occludens protein-1 (ZO-1). To date, little is known about the regulation of tight junction components in epidermis. p180 pathway is one of the mitogen-activated protein kinase pathways, which controls cell growth, differentiation, and apoptosis. We have investigated the role of p180 signaling pathway in the regulation of selected desmosomal, adherens and tight junction components in human primary keratinocytes during Ca²⁺-induced differentiation. The results showed that specific inhibition of p180 function by chemical inhibitor, adenovirally delivered dominant negative mutant or siRNA led to markedly decreased levels of ZO-1 protein in keratinocytes, as demonstrated by western blotting. The expression of other skin barrier related junctional proteins such as desmoplakin, beta catenin, occludin, claudins-1 and 4 was not regulated by p180. Immunolocalization of ZO-1 revealed that in p180 inhibited cells the intercellular junction areas were depleted from ZO-1. p180 inhibition did not affect the relocalization of desmoplakin, occludin or claudins in the junction areas. These results show for the first time a role for p180 signaling pathway and specifically p180 in regulation of a tight junction component. The results also indicate that tight junction components are differentially regulated. Since ZO-1 is an integral component of functional tight junctions, various pathological processes affecting signaling via p180 may also interfere with epithelial maturation and the formation and function of tight junctions as a secondary epidermal barrier.

**Proteomics Analysis Reveals Phenotypic Alterations in Scleroderma Fibroblasts**

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Scleroderma (SSc), the prototype of fibrotic skin diseases, is characterized by excessive deposition of collagen and other extracellular matrix components in human primary keratinocytes during Ca²⁺-induced differentiation. The results showed that specific inhibition of p180 function by chemical inhibitor, adenovirally delivered dominant negative mutant or siRNA led to markedly decreased levels of ZO-1 protein in keratinocytes, as demonstrated by western blotting. The expression of other skin barrier related junctional proteins such as desmoplakin, beta catenin, occludin, claudins-1 and 4 was not regulated by p180. Immunolocalization of ZO-1 revealed that in p180 inhibited cells the intercellular junction areas were depleted from ZO-1. p180 inhibition did not affect the relocalization of desmoplakin, occludin or claudins in the junction areas. These results show for the first time a role for p180 signaling pathway and specifically p180 in regulation of a tight junction component. The results also indicate that tight junction components are differentially regulated. Since ZO-1 is an integral component of functional tight junctions, various pathological processes affecting signaling via p180 may also interfere with epithelial maturation and the formation and function of tight junctions as a secondary epidermal barrier.

## Identifying Biomarkers for Scleroderma Fibroblasts

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**The α7 nicotinic acetylcholine receptor mediates suppression of collagen synthesis in human dermal fibroblasts – a novel target for anti-fibrotic strategies**

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**Identification of cell cycle and cancer-associated gene networks activated by Dsg2 using cdNA microarray: evidence of cytA A deregulation**

S Addya, S Prasad, S Saha, S Datta, J Paul, I Chatterjee, N Bose, A Roy, S Panda, 1 Department of Dermatology, Calcutta Medical College and Hospital, Kolkata, West Bengal, India and 2 Department of Dermatology, King’s College Hospital, London, UK.

Epidermal growth factor (EGF) receptor is a tyrosine kinase that plays a central role in the proliferation of keratinocytes. However, its role in the proliferation of human normal skin epidermal cells is not well understood. In the present study, we investigated the influence of EGF receptor on cell proliferation in human normal keratinocytes using flow cytometric analysis. We observed that EGF receptor promotes cell proliferation in normal human keratinocytes. Western blot analysis revealed that EGF receptor is upregulated in normal human keratinocytes as compared to human normal fibroblasts. Immunohistochemical analysis showed that EGF receptor is highly expressed in normal newborn mouse skin, whereas it is not expressed in normal newborn mouse fibroblasts. The results suggest that EGF receptor may play a role in the proliferation of normal human keratinocytes.

**Septins, a conserved family of GTP/GDP-binding proteins, are present in organisms as diverse as yeast and mammals**

S Khabibullin, D Khabibullin, A Grishina, I Krymskaya, M Beis, N Wang, Z Chen, T Crosnier, 1 Department of Cell Biology, University Medical Center Freiburg, Freiburg, Germany and 2 Department of Cell Biology, University Medical Center Freiburg, Freiburg, Germany.

Septins are a conserved family of GTP/GDP-binding proteins, which are present in organisms as diverse as yeast and mammals. Septins assemble in large and persistent stress fibers which are thought to function as scaffolds and/or diffusion barriers controlling and maintaining the localization of diverse proteins. In this study, we defined a biological characterization of septins, focusing on septin-1 in human skin tissues and a human squamous cell carcinoma (SCC) cell line. In Western blotting, septin 1 was detected with other septins in normal human epidermis, SCC and D1A-1 cells. In immunoblotting analysis, septin 1 was detected with other septins in normal human epidermis, SCC and D1A-1 cells. In immunoblotting analysis, septin 1 was detected with other septins in normal human epidermis, SCC and D1A-1 cells. In immunoblotting analysis, septin 1 was detected with other septins in normal human epidermis, SCC and D1A-1 cells. In immunoblotting analysis, septin 1 was detected with other septins in normal human epidermis, SCC and D1A-1 cells. In immunoblotting analysis, septin 1 was detected with other septins in normal human epidermis, SCC and D1A-1 cells.
262 Impact of c-Rel on proliferation, migration and adhesion of HaCaT cells


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Characterization of a Novel Hyaluronan-Depolymerizing System Mediated by KIAA1199

A. Nagase, Y. Hashida, A. Asaka-Kikushima, M. Ishikawa, K. Kawanishi, T. Sayo, S. Saka, T. Sugiyama, M. Arata, T. Tanaka, M. Hyaluronan (HA), a nonsulfated linear glycosaminoglycan (GAG) composed of repeating disaccharide units of N-acetylgalactosamine (GalNAc) and glucuronic acid, has an extraordinarily high affinity for sodium-dependent hyaluronan-binding receptors expressed on the surfaces of several cell types. These receptors, including CD44 and syndecan, recognize the terminal sialylated disaccharide sequences of HA. In this study, we sought to identify novel HA-degrading enzyme(s) and explore their role in tumor progression. Here we have analyzed the properties of the hyaluronan-depolymerizing system mediated by KIAA1199. The ectopic expression of full-length KIAA1199 cDNA endows HEK293 cells with the ability to depolymerize high molecular weight HA (>1,000 kDa) in a similar manner to skin fibroblasts. Next, we established stable transformants that are capable of degrading HA with an efficiency similar to skin fibroblasts. These results strongly imply that KIAA1199 can be exploited as a potential therapeutic target for HA-mediated diseases.

264 Environmental factors drive the cross-linking of structural proteins in ageing skin


School of Medicine, Osaka University Graduate School of Medicine, Suita, Japan

265 Papillary and reticular human fibroblasts: distinct characteristics and contribution to clonal growth of human keratinocytes progenitors


School of Medicine, Osaka University Graduate School of Medicine, Suita, Japan

266 Non-invasive study of human cutaneous innervation in an re-innervated skin explant

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267 Local cortisol activation by 11β-HSD1 progresses skin atrophy through reducing the proliferation of fibroblasts in adult mouse skin


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Glucoconoids (GCs) are one of the most effective anti-inflammatory drugs to treat acute and chronic inflammatory diseases. However several studies show that GCs alter collagen metabolism and induce skin atrophy. In this study, we have shown that 11β-HSD1 progresses skin atrophy through reducing the proliferation of fibroblasts in adult mouse skin.
Remodeling dermal ECM organization by dermatopontin reveals anti-aging properties. Ex vivo studies showed that, after a 1% IV09021 treatment on skin biopsies, elastic fibers were extended and the expression of their major components (Tropoelastin, Fibrillin-1 and Fibulin-5) was significantly increased in the dermis. The anti-aging effect of IV09021 formulated at 1% was evaluated in vivo, by measuring expression changes of the papillary and reticular markers we showed that papillary fibroblasts represent an undifferentiated phenotype, while reticular fibroblasts represent a more differentiated phenotype. We have investigated the differentiation process of both fibroblast types to understand their role in skin aging. Because no markers were available for reticular or papillary fibroblasts, we performed gene expression analysis on in vitro cultures of both fibroblasts. We identified and validated 13 differently expressed genes on mRNA level and 4 biomarkers on protein level. By measuring expression changes of keratinocytes, HA/CD44 interaction and cell sensitivity to epidermal growth factors, we showed that papillary fibroblasts can differentiate into reticular fibroblasts after prolonged culture. These observations showed that papillary fibroblasts can differentiate into reticular fibroblasts. Since both fibroblast populations have distinct effects on skin physiology, such as on matrix generation and epidermal differentiation, we propose that the differentiation of papillary to reticular fibroblasts is a mechanism of skin aging and a potential new target for anti-aging strategies.

Characteristics of reticular and papillary fibroblasts and their potential functional implications in skin aging

A Novel Hyaluronan-Degradating System Mediated by KIAA1199 Independent of CD44

In the literature, many studies discuss the distinction of reticular fibroblasts from papillary fibroblasts. We hypothesized that papillary fibroblasts represent an undifferentiated phenotype, while reticular fibroblasts represent a more differentiated phenotype. We have investigated the differentiation process of both fibroblast types to understand their role in skin aging. Because no markers were available for reticular or papillary fibroblasts, we performed gene expression analysis on in vitro cultures of both fibroblasts. We identified and validated 13 differently expressed genes on mRNA level and 4 biomarkers on protein level. By measuring expression changes of keratinocytes, HA/CD44 interaction and cell sensitivity to epidermal growth factors, we showed that papillary fibroblasts can differentiate into reticular fibroblasts after prolonged culture. These observations showed that papillary fibroblasts can differentiate into reticular fibroblasts. Since both fibroblast populations have distinct effects on skin physiology, such as on matrix generation and epidermal differentiation, we propose that the differentiation of papillary to reticular fibroblasts is a mechanism of skin aging and a potential new target for anti-aging strategies.

Evaluation of the effect of Rho-kinase inhibitor Y-27632 on the lifespan of dermal fibroblasts

Bioactive in dermatopontin revealed anti-aging properties. Ex vivo studies showed that, after a 1% IV09021 treatment on skin biopsies, elastic fibers were extended and the expression of their major components (Tropoelastin, Fibrillin-1 and Fibulin-5) was significantly increased in the dermis. The anti-aging effect of IV09021 formulated at 1% was evaluated in vivo, by measuring expression changes of the papillary and reticular markers we showed that papillary fibroblasts can differentiate into reticular fibroblasts after prolonged culture. These observations showed that papillary fibroblasts can differentiate into reticular fibroblasts. Since both fibroblast populations have distinct effects on skin physiology, such as on matrix generation and epidermal differentiation, we propose that the differentiation of papillary to reticular fibroblasts is a mechanism of skin aging and a potential new target for anti-aging strategies.

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The Use of Two Photon Laser Microscopy for Diagnosis and Imaging in Dermatology

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Two photon excitation fluorescence microscopy is a useful tool for the non-invasive evaluation of cellular and molecular structure of the skin. This novel approach uses femtosecond lasers in the near infrared (650-1300nm) spectral range that allows a superior degree of penetration and high resolution imaging. Various methods such as Second Harmonic Generation, Fluorescence Lifetime Imaging Microscopy or Coherent anti-Stokes Raman Spectroscopy have been used to study aging of the skin or diagnosing different skin diseases. We investigated a various set of human ex vivo skin samples. Morphological and spectroscopic differences were found between healthy and pathological skin samples. In particular, we examined tissue samples from young and old patients, diabetic patients, melanocytic nevi and skin tumors, including basal cell carcinoma. By using combined two-photon microscopy with SHG imaging we investigated morphological features of different skin specimens. The results indicate that two photon autofluorescence and SHG imaging could be used for in vivo noninvasive imaging and diagnosis of diseased skin including malignant skin tumors.

Cutaneous cells exhibit contractile properties stimulated by a cereal rye extract

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Reduction of skin elasticity is a characteristic feature of chronologically aged skin. At the cellular level, when a mechanical stress is applied to the skin it results in a change in the structure of the cytoskeleton. The cells produce a large number of stress fibers composed of SMA (alpha Smooth Muscle Actin), which play an active role in the transmission of the mechanical tensile signal. The aim of the study was first to highlight the decrease of tensile properties of aged dermal fibroblasts by measurement of strengths exerted by cells embedded in collagen lattice during isometric dermal remodeling (GlaSbox® device) and by evaluation of the expression of SMA by immunofluorescence. We have selected a cereal rye extract able to restore the synthesis of stress fibers and the tensile properties of aged dermal fibroblasts. We previously demonstrated that adipose tissue-stroma vascular-derived cells (SVC) treated in vitro with TGF were able to express, as fibroblasts do, the phenotypic smooth muscular cell. This study investigates the contractile properties of SVC and their SMA expression compared to dermal fibroblasts obtained from the same individual, skin location and culture conditions. The SVC embedded in collagen lattices exhibit equivalent tensile strengths as dermal fibroblasts. Moreover, treatment with the extract significantly stimulated SVC isometric forces and SMA expression. We conclude that the adipose tissue stroma vascular fraction is a reservoir of fibroblast-like cells able to reorganize and tense in depth the dermal collagen matrix. In vivo, this vegetable extract firms skin. These results illustrate the ability of this vegetable extract to modulate the dermal fibroblasts and SVC plasticity and its regulating role in the mechanical resistance and stretching of aged skin.

Improvement of Skin Aging through Stimulation of Microfibril-Associated Glycoprotein 1

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Skin aging is a continuous process that affects skin function and appearance. Chronological aging, environmental damage, and life style, which are the major contributors to the skin aging process, can lead to reduction and damage of skin matrix. A dysfunctional skin matrix can eventually cause facial wrinkles, sagging and other signs of skin aging. Previously, we have identified that a matrix component, microfibril-associated glycoprotein 1 (MAGP-1), declines with age in skin. We further demonstrated that Cortisol, a bio-marker of emotional stress, can also decrease the expression of MAGP-1. In order to identify active ingredients that can influence the expression of MAGP-1 in skin cells, and potentially have a positive impact on the visual appearance of aged skin, we initiated a screening program. In this report, we reveal the identification of several novel ingredients that increase the expression of MAGP-1 in skin cells in vitro. Topical skin care formulations containing these novel MAGP-1 stimulators can counteract emotional stress-induced reduction of MAGP-1 in vitro and help improve the appearance of aging skin in vivo.