144

Desmoplakin (DP) mutations impair gap junctions by disrupting a novel DP-EB1 Interaction

DM Patel and KJ Green *Pathology and Dermatology, Northwestern University, Chicago, IL*

Desmoplakin (DP) is an essential desmosomal protein that imparts mechanical integrity to skin and heart by tethering intermediate filaments (IF) to sites of strong cell-cell adhesion. DP mutations cause a range of cutaneous and cardiac disorders including lethal skin blistering diseases and arrhythmogenic cardiomyopathy (AC). Disease pathogenesis has been proposed to be due to an inability of mutated DP to tether IFs to junctions. However, the existence of a mutation hotspot in the DP N-terminus, downstream of the known armadillo protein binding region and upstream of the C-terminal IF binding domain, raises the possibility that impairment of non-canonical DP functions contributes to disease. To address this possibility we carried out a Yeast-two-hybrid screen and identified a novel interaction between DP and the microtubule (MT)-associated protein End-Binding 1 (EB1), which enables DP to act as a component of the MT cortical capture machinery. Utilizing biochemical and *in situ* protein-protein interaction assays we showed that N-terminal DP mutants associated with AC and skin fragility/woolly hair syndrome interfere with MT capture in one of two ways: AC mutants localize to desmosomes but are unable to bind EB1, whereas the skin fragility mutant fails to localize to junctions. As EB1 was previously reported to play a role in delivery of connexins to sites of gap junction assembly, we asked whether mis-regulation of DP-EB1 complexes impaired gap junctions. For both types of mutants, destabilization of MT plus-ends led to decreased Cx43 plasma membrane localization in cardiac myocytes and keratinocytes grown in submerged or 3D cultures. Cx43 mis-localization was associated with loss of gap junction coupling, as assessed by dye transfer assays. Our results identify a novel function for DP in binding EB1 to regulate MTs, and reveal a mechanism by which DP mutations contribute to the development of cardiac and cutaneous diseases through impairment of gap junctions.

145

RNA-Seqing the skin basement membrane

S Marsh,[1] V Martins,[1] M Caley,[1] M Barnes,[2] M Donaldson[3] and E O'toole[1] *1 Centre for Cutaneous Research, Barts and the London SMD, Queen Mary University of London, London, United Kingdom, 2 Genome Centre, Barts and the London SMD, Queen Mary University of London, london, United Kingdom and 3 Stiefel, a GSK company, Uxbridge, United Kingdom*

The basement membrane plays an important role in wound healing and cancer invasion. Mutations in genes encoding basement membrane proteins lead to the severe blistering disease, Epidermolysis Bullosa (EB), which can increase predisposition to cancer. In this study, siRNA knockdown (KD) of basement membrane proteins, type IV, VII and XVII collagens and the α3 chain of laminin 332 was performed in primary neonatal foreskin keratinocytes. The transcriptome of siRNA transfected keratinocytes was analysed by RNA-Seq. Loss of type IV collagen produced relatively few gene expression changes compared to knockdown of the other basement membrane proteins. Gene expression changes that overlapped in keratinocytes with knockdown of type VII collagen, type XVII collagen, and the α3 chain of laminin 332 were analysed by Ingenuity Pathway Analysis (IPA). Overlapping genes validated by QPCR included *ADAM19*, *MMP15*, *IL33*, *SPARC* and *TNF*, important in extracellular matrix remodelling, the immune system, and cancer progression. Loss of type VII collagen resulted in differential expression of genes important in regulation of the cell cycle which were validated by Western blotting. IPA predicted that TGFβ was an upstream regulator of keratinocytes with loss of type VII collagen and increased phospho-Smad2 was observed in these cells. The altered gene signature of keratinocytes with loss of type VII collagen predicted reduced cell proliferation, which was confirmed functionally with a proliferation assay over a 72 hour time period. In conclusion, the increased expression of proteases, *SPARC* and pro-inflammatory cytokines suggests that loss of the basement membrane leads to a pro-tumourigenic microenvironment. In addition, loss of type VII collagen results in increased TGFβ signalling, cell cycle dysregulation and reduced proliferation, which may inhibit wound healing and contribute to cancer progression in EB patients with mutations in *COL7A1*

146

Non-structural functions of the Cartilage Oligomeric Matrix Protein in the dermis

J Schulz,[1] F Zaucke,[2] W Bloch,[3] A Niehoff,[4] J Nüchel,[2] S Hayashi,[1] T Krieg[1] and B Eckes[1] *1 Dermatology, University of Cologne, Cologne, Germany, 2 Biochemistry, University of Cologne, Cologne, Germany, 3 German Sport University, Cologne, Germany and 4 Cologne Center for Musculoskeletal Biomechanics, Cologne, Germany*

We recently showed that the extracellular matrix protein Cartilage Oligomeric Matrix Protein (COMP), thought to be exclusively expressed in cartilage and force-bearing parts of tendons and ligaments, is deposited by fibroblasts in skin and highly elevated in pathologic fibrosis. As COMP binds to fibrillar collagens and procollagens, we asked whether it assists in the spatial organization of a functional dermal collagen network. Using plasmon resonance spectroscopy, we identified the fibril-associated collagens XII and XIV as high affinity binding partners for COMP and showed co-localization in the dermis. Interestingly, we found strongly reduced collagen XII levels in skin of mice with a targeted deletion of COMP. The skin of these animals displayed altered biomechanical properties and ultrastructural analysis revealed collagen fibrils with highly asymmetric diameters and abnormal spatial organization. In addition, COMP-deficient fibroblasts showed retention of collagen I and XII in the endoplasmic reticulum (ER), resulting in ER stress, illustrated by dilated ER cisternae in vivo and elevated levels of the ER stress marker CHOP in vitro. Based on these findings we hypothesized that COMP is required to interact with collagens, forming intracellular complexes that are subsequently targeted for secretion, while incompletely formed complexes, e.g. lacking COMP, are retained within the ER of stressed fibroblasts. To test this concept, we used bleomycin injections to induce high collagen production leading to pathologic fibrosis in COMP-null mice. This led to massive apoptosis of fibroblasts in the dermis and consequently to significantly reduced fibrosis in comparison to control mice. Our results identify a hitherto unrecognized non-structural role of COMP, which might represent a potential target for antifibrotic strategies, and they reveal a novel mechanism for the extrusion of collagen from dermal fibroblasts.

147

Lrig1 regulates epidermal homeostasis by repressing Arhgap19 necessary for a functional hyalurosome associated with EGFR signaling

L Barnes,[1] B Darbellay,[1] J Saurat[2] and G Kaya[1] *1 University Hospital of Geneva Dermatology, University of Geneva, Geneva, Switzerland and 2 Swiss Center of Applied Toxicology, University of Geneva, Geneva, Switzerland*

The interaction of hyaluronate (HA) with its receptor CD44 in the interstitial space of epidermis was previously shown to regulate the epidermal homeostasis. As this interaction is disrupted in CD44KO mice, we explored their epidermal phenotype. HA is no longer detectable in the epidermis and the expression pattern of EGFR and Lrig1 was significantly disturbed in CD44KO mice. To understand the relationship between the interaction HA/CD44 and the expression of EGFR and Lrig1, we analyzed the previously described hyalurosome platform. In this model, the EGFR response is tightly dependent on cell protrusions raised by F-actin spines, also called filopodia. HA secretion as well as HA/CD44 interaction are necessary for filopodia stability. To further understand the relationship of the hyalurosome components with Lrig1 expression, we constitutively expressed a GFP-Lrig1 fusion peptide in human keratinocytes in vitro. As a functional inhibitor of EGFR, Lrig1 inhibited EGFR responsiveness as expected, but also filopodia and CD44 and HAS3 expression, two components of the hyalurosome platform. A gene expression profile experiment performed on these keratinocytes indicated that Lrig1 constitutive expression inhibited Arhgap19, a RhoGAP protein involved in the plasticity regulation of the actin cytoskeleton. RNA silencing experiments further confirmed that Arhgap19 was necessary for filopodia growth, HA secretion and EGFR signaling. On the other hand, silencing of Lrig1 activated the expression of hyalurosome components. Taken together, these results demonstrate that Lrig1, also considered as an epidermal stem cell marker, may regulate the quiescence by repressing Arhgap19 and F-actin modifications which are necessary for the hyalurosome platform activation and epidermal EGFR signaling. HA/CD44 interaction which is important for the cytoskeleton plasticity may also play a crucial role in stem cell recruitment by regulating EGFR signaling.

148

Loss of Adhesion in Pemphigus Vulgaris Occurs without Apoptosis yet Involves Transient Low-level Activation of Caspase 3

A Galichet, C Luyet, B Sayar, K Schulze, D Howald, M Suter and E Müller *Animal Pathology, University of Bern, Bern, Switzerland*

Apoptosis has been proposed as a relevant pathogenic event in the autoimmune blistering disease Pemphigus vulgaris (PV). Although the presence of apoptotic cells in PV patient's skin has been controversially debated for many years and a recent survey of four PV patients did not confirm this finding, support for the implication of cell death in PV acantholysis came from the observation that caspase-3 inhibitors prevented epidermal blisters in the neonatal PV mouse model. This prompted us to specifically evaluate the involvement of apoptosis in correlation with caspase-3 activation in PV. We used a combination of cultured keratinocytes, established PV mouse models and biopsies from PV patients to firstly investigate the occurrence of apoptosis and secondly, caspase-3 activation in response to the Dsg3-specific pathogenic antibody AK23 or purified PV patient's IgG. Our data demonstrate that acantholysis in PV occurs without implicating apoptosis and robust activation of apoptotic pathway components. However, an early, transient and low-level caspase-3 activation was revealed which functionally contributes *in vitro* and *in vivo* to major pathological events in the acantholytic process, such as increased expression of proliferation markers including c-Myc, p38 activation, cleavage of Dsg3, keratin retraction as well as loss of intercellular adhesion and blister formation. Our results support a redefined PV model in which caspase-3 activation is not a trigger for apoptosis but contributes as an important early event to PV blistering, thus representing a promising therapeutic target in PV.

149

Subcutaneous adipose layer influences dermal layer structure and function via secreted factors

T Ezure and S Amano *Shiseido Co. LTD, Yokohama, Japan*

Obesity is a significant risk factor of skin disease, including ulcers and delayed wound healing. Subcutaneous adipose tissue (SAT) mass beneath the dermal layer increases drastically in obesity. This study aimed to clarify whether and how SAT, usually regarded as inert fat storage tissue, influences dermal condition. Dermal elasticity (measured with a cutometer) in subjects with a wide range of SAT mass decreased with increment of SAT. Histology of human abdominal skin indicated that increment of SAT mass is associated with a decrease of elastic fibers in the dermal layer. As increased SAT mass was also associated with adipocyte enlargement, we prepared enlarged adipocytes by long-term culture of adipocytes induced from 3T3-L1 fibroblasts. Microarray analysis revealed that gene expression of MMP-9, which has elastase activity, was increased in enlarged adipocytes, and MMP-9 secretion was confirmed by ELISA. These changes were blocked by inhibitors of the extracellular-signal-regulated kinase (ERK) pathway. Nuclear translocation of transcriptional factor AP-1 (a MMP-9 regulator), a downstream factor in the ERK pathway, was also observed. MMP-9 protein was immunohistochemically confirmed to be increased in SAT from obese subjects. These results suggest that SAT influences dermal condition by secreting factors (e.g., MMP-9) that directly degrade dermal matrix. Next, we co-cultured dermal fibroblasts with small adipocytes (representative of normal subjects) and enlarged adipocytes (representative of obese subjects) in a system where the two cell types were separated by a permeable membrane, through which secreted factors could pass. Enlarged adipocytes decreased expression of elastin and other matrix-related genes in fibroblasts, whereas small adipocytes increased expression of some of these genes. This suggests that enlarged adipocytes also negatively influence dermal condition indirectly by modulating dermal fibroblast gene expression. We propose that subcutaneous adipose tissue influences dermal structure and function both directly and indirectly via multiple secreted factors, depending on adipocyte size.

150

Comparative study of transcriptome and miRNome profiles of normal and senescent cells after exposure to solar simulator

S Bordes, P Rouaud, V Barruche, D Boudier and B Closs *Silab, St-Viance, France*

Understanding the transcriptomic modulations has become a growing priority, especially in the field of cutaneous senescence. In this context, we studied on the same in vitro model of cellular senescence, the modulations of microRNA profile (miRNome) and their association with modifications of mRNA profile (transcriptome). First, dermal fibroblasts were submitted to repetitive doses of solar simulator in order to induce a senescent state. Then, this phenotype was validated by analyzing aging markers (β-galactosidase coloration, quantification of p21 expression and cellular cycle monitoring) and evaluating histones modulations (H3K9me3 by western blotting and gH2AX by immunocytofluorescence). Finally, miRNomes were monitored by microarray and compared to transcriptomic profiles to determine their targets. Datas were refined and validated by QPCR. After treatment, aging markers were enhanced since p21 expression and β-galactosidase activity were increased by 384% and 417% respectively whereas cellular cycle detection was reduced by 85%. H3K9me3 and gH2AX were also increased by 303% and 471% respectively. Once our in vitro model of senescence validated, the comparison of miRNome and transcriptome profiles highlighted the modulation of different biological processes. Our more accurate QPCR analysis confirmed that genes involved in chromatine methylation were upregulated whereas genes involved in acetylation were down-regulated. Genes coding matrix proteins were also down-regulated. All these results were inversely correlated with miRNA profiles. In conclusion, we designed an in vitro model of senescence which is characterized by aging markers modulations correlated to an increase of histones modifications. This phenotype is linked to a modification of miRNome, associated with a modification of transcriptome. This is the first study assuming that senescence is associated with a modulation of miRNAs regulating the expression of genes coding for matrix components. These results will support the study of molecules working on the modulations observed.

151

Dissecting the interaction of plakins with epidermal keratins: role of plakins C-terminal domains, type I keratins coil 1 and keratins quaternary structure

B Favre,[1] J Bouameur,[1] L Fontao[2] and L Borradori[1] *1 Dermatology-Clinical Research, Bern University Hospital-Inselspital, Bern, Switzerland and 2 Dermatology, Geneva University Hospitals, Geneva, Switzerland*

The plakin protein family is composed of cytolinkers connecting elements of the cytoskeletal system to each other and to various membrane sites. Thereby plakins confer to cells critical resilience to mechanical stress in distinct tissues, such as the epidermis. To gain further insight into the connection of the plakins BPAG1-e, desmoplakin and plectin with the basal epidermal keratins 5 (K5) and 14 (K14) or suprabasal keratins 1 and 10, we have characterized their interactions. For this purpose we used a variety of full-length and truncated proteins in yeast-three hybrid assays, cell transfection studies and sensitive fluorescent-binding assays. The results showed that: 1) desmoplakin and plectin interact much better with monomeric type I than type II keratins, while BPAG1-e interacts only with heterodimeric K5/K14, K6/K17 and heterotypic combinations of them; 2) the coil 1 domain of type I keratins contains sequences essential for their binding to plakins; 3) the quaternary structure induced by hetero-polymerization of keratins increases the number of binding sites for plakins; 4) serial truncations of the COOH-extremity of desmoplakin or plectin significantly decrease their binding to keratins; and 5) recombinant plakins carrying distinct pathogenic mutations identified in human diseases associated with cell fragility exhibit weaker binding properties than their normal counterparts. Our findings indicate for the first time that distinct mutations within the C-terminus of BPAG1-e, plectin or desmoplakin identified in epidermolysis bullosa simplex or palmoplantar keratoderma patients are pathogenic by affecting the interaction of plakins with the keratin intermediate filaments.

152

20-O- β-D-glucopyranosyl-20(S)-protopanaxadiol Suppresses stress-induced cellular senescence in skin cells

H Choi, J Lee, J Lee and H Lee *Skin Research Institute, R&D Center, AmorePacific Corporation, Yongin-si, Republic of Korea*

Photoaging of skin is due to accumulative effect of Ultraviolet (UV) irradiation. UV irradiation promotes extracellular matrix (ECM) breakdown in dermis by inducing matrix metalloproteinase (MMP)-1 expression. It generates severe oxidative stress in skin cells resulting in DNA damage and premature cellular senescence. Collagen synthesis in senescent dermal fibroblasts do not recover ECM breakdown. Ginseng is one of the most widely used herbal medicines in Asia. Ginseng's efficacy is based on ginsenosides. 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (Compound K) is a major ginsenoside metabolite of ginseng extract after oral administration. Compound K has been used to improve aged skin. Compound K inhibits MMP-1 expression induced by UV irradiation through AMPK activation. However, the effect of compound K on premature cellular senescence was unknown. To investigate inhibitory effect of compound K on cellular senescence, we established premature senescent dermal fibroblasts and epidermal keratinocytes induced with 100uM hydrogen peroxide. Senescence-associated β-Galactosidase (SA-β-Gal) staining was used for detecting senescent cells. Premature senescent skin cells showed strongly SA-β-Gal positive with intense staining compared to normal skin cells. The number of SA-β-Gal positive cells was decreased in compound K treated cells compared with control cells under oxidative stress (dermal fibroblasts; 50.5±2.7% vs. 74.7±11.1%_19.2±7.1%, epidermal keratinocytes; 51.7±5.2% vs. 76.4±3.3%_10.9±7.1%). Compound K reduced p21 protein expression induced by oxidative stress in epidermal keratinocytes. Here, we report that compound K inhibits stress-induced cellular senescence through p21 inhibition in skin cells. These findings showed the mechanisms of the anti-aging effects of compound K against photoaged skin.

153

Alpha-melanocyte-stimulating hormone reduces bleomycin-induced mediated collagen synthesis via catalase in dermal fibroblasts

M Böhm, M Apel, T Luger and A Stegemann *Dept. of Dermatology, University of Münster, Münster, Germany*

Neuroendocrine mediators such as melanocortins, serotonin or endocannabionoids are emerging as endogenous regulators of collagen synthesis and skin fibrosis. We previously reported that alpha-melanocyte-stimulating hormone (alpha-MSH) suppresses bleomycin (BLM)-induced collagen synthesis in human dermal fibroblasts (HDFs) in vitro via reduction of oxidative stress. The relevance of this finding was confirmed in vivo in the BLM model scleroderma in which alpha-MSH attenuated the extent of experimentally induced skin fibrosis. To clarify the molecular mechanism behind this effect of alpha-MSH we examined the expression and activity of various antioxidative enzymes in HDFs. Interestingly, catalase activity but not mRNA and protein expression was time-dependently increased by alpha-MSH. In support of this observation exogenous catalase abrogated the inductive effect of BLM on COL(I) expression. Gene knock-down of catalase by siRNA neutralized the impact of alpha-MSH on BLM-induced COL(I) expression. A functional melanocortin 1 receptor (MC_1) was essential for the suppressive effect of alpha-MSH on BLM-induced collagen synthesis since HDFs carrying loss of function alleles of *Mc1r* did not react to the neuropeptide. To finally assess the role of a functional MC_1 in the context of BLM-induced collagen synthesis in vivo we injected mice with signalling-deficient MC_1 (recessive yellow C57BL/6-Mc1re/e mice) with BLM. Wild-type C57BL/6J mice were previously shown to be BLM-insensitive. Notably, only in C57BL/6J-Mc1re/e mice BLM induced skin fibrosis. In summary, our findings show that alpha-MSH via MC_1 and catalase attenuates BLM-induced collagen synthesis in HDFs in vitro. Expression of functional MC_1 in vivo protects against BLM-induced skin fibrosis.

154

Analysis of the pigmentation defect in mice with keratinocyte specific deletion of the laminin γ 1 chain

Y Üstün,[1] A Fleger-Weckmann,[1] M Reibetanz,[1] B Brachvogel,[2] Z Chen,[3] L Langbein,[4] L Sorokin[5] and R Nischt[1] *1 Dermatology, University Hospital of Cologne, Cologne, Germany, 2 Medical Faculty, Biochemistry, University of Cologne, Cologne, Germany, 3 Laboratory of Neurobiology and Genetics, The Rockefeller University, New York, NY, 4 German Cancer Research Center, Heidelberg, Germany and 5 Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Münster, Germany*

Laminins, one of the major basement membrane (BM) components, are a family of heterotrimeric glycoproteins. Each heterotrimer is composed of an α, β and γ chain, with the γ1 chain being the most abundant γ chain. To analyze the function of the γ1 chain containing laminin isoforms in skin, mice with keratinocyte-specific deletion of the γ1 chain were generated using the Cre/*loxP* system. This deletion ($LAMC1^{EKO}$) results in the loss of laminin-511 from the BM and ectopic deposition of laminin-211, identifying keratinocytes as the source of laminin-511 and fibroblasts as the source of laminin-211 expression. $LAMC1^{EKO}$ mice display a delay of hair follicle differentiation and pigmentation among other phenotypes. Hairless regions like ears and nose are normally pigmented, however, in hairy skin regions pigmentation is impaired. Analysis of skin sections reveals epidermal retention of Tyrosinase-positive melanocytes in $LAMC1^{EKO}$ mice, indicating a defect in melanocyte migration into the hair follicle. Further, expression of the enzymes Trp1 and Trp2 is significantly reduced in $LAMC1^{EKO}$ mice although FACS analysis ($CD45^{-}/cKit^{+}$) shows comparable numbers of melanocytes in the epidermis of both $LAMC1^{EKO}$ and control mice. *In vitro* melanocytes isolated from control and $LAMC1^{EKO}$ mice show comparable migratory and adhesive activities on laminin-511 and -211. This suggests that the pigmentation defect might be linked to an impaired crosstalk either directly between keratinocytes and melanocytes or indirectly between cells of the hair follicles and melanocytes in $LAMC1^{EKO}$ skin due to the changes in BM composition. This is currently under investigation.

155

Anti-aging effect of undifferentiated *Argania spinosa* cells extract on dermal-epidermal junction in both human keratinocytes and photodamaged full-thickness skin substitutes

H Hernandez-Pigeon, D Bacqueville, N Steward, L Duprat, A Caruana, T Nguyen, N Castex-Rizzi, H Duplan and S Bessou-Touya *Pierre Fabre Dermo-Cosmétique, Toulouse, France*

Skin aging is characterized by wrinkling and laxity, and displays a hyaluronic acid (HA) deficit and a flattening of the dermal-epidermal junction (DEJ). Skin photoaging is induced by an excessive ultraviolet (UV) exposure. The aim of this study was to evaluate the anti-aging properties of an extract obtained from plant cell culture of *Argania spinosa* (Argan cell extract (CE)). Its efficacy was evaluated on HA and DEJ in human keratinocytes and an *in vitro* photodamaged full-thickness human skin model following topical application. Keratinocytes were cultured 48 hours with increasing concentrations of Argan CE from 0.2 to 1 mg/mL, and assayed by ELISA and immunolabeling for the expression of mediators involved in epidermal regeneration and DEJ structure. The results showed that the Argan CE strongly induces the expression of HA, but also collagen IV and laminin 5. Confocal microscopy revealed that chronic UVA exposure (3x4 J/cm^2) disorganizes the DEJ network and alters both collagen IV and perlecan staining in irradiated-skin substitutes. In contrast to a placebo, the topical application of an Argan CE formulation at 5 mg/cm^2 efficiently protected skin and well preserved the DEJ network from UVA damage. Thus, the Argan CE presented anti-aging properties on both keratinocytes and *in vitro* reconstructed skin models and was able to prevent UVA-induced photoaging, suggesting that it may be useful for the development of new anti-aging dermo-cosmetic products.

Download English Version:

https://daneshyari.com/en/article/3215380

Download Persian Version:

https://daneshyari.com/article/3215380

Daneshyari.com