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362

Deletion of the glucocorticoid receptor chaperone Fkbp5 unexpectedly prevents development of glucocorticoid-induced cutaneous atrophy via Akt activation

G Baida,[1] P Bhalla,[1] H Chen,[2] W Shou,[2] E Sanchez[3] and I Budunova[1] *1 Dermatology, Northwestern University, Chicago, IL, 2 Physiology & Pharmacology, University of Toledo College of Medicine, Toledo, OH and 3 Wells Center For Pediatric research, Indiana University School of Medicine, Indianapolis, IN*

Fkbp5 (FK506 binding protein 51) is a known co-chaperone and regulator of glucocorticoid receptor (GR), which attenuates GR activity by preventing its nuclear translocation. We and others showed previously that Fkbp5 is one of the major GR target genes in epidermis/skin. However, its role in clinical effects of topical glucocorticoids in the skin has not been studied. Here, we used Fkbp5 knockout mice to determine the role of Fkbp5 in the major adverse effect of topical glucocorticoids, skin atrophy. We discovered that adult Fkbp5 KO mice have significant skin phenotype including epidermal hyperplasia and frequent follicular cysts suggesting the important role of Fkbp5 in epidermis maintenance and hair development. Importantly, Fkbp5 KO animals appeared much more resistant to skin atrophy induced by chronic treatment with glucocorticoid fluocinolone acetonide (FA) which coincided with protection of CD34+ keratinocyte stem cell population in the hair follicle bulge niche in Fkbp5 KO mice. Paradoxically, despite the lack of inhibitory Fkbp5, the activation of some GR target genes was attenuated in vitro and in vivo. Among them was REDD1 which we have recently identified as a marker of steroid-induced skin atrophy. GR activation by glucocorticoids results in both genomic and non-genomic effects, including the inhibition of Akt, a major mediator of proliferative signaling in the skin. It is known that Fkbp5 negatively regulates Akt phosphorylation and activity. Importantly, we found drastic increases in Akt Ser473 phosphorylation in the epidermis of both vehicle- and FA-treated Fkbp5 KO mice compared to wt animals as well as in shFkbp5 keratinocytes in vitro. Overall, our results suggest that Akt activation maybe responsible for the resistance of Fkbp5 KO mice to atrophic effects of topical glucocorticoids.

363

c-Src/Cav1-dependent activation of the EGFR by Dsg2

KP McGuinn,[1] F Cooper,[1] DM Brennan-Crispi,[1] JK Wahl[2] and MG Mahoney[1] *1 Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA and 2 Oral Biology, University of Nebraska Medical Center, Lincoln, NE*

Squamous cell carcinoma (SCC) is the second most common skin cancer and incidence continues to rise. Mutations leading to EGFR overexpression and activation contribute to the development and progression of SCC. We recently showed that the desmosomal cadherin desmoglein 2 (Dsg2) is upregulated in SCC and activates mitogenic signaling including the MAPK/ERK pathway. Here we investigate the crosstalk between the EGFR and Dsg2 signaling pathways that may provide a mechanism by which Dsg2 stimulates epithelial proliferation, migration and survival. We generated stable *Dsg2* knockdown in HaCaT cell lines by shRNA and showed that loss of Dsg2 reduced EGFR expression and activation in response to EGF ligand. With loss of Dsg2 the level of activated c-Src, a non-membrane tyrosine kinase proto-oncogene, was also abrogated. Pharmacological inhibition of c-Src with PP2, reduced phosphorylation of EGFR independent of EGF ligand, suggesting EGFR activation is mediated in part by c-Src. We recently established Dsg2 binds to caveolin 1 (Cav1), the major component of caveolae. These specialized membrane lipid rafts regulate cell communication by compartmentalizing signaling proteins. c-Src has been shown to phosphorylate Cav1, which in turn binds c-Src rendering it inactive. Here we show that in absence of Dsg2, c-Src and Cav1 localize to the raft fractions with concomitant increase in phospho-Cav1 indicating c-Src is inactive. In the presence of Dsg2, Cav1 is sequestered outside the rafts releasing its negative effects on c-Src, thus providing a mechanism for Dsg2 to regulate cell signaling. Perturbation of lipid rafts with the cholesterol-chelating agent MβCD disrupted cell-cell adhesion, which was rescued by MAPK/ERK and c-Src inhibitors, suggesting that cell adhesion is modulated in part by cell signaling. As such, knockdown of Dsg2 also protected cells from MβCD-mediated disadhesion. Taken together, these observations suggest that Dsg2 may enhance tumor development by positively regulating EGFR level and signaling.

364

Significant birth weight advantage of Melanocortin–1–Receptor variants in humans, an evolutionary pressure?

AC Thomas,[1] C Santos,[2] GE Moore[1] and VA Kinsler[1] *1 Genetic and Genomic Medicine, UCL Institute of Child health, London, United Kingdom and 2 Birth Defects Research Centre, Institute of Child Health, London, United Kingdom*

The Melanocortin-1-Receptor (*MC1R*) gene encodes a G-Protein-Coupled Receptor key to the production of melanin, and is involved in normal pigmentation, pigmentary disorders including melanoma, and opioid receptor pathways. Recent evidence that *MC1R* variants may play a role in fetal growth led us to investigate the effect of this gene on birth weight in a large cohort of healthy children. The genotype for previously implicated variants R151C and V92M was established for 9139 and 962 children respectively from the UK ALSPAC study cohort. This was accompanied by detailed maternal phenotyping data. Using linear regression modeling, a striking association was found between both variants separately and birth weight (R151C resulted in babies 106.9g heavier with a p-value of 0.011, V92M resulted in babies 90.4g heavier with a p-value of 0.035), independent of known birth weight modifiers. This strongly suggests that the evolution of these common *MC1R* variants could be in part due to a survival advantage from increased birth weight, independent of maximisation of vitamin D production in low UVB areas. Possible mechanisms underlying this effect were studied in human tissue, using qRT-PCR of MC1R in 30 term placental samples with matched genotype, and in situ hybridisation in human embryonic tissue at different stages. No differences were found in placental expression between wild-type and variant genotype. MC1R expression was detected in fetal tissue, in areas involved in bone growth and neuronal development, providing a tentative mechanism for the effect of genotype on birth weight.

365

A disintegrin and metalloproteinase 17 (ADAM17) dependent EGFR signaling modulates epidermal barrier maintenance through PKC

C Cobzaru, L Bruckner-Tuderman and C Franzke *Dermatology, University Medical Center Freiburg, Freiburg, Germany*

The multilayered epidermis builds a vitally important skin barrier. Weakening of this barrier seems to be tightly connected to the pathogenesis of chronic inflammatory skin diseases. We have previously shown that conditional keratinocyte specific loss of ADAM17, the principal EGFR ligand sheddase, results in postnatal skin barrier defects with decreased transglutaminase (TGM) activity, followed by massive immune cell infiltrations. These effects were most likely caused by strongly repressed EGFR signaling in the keratinocytes, since *in vitro* differentiated *Adam17*$^{-/-}$ and *Egfr*$^{-/-}$ keratinocytes revealed a cell autonomous decrease in TGM1 expression and activity, while TGM activity was restored in differentiated *Adam17*$^{-/-}$ keratinocytes by addition of EGFR ligand TGFα. To identify the pathways by which the ADAM17/EGFR axis regulates TGM activity, we have analyzed the MAPK, PI3K/Akt and PLC/PKC signaling as well known EGFR downstream pathways. Western blot analysis of *Adam17*$^{-/-}$ keratinocytes and epidermis revealed reduced phosphorylation of ERK and PLCγ1 as well as decreased expression of PKCη. Using TGFα-stimulated differentiated *Adam17*$^{-/-}$ keratinocytes as a model for EGFR dependent TGM activity, the addition of either the PLCγ1 inhibitor U73122 or the PKC inhibitor bisindolylmaleimide strongly repressed TGM activity. In addition, the strongly reduced TGM activity in differentiated *Adam17*$^{-/-}$ keratinocytes was completely restored by treatment with the phorbol ester TPA *in vivo* and *in vitro*. Our results suggest the ADAM17/EGFR axis as modulator of TGM activity through PLC/PKC signaling and highlight the importance of the EGFR/PLC/PKC pathway for terminal differentiation in keratinocytes. These findings may help to identify new therapeutic targets for inflammatory skin diseases, like atopic dermatitis or psoriasis.

366

B-Myb Enhances Proliferation and Suppresses Differentiation of Keratinocytes in Three-dimensional Cell Culture

H Maruyama,[1] Y Ishitsuka,[1] Y Fujisawa,[1] J Furuta,[1] Y Kawachi,[1] M Sekido[2] and M Fujimoto[1] *1 Dermatology, University of Tsukuba, Tsukuba, Japan and 2 Plastic Surgery, University of Tsukuba, Tsukuba, Japan*

B-Myb (Mybl2) is a member of the Myb gene family of transcription factors involved in the control of cell growth, differentiation, and apoptosis. The effects of B-Myb on keratinocyte proliferation and differentiation have not yet been clarified. The present study was performed to examine the role of B-Myb in proliferation and differentiation of the spontaneously immortalized human skin keratinocyte cell line HaCaT and normal human keratinocytes with formation of a stratified epidermoid structure in air-liquid interface 3-dimensional culture. B-Myb was expressed specifically in undifferentiated normal keratinocytes and downregulated during differentiation. The constitutive overexpression of B-Myb in HaCaT cells during air exposure-induced differentiation resulted in an undifferentiated phenotype, i.e., thickening of the stratified layers, suppression of differentiation marker expression, and retention of proliferative activity with activation of cell cycle regulatory proteins in the S and G2/M phases. In contrast, suppression of B-Myb caused their downregulation and constrained proliferation with retention of differentiation capacity. These findings suggested that B-Myb may play an important role in maintenance of the undifferentiated phenotype of keratinocytes in the basal epidermal layer.

367

Endocannabinoids and growth factors cross-talk in the process of wound healing

G Cardinali,[1] D Kovacs,[1] E Flori,[1] S Briganti,[1] R Bertorelli,[2] D Piomelli[2] and M Picardo[1] *1 Laboratory of Cutaneous Physiopathology and Integrated Center of Metabolomics Research, San Gallicano Dermatologic Institute, Rome, Italy and 2 Drug Discovery and Development, Italian Institute of Technology, Genoa, Italy*

The interplay among the pathways triggered by growth factors and lipid mediators is crucial in the healing process. Among the lipid messengers, the endocannabinoids (eCBs) exert proliferative and migratory effects via both their specific G protein-coupled receptors and the trans-activation of growth factor tyrosine kinase receptors. To analyse the biological effects induced by the modulation of endogenous eCB levels in the wound repair we used an inhibitor of the fatty-acid amide hydrolase, (URB 597), an enzyme that catalyzes the anandamide (AEA) intracellular hydrolysis, on human primary cultures of keratinocytes and fibroblasts. The inhibitor (0.1-1.0 μM) induced a dose dependent increase of AEA at intracellular and extracellular levels (from 100 to 500 % and from 50 to 200% respectively). No effect on keratinocyte proliferation, but a decrease (10-40%) of keratin 1 and an increase (20%) of keratin 16 expression were observed at 1 μM indicating the induction of a de-differentiation and activation process. A migration promotion was demonstrated by the scratch assay (30-40% reduction area). Increased intracellular levels of AEA induced the phosphorylation of the epidermal growth factor receptor (up to 2.3 fold increase). In turn, treatment with epidermal growth factor augments the levels of eCBs. Treated fibroblasts displayed a trans-differentiation in myofibroblasts with an increased expression of alpha-smooth muscle actin (20-30%) and of collagen type I production (20-40%). Moreover an increased hepatocyte growth factor expression was detected (40-60%). Our results demonstrate that the signaling pathways mediated by lipids and growth factors work in concert favouring cellular processes involved in the wound repair.The knowledge of the network generated might open important therapeutic perspectives to specifically manage conditions of impaired wound healing.

368

Insulin Activates Phosphoinositide–3–Kinase/ Akt/ Forkhead box-O1 Pathway in SZ95 Sebocytes *in Vitro*

Y Mirdamadi,[1] A Thielitz,[1] A Wiede,[1] SR Quist,[1] M Lotzing,[1] E Papakonstantinou,[1] L Simeoni,[2] CC Zouboulis[3] and H Gollnick[1] *1 Department of Dermatology and Venereology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany, 2 Institute of Molecular and Clinical Immunology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany and 3 Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, Germany*

A recent hypothesis in the pathogenesis of acne suggests that nutrition related acne-inducing factors exert their action by reducing nuclear transcription factor Forkhead box-O1 (FoxO1) levels via phosphoinositide-3-kinase (PI3K)/ Akt pathway. The aim of our study was to confirm in vitro the yet hypothetical nutrigenomic regulation of insulinotropic diet and thereby support the role of FoxO1 as a key molecule in the acne pathogenesis. SZ95 sebocytes were treated with insulin in a time and dose dependent manner and nuclear and cytoplasmic expression levels of p-Akt and p-FoxO1 were analyzed by western blot. FoxO activity was determined by dual luciferase reporter assay. Proliferation of sebocytes was measured by [^{3}H] thymidine incorporation assay and differentiation by semiquantitative analysis of lipid droplet accumulation using Oil Red O staining. Western blotting results revealed an activation of the PI3K /Akt /FoxO1 pathway with a cytoplasmic up-regulation of p-Akt (60 minutes) and p-FoxO1 (90 minutes) after stimulation with 1μM insulin. FoxO activity detected after 30, 60 and 90 minutes was decreased upon 1μM and 0.1μM insulin incubations. The DNA synthesis was suppressed and in contrast sebocytes differentiation was increased after insulin incubation. Our data support the role of insulin as an activator of p-FoxO1 via nuclear mobilization of FoxO1 after cytoplasmic activation of PI3K / Akt pathway. We showed FoxO1 acted as a mediator for the regulation of insulin-induced effects on sebaceous lipogenesis and proliferation in vitro. Our data support the potential role of FoxO1 as a key molecule in pathogenesis of acne.

369

KGF influences EDA$^+$FN production in fibroblasts through the MAPK cascade

B Gubán Konczné,[1] K Vas,[1] A Bebes,[1] G Groma,[1] M Széll,[2] L Kemény[1] and Z Bata-Csorgo[1] *1 Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary and 2 Department of Medical Genetics, University of Szeged, Szeged, Hungary*

In our previous work we showed that the fibronectin splice variant extradomain-A (EDA$^+$FN), its receptor the alpha5-integrin, the keratinocyte growth factor (KGF, FGF-7), and its receptor (FGFR2) are overexpressed in psoriatic uninvolved skin compared to healthy skin. We also found a putative autocrine regulatory loop between KGF and EDA$^+$FN in fibroblasts. In our current work we aimed to elucidate the signaling mechanisms leading to the KGF mediated changes in EDA$^+$FN expression. For that we opted to use inhibitors of the major KGF induced signaling pathways, the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (Akt) pathways known to act downstream of FGFR2. Cultured normal human fibroblasts were treated with human recombinant KGF. We carried out RTq-PCR and flow cytometric measurements to analyze EDA$^+$FN production 24 hours after applying exogenous KGF and inhibitors of MEK-1 and Akt ½. EDA$^+$FN protein expression increased significantly following exogenous KGF treatment (n=7, p<0.01), which was inhibited by a specific MEK-1 inhibitor (PD 098059), indicating that MAPK cascade activation is essential for the effect (n=7, p<0.05). Blocking the Akt ½ kinase resulted in a notable induction of EDA$^+$FN protein expression in fibroblasts (n=6) that was not influenced by the addition of exogenous KGF. These data indicate the existence of a previously unknown regulatory feedback loop of KGF in fibroblasts affecting EDA$^+$FN production, which may be relevant in psoriasis pathomechanism.

370

Melanocytes: Potential role in psoriasis?

Z Balog, G Groma, A Bebes, L Kemény and Z Bata-Csörgö *Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary*

Immune cells and keratinocytes contribute substantially to the formation of skin lesions in psoriasis, however the role of melanocytes, another important epidermal resident cell type is not fully understood. We showed that the keratinocyte growth factor (KGF) as well as its receptor (FGFR2) and the fibronectin (FN1) splice variant EDA$^+$FN, are overexpressed in psoriatic uninvolved skin compared to normal skin. Here, we aimed to elucidate whether KGF could influence the expression of FN1 and EDA$^+$FN in melanocytes. We determined that melanocytes express the FGFR2IIIb splice variant of KGF receptor. We also compared the adhesion ability of cultured human melanocytes and peripheral blood mononuclear cells (PBMCs) derived from psoriatic patients to healthy controls. Human melanocytes derived from healthy and psoriatic uninvolved skin were cultured in AIMV and Keratinocyte –SFM (1:1) media mixture. Melanocytes in 3rd passage were treated with human recombinant KGF and after 24 and 48 hours EDA$^+$FN protein levels were measured by flow cytometry. Unlike keratinocytes, melanocytes expressed fibronectin and EDA$^+$FN at comparable levels to that of fibroblasts in culture. KGF treatment did not alter FN1, EDA$^+$FN and FGFR2 protein levels, as determined by flow cytometry. Cell adherence was measured using xCELLigence real time cell detection system with or without FN coating on the culture plates. We observed that melanocytes and PBMCs of psoriatic patients showed higher adhesion to fibronectin coated plates compared to healthy controls. The altered adhesive properties of melanocytes and PBMCs in psoriatic patients may contribute to psoriasis patomechanisms.

371

Neurite elongation of a neuro-sensory cell line was promoted by piperine, an alkaloid extract from *Piper Nigrum*

Z Zarei,[2] R Leschiera,[1] J Carré,[1] L Misery[1] and N Lebonvallet[1] *1 Laboratoire des neurosciences de brest, Brest, France and 2 Laboratoire de Biochimie & de Génie Enzymatique des Lipases, Sfax, Tunisia*

Black pepper or *Piper Nigrum* is a vegetal of the family of Piperacea. It is traditionally used as a flavoring agent in foods. It also has biological activities and is often used in traditional medicine. Anti -inflammatory, antioxidant and analgesic activity were recognized. One of its active ingredients is piperine which is an alkaloid. In this work, neurite outgrowth/elongation of PC12 cells, was studied. Piperine was diluted in diméthylsulfoxide. The effects of piperine at the concentrations of 0.05, 0.5, 5 and 50 mg/mL were assessed. The PC12 cell line, used as a model of sensory neurons, was cultured at 50 000 cells per well in medium containing nerve growth factor (NGF) as inductor of differentiation. Cells were incubated with piperine for 5 days and then the length of the neurites was assessed. We shown that neurite length was increased by 30% with 5 mg / mL of piperine. Furthermore, piperine did not induce cytotoxic activity evaluated by lactate deshydrogenase and MTT test and perturbation of mitochondrial activity until 50 mg/ml. This work is interesting because it highlighted a previously unknown biological property of piperine. We suggested that piperine may be tested in circumstances where neuronal growth is inhibited, as for example in burns.

372

The NADPH oxidase isoform 4 controls TGF-beta 1-mediated activation of human dermal fibroblasts - a promising new target for the treatment of systemic sclerosis?

H Dosoki,[1] A Stegemann,[1] M Taha,[2] HJ Schnittler,[2] K Hashimoto,[3] J Kudla,[3] T Luger,[1] C Kerkhoff[4] and M Böhm[1] *1 Department of Dermatology, University of Münster, Münster, Germany, 2 Department of Anatomy, University of Münster, Münster, Germany, 3 Institute of Biology and Biotechnology of Plants, University of Münster, Münster, Germany and 4 Department of Biomedical Sciences, University of Osnabrück, Osnabrück, Germany*

The pathogenesis of systemic sclerosis is still incompletely understood. Transforming growth factor-β1 (TGF-beta1)-mediated fibroblast activation plays a central role in this disease and oxidative stress has long been implicated in the development of tissue fibrosis. However, the precise molecular interplay between TGF-beta1 and oxidative stress generating intracellular enzymes remains largely unexplored in human dermal fibroblasts (HDFs). We showed that the NADPH oxidase isoform Nox4 is expressed in neonatal and adult HDFs as well as in HDFs from SSc patients. Stimulation of normal HDFs with TGF-beta1 resulted in a time- and dose-dependent induction of Nox4 mRNA and protein. This effect of TGF-beta1 was mediated by transcriptional induction and associated with increased NADPH activity in membrane fractions of HDFs. Immunofluorescence analysis with laser confocal microscopy studies further revealed that Nox4 localizes to the endoplasmic reticulum as demonstrated by double staining with protein disulfide isomerase. Importantly, pharmacological inhibition of NADPH oxidase activity or Nox4-specific knock-down not only suppressed TGF-beta1-mediated expression of collagen type I COL(I) but also induction of both alpha-smooth muscle actin and fibronectin 1 in normal HDFs. Likewise, alpha-melanocyte-stimulating hormone, a neuropeptide with antioxidative and antifibrogenic effects, suppressed TGF-beta1-mediated expression of Nox4 in HDFs. In summary, our findings highlight Nox4 as a novel intracellular nodal point that mediates fibroblast activation. Targeting this oxidative stress-generating enzyme may become a novel approach for the treatment of fibrotic skin diseases.