607 Phenotypical characterisation of mice deficient for the channel activating protease 2/Tmprss4
A Koppener, ID Andreasen, A Merillat and E Hummelke. Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland.

Channel activating protease 2 (CAP2/Tmprss4) is a member of the family of membrane-bound serine proteases and is highly expressed in skin. CAP2 belongs to the family of serine proteases being expressed in various organs, with highest expression in digestive tract and skin. We recently identified membrane-bound serine proteases as upstream activators of the protease-activated receptor PAR2 being involved in inflammation, with barrier function. To study the effect of CAP2 in vivo, we generated mutant mice for CAP2/Tmprss4, in which the histidine and aspartate of the catalytic triad have been deleted. These CAP2 knock-out mice (CAP2/ΔΔ mice) do not show abnormal phenotype, they are viable and fertile, and their pups are born according to the expected Mendelian distribution. Moreover, body weight and histological analysis showed no particular difference between control and knock-out littermates. These mice are now analysed with respect to their epidermal barrier function and their capacity to induce ENEA-mediated sodium currents in vivo e.g., in colon. This work is supported by the Swiss National Science Foundation to E. Hummelke.

608 Phosphorylation of the keratin K10 plays an important role in maintaining the physical properties of the stratum corneum
N Nakagawa, N Shirrimi and S Sakai. Innovative Beauty Science Laboratory, Kanebo Cosmetics Inc., Kanagawa, Japan.

We previously demonstrated that potassium lactate—a natural moisturizing factor (NMF)—increases the water-holding capacity of the stratum corneum (SC) by increasing interaction between water molecules and the CH2 group of SC keratin, suggesting that the modification of serine residues in SC keratin may affect the physical properties of the SC. In general, some serine residues in SC keratin are phosphorylated; moreover, acid phosphorylase activity in the SC of patients with atopic dermatitis and psoriasis is higher than in the SC of healthy subjects. However, no quantitative analysis of the relationship between phosphorylation of the SC keratin and the physical properties of the SC has been conducted. We therefore investigated whether phosphorylation of keratin K10 in SC keratin correlated positively with the physical properties of the SC. The Fourier of 40 healthy male subjects was treated with water for 5 minutes to reduce the effects of NMFs. After 20 minutes, the SC water content and SC stiffness (st) were measured using a conical Raman spectrometer and Venustron tactile sensor, respectively. SC protein was extracted from tape-stripped SC, and the bands of keratin K10 and phosphorylated protein were detected using western blotting with anti-K10 and anti-phosphopeptide antibodies, respectively. The rate of phosphorylation of K10 was increased by the intensity of this method. In conclusion, this is the first step in seeking alternatives for the classical tar therapy and may aid in drug discovery and development for skin diseases with disturbed epidermal differentiation.

609 Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis: The search for the active ingredient(s)
J van den Bogaard,1,2 J Bergsone,1 van Vlijmen-Willems,1 S Hats,1 P van der Valk,1 JM Schreuder,1 J Koerten,1 P Zeese1 and JS Schalkwijk.1 1Department of Dermatology, Rijnstate, Nijmegen, Netherlands, 2Lab of Med Immunology, Rijnstate, Nijmegen, Netherlands, 3Tumor Immunology, Rijnstate, Nijmegen, Netherlands and 4Cardiologie, Univ Hospital of Schleswig-Holstein, Kiel, Germany.

Topical application of coal tar is one of the oldest therapies for atopic dermatitis (AD). A TH2-like response in skin is characteristic of AD. We identify and characterize the active ingredient present in coal tar which would possibly circumvent some of the cosmetic and putative safety problems associated with coal tar. In addition, we will facilitate the development of mechanism-based drugs for AD patients. Using qPCR and cell-based ELISAs we analyzed known coal tar ingredients and coal tar fractions for the ability to promote the expression of differentiation-related genes in primary keratinocytes. We identified several polycyclic (hetero)aromatic compounds that induced filaggrin expression, and a mixture of the top 10 compounds present in coal tar induced differentiation. We observed a dramatic increase in binding of EGF to synthetic and physiological membranes with the addition of ACML. With a ratio of approximately 0.1% AHR/CYP11B1, we increased the expression of CYP11B1 and IL-1β leading to a decrease in the expression of CYP11B1 and IL-1β.

610 Modifying cellular membranes modulate cosmetic improvements in the skin
WC Smith, Dermam, Wellington, Fiji.

We previously demonstrated that potassium lactate—a natural moisturizing factor (NMF)—increases the water-holding capacity of the stratum corneum (SC) by increasing interaction between water molecules and the CH2 group of SC keratin, suggesting that the modification of serine residues in SC keratin may affect the physical properties of the SC. In general, some serine residues in SC keratin are phosphorylated; moreover, acid phosphorylase activity in the SC of patients with atopic dermatitis and psoriasis is higher than in the SC of healthy subjects. However, no quantitative analysis of the relationship between phosphorylation of the SC keratin and the physical properties of the SC has been conducted. We therefore investigated whether phosphorylation of keratin K10 in SC keratin correlated positively with the physical properties of the SC. The Fourier of 40 healthy male subjects was treated with water for 5 minutes to reduce the effects of NMFs. After 20 minutes, the SC water content and SC stiffness (st) were measured using a conical Raman spectrometer and Venustron tactile sensor, respectively. SC protein was extracted from tape-stripped SC, and the bands of keratin K10 and phosphorylated protein were detected using western blotting with anti-K10 and anti-phosphopeptide antibodies, respectively. The rate of phosphorylation of K10 was increased by the intensity of this method. In conclusion, this is the first step in seeking alternatives for the classical tar therapy and may aid in drug discovery and development for skin diseases with disturbed epidermal differentiation.

611 (Klotho is required for the epidermal differentiation, and its expression is suppressed in human nonmelanoma skin cancer
K Nakagawa,1 S Suea,1 R Haba,1 Y Kushida,1 N Katsuki,1 Y Hosokawa,1 T Moriea,1 K Noneda,1 H Kodama,1 and Y Kudaka1 1Department of Dermatology, Kagawa University, Kitagata, Japan, 2Institute of Innovative Science and Technology, Tokai University, Ibaraki, Japan, 3Diagnostic Pathology, Kagawa University, Kitagata, Japan and 4Cardiovascular Physiology, Kagawa University, Kitagata, Japan.

Mice deficient in the Klotho gene (Δkl/Δkl mice) display the phenotypes of human aging. However, the keratin K10, a Klotho gene-encoded protein, in skin is unclear. Using immunohistochemistry and Western blotting, we found that the expression of Klotho, a family of Klotho, and epidermal differentiation-associated factors (keratin 1, keratin 10, filaggrin, and loricrin) was lower in the skin of Δkl/Δkl mice than that of wild type mice. In vitro experiments showed that Klotho expression was induced concomitantly with the differentiation of normal human epidermal keratinocytes and an immortalized human epidermal keratinocyte cell line (HaCaT cells) when they were cultured in an air liquid interface. Klotho knockdown by small interfering ribonucleic acid suppressed the expression of the above differentiation-associated factors but activated p44/42 mitogen-activated protein kinase and Wnt pathways in HaCaT cells, resembling a human epithelial carcinoma cell line (A549 cells). Immunohistochemical analysis demonstrated that Klotho was expressed not only in normal human skin epidermis, but the expression of Klotho was suppressed in human skin squamous cell carcinoma and basal cell carcinoma. These findings suggest that Klotho is required for the differentiation of epidermal keratinocytes, and Klotho possibly suppresses epidermal tumorigenesis in human skin.

612 Low environmental humidity induces synthesis and release of cortisol from skin equivalent model
K Takahashi1, M Dievlev1,2,3 S Dievlev1,2 and J Kwak2 1Shinseido Research Center, Yokohama, Japan and 2Japan Science and Technology Agency, Tokyo, Japan.

We previously demonstrated that dry environmental conditions induce a variety of skin pathologies, such as epidermal proliferation and inflammation. A recent report that cortisol synthesis in epithelial cells is low was increased during wound healing led us to hypothesize that environmental dryness might induce increased cortisol secretion in the epidermis. In the present study, we incubated a skin equivalent model under dry (relative humidity: less than 10%) and humid (relative humidity: approximately 70%) conditions for 48 hours and measured cortisol secretion and mRNAs expression of cortisol-synthesizing enzyme (steroid 11β-hydroxylase, CYP11B1) and interleukin 1 beta (IL-1β) under dry condition. We previously demonstrated that dry environmental conditions induce a variety of skin pathologies, such as epidermal proliferation and inflammation. A recent report that cortisol synthesis in epithelial cells is low was increased during wound healing led us to hypothesize that environmental dryness might induce increased cortisol secretion in the epidermis. In the present study, we incubated a skin equivalent model under dry (relative humidity: less than 10%) and humid (relative humidity: approximately 70%) conditions for 48 hours and measured cortisol secretion and mRNAs expression of cortisol-synthesizing enzyme (steroid 11β-hydroxylase, CYP11B1) and interleukin 1 beta (IL-1β) under dry condition. We previously demonstrated that dry environmental conditions induce a variety of skin pathologies, such as epidermal proliferation and inflammation. A recent report that cortisol synthesis in epithelial cells is low was increased during wound healing led us to hypothesize that environmental dryness might induce increased cortisol secretion in the epidermis. In the present study, we incubated a skin equivalent model under dry (relative humidity: less than 10%) and humid (relative humidity: approximately 70%) conditions for 48 hours and measured cortisol secretion and mRNAs expression of cortisol-synthesizing enzyme (steroid 11β-hydroxylase, CYP11B1) and interleukin 1 beta (IL-1β) under dry condition. We previously demonstrated that dry environmental conditions induce a variety of skin pathologies, such as epidermal proliferation and inflammation. A recent report that cortisol synthesis in epithelial cells is low was increased during wound healing led us to hypothesize that environmental dryness might induce increased cortisol secretion in the epidermis. In the present study, we incubated a skin equivalent model under dry (relative humidity: less than 10%) and humid (relative humidity: approximately 70%) conditions for 48 hours and measured cortisol secretion and mRNAs expression of cortisol-synthesizing enzyme (steroid 11β-hydroxylase, CYP11B1) and interleukin 1 beta (IL-1β) under dry condition.
613 External negative electrical potential accelerates exocytosis of lamellar bodies in human skin tissue explant culture

[...]

Characterisation of Spink6, a conserved inhibitor of Kallikrein-related peptidases, during barrier injury


Department of Dermatology, University Hospital Heidelberg, Germany

The proteolytic regulation of the desquamation process by kallikrein-related peptidases (KLK)s is crucial for epidermal barrier function and elevated KLK levels have been reported in atopic dermatitis. KLKs are controlled by specific inhibitors of the serine protease inhibitor Kazal-type (spink) family. Recently, SPINK6 was shown to be present in human stratum corneum. In order to investigate its role in epidermal barrier function, we studied mouse Spink6. Sequence alignment revealed that the Kazal-domain of Spink6 is highly conserved in animals. Recombinant Spink6 efficiently blocked mouse KL5 and human KL2, KL4, KL5, KL6, KL7, KL12, KL13 and KL14, whereas human KL1 and KL8 were not inhibited. Spink6 was expressed in mouse epidermis in the stratum granulosum and stratum corneum, in sebaceous glands and the inner root sheath of hair follicles. Stimulation with flagellin, TNF-α/IFN-γ induced Spink6 mRNA expression, whereas stimulation with all-trans retinoic acid resulted in a significant downregulation of Spink6 mRNA expression in cultured primary mouse keratinocytes. Mechanically and metabolically induced skin barrier dysfunction results in changes of Spink6 mRNA- and immuno-expression. Our study indicates that Spink6 is a potent inhibitor of KLKS and involved in skin barrier function.

616 In vivo and in vitro inhibition of the membrane-bound serine protease CAP1/Prss8 by serpins

U. Cicerone, C. Sierig, A. Merillat and E. Hummler

Department of pharmacology and dermato-venereology, University of Lausanne, Lausanne, Switzerland

Serine proteases are involved in the regulation of many biological processes like e.g., blood coagulation, wound healing, digestion, immune response and channel activation. This requires a tight regulation that may be achieved by specific serine protease inhibitors (serpins) and any alteration of this balance may lead to diseases. Using the in vivo Xenopus oocyte expression system, we identified a serpin that was able to block CAP1-induced Dna- mediated sodium currents. To verify its inhibitory effect in vivo, we generated mice transgenic for this serpin and crossed those with mice transgenic for CAP1/Prss8 in the skin that exhibit a scaly skin phenotype, an increased epidermal intracellular Ca2+, increased inflammation and an excessive water loss through the skin (Frateschi et al., Nat Comm 2011). Strikingly, in double CAP1/Prss8/Serpin- transgenic mice, this phenotype can be prevented strongly suggesting that the effects through CAP1/Prss8 over-expression is efficiently blocked by this inhibitor. In conclusion, we achieved an inhibition of CAP1/Prss8 that may well be implicated in the regulation of CAP1/Prss8 activity in skin as well as in various organs.

617 A regulation of keratinocyte differentiation and proliferation by transcription factors Runx1 and Runx3


Department of dermatology, Shimizu university, Matsumoto, Japan, 2 Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan, 3 National University Singapore, Singapore, Functional and 4 Center for Interdisciplinary Research, Tohoku University, Sendai, Japan

Runx-related transcription factor (Runx) family is implicated in stem cell regulation, tissue development, and oncogenesis. Runx1 and Runx3 play important roles in several organs, including blood, muscle, gastric mucosa, the nervous system, and hair follicles by affecting cell survival, proliferation, and differentiation. However, it is unknown whether and how Runx1 and Runx3 regulate in the epidermis. We investigated the functions of Runx1 and Runx3 in the epidermis. First, we over-expressed Runx1 and Runx3 in primary mouse keratinocyte by using adenovirus vectors. The expression of keratin 1 (K1) and keratin 10 (K10) were increased by Runx overexpression. Next, we decreased the expression of Runx1 and Runx3 by using sequence-specific siRNAs. The expression of K1 and K10 were inhibited by the Runx knockdown. These results suggested that both Runx molecules regulate expression of K1 and K10. Further, Runx regulates keratinocyte growth. Overexpression of Runx1 and Runx3 suppresses keratinocytes proliferation. However, the knockdown of Runx1 and Runx3 induced proliferation in keratinocytes. It is possible that Runx1 and Runx3 inhibit both differentiation and proliferation in keratinocyte.
619 
Cholesterol depletion induces similar cell responses in keratinocyte monolayers and reconstituted human epidermis.

F. De Vuyst,1 J. Desède,1 C. Lambert de Rouvoet,1 S. Bessou-Touza,2 H. Duplan,2 M. Salomon1 and Y. Plouay1 1 Cell and Tissue Laboratory-URFHP-Paris, University of Nancy, Nancy, Belgium, 2 Laboratory of Clinical and Experimental Pharmacology, Toulouse, France and 3 StrattaCell, Namur, Belgium

Cholesterol-enriched plasma membrane micromembranes, namely “lipid rafts”, have been shown to play an important role as keratinocytes signaling platforms. They contain integral proteins such as EGF receptor (EGFR) that contributes to the activation of pathways regulating keratinocytes proliferation, migration and differentiation. The importance of membrane cholesterol in normal epidermal keratinocytes cultured as monolayers has been previously studied in our laboratory using methyl-β-cyclodextrin (MβCD) to extract cholesterol. Cholesterol depletion induced activation of several signalling molecules such as EGFR, MAPK(JNK s) and ERK, alterations in the expression of differentiation markers (PNa and FGMD), of growth factors (HB-EGF) and cytokines (IL-8). In the present study, we investigated whether these changes were applicable to a three dimensional culture model that mimics more closely the in vivo epidermis, harboring a cornified barrier made of fully differentiated keratinocytes. Reconstructed human epidermis (RHE) were cultured for 11 days after seeding human keratinocytes on polycarbonate filter, leading to typical in vivo morphology. RHE were treated with cholesterol depletion in the culture medium,国际机场, under development. The morphology was transiently altered immediately after the MβCD treatment, but the RHE were thereafter able to recover, recovering morphology similar to the control RHE at day 11. Upon supplementation of the culture medium, the RHE allowed to counteract the effects of MβCD by re-differentiation, the skin treatment with AP avoided the UV-induced downregulation of the two main markers of skin barrier, transglutaminase proteins expression and also to induce hyaluronic acid synthesis. Then in models of defective skin barrier, we have developed an active ingredient from avocado cake, according to optimised, secured and eco-friendly approach combining very-high magnification cryo-electron microscopy of vitreous skin sections (CIVEMO) delocous-serial, molecular modelling and electron microscopy simulation. The lipids are organized in an arrangement not previously described in a biological system – stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingo- myelin. This arrangement rationalizes the skin’s low permeability towards both hydrophilic and lipophilic substances, as well as the skin barrier’s robustness towards hydration, environmental temperature and pressure changes, stretching, compression, bending and shearing (Iwai et al, Invest. Dermatol. 132:2215–2225).

622 
Dlx3 is a cell cycle modulator during keratinocyte differentiation and tumor development

F. Polanco,1 P.Velazta, C. Annan,1 N.Rajada,1 M.Blumentghi,1 J.Kim,1 A.Kyryasch,2 C.Catacaos2 and M.Morata1 1 Developmental Biology Section Unit, NIH/NIAMS, Bethesda, MD, 2 Laboratory of Cancer Biology and Genetics, NIH/NCI, Bethesda, MD and 3 Department of Dermatology, New York University, New York, NY

Avocado perseeose, a biomimetic active ingredient for the protection and accompaniment of infants’ skin

S. Lecleire-Buffiere,1 J. Rochetou,1 S. Bédif,1 C. Baudouin,1 A. Saumis and P. Meika Innovation, R&D Direction, Laboratories Experience, France

Avocado perseeose is a rare 7-carbon sugars (perseitol and mannoheptulose) concentrate whose molecular organization of the lipid matrix in-situ, in its near-native state, using a novel methodological approach combining very-high magnification cryo-electron microscopy of vitreous skin sections (CIVEMO) delocous-serial, molecular modelling and electron microscopy simulation. The lipids are organized in an arrangement not previously described in a biological system – stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingomyelin. This arrangement rationalizes the skin’s low permeability towards both hydrophilic and lipophilic substances, as well as the skin barrier’s robustness towards hydration, environmental temperature and pressure changes, stretching, compression, bending and shearing (Iwai et al, Invest. Dermatol. 132:2215–2225).

623 
Avocado perseeose, a biomimetic active ingredient for the protection and accompaniment of infants’ skin

S. Lecleire-Buffiere,1 J. Rochetou,1 S. Bédif,1 C. Baudouin,1 A. Saumis and P. Meika Innovation, R&D Direction, Laboratories Experience, France

In vivo and in vitro investigations of infants’ skin, focused on the stratum corneum and epidermis, have provided some evidence concerning its development, organization and its biological signature as a barrier function in maturation, water handling capacity in fluctuation, inflammation signals and cellular resource to preserve. In order to accompany the development of infants’ skin, we have selected and isolated an ingredient from avocado cake, called Avocado perseeose. Other studies have shown that this ingredient presented such range of activity and also a good tolerability. First we have measured the avocado perseeose (AP) potential on water barrier in normal human keratinocytes (NHK) and skin explants and we have demonstrated that AP is able to stimulate ceramides production, involucrin and transglutaminase proteins expression and also to induce hyaluronic acid synthesis. Then in models of LPS or PMA-stressed NHK, AP downregulated the release of inflammatory mediators as IL-8, IL-1, TNFα. The specific induction of the lipid replenishing balm with SO enables us to counteract defects of AD skin at the level of the skin barrier, inflammatory response and itch signaling. Thus this balm could soothe dryness and itching sensations in atopic skin.

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Molecular biomarkers’ analysis to characterize epidermis of infants

S. Baubock,1 S. Bédif,1 N. Pedretti,1 C. Barnaut,1 FA Bernard1 and P. Meika1 Innovation, R&D Direction, LABORATOIRES ESPANSIONE, EPFRON, France and 2 BIOSCIENTIA, GENCAY, France

The functional skin adaptation is a continuous process that takes place in the first year of postnatal life. The mechanisms of skin hydration, water handling properties and permeability barrier under development. The skin treatment with AP avoided the UV-induced downregulation of the two main markers of skin barrier, transglutaminase proteins expression and also to induce hyaluronic acid synthesis. Then in models of defective skin barrier, we have developed an active ingredient from avocado cake, according to optimised, secured and eco-friendly approach combining very-high magnification cryo-electron microscopy of vitreous skin sections (CIVEMO) delocous-serial, molecular modelling and electron microscopy simulation. The lipids are organized in an arrangement not previously described in a biological system – stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingomyelin. This arrangement rationalizes the skin’s low permeability towards both hydrophilic and lipophilic substances, as well as the skin barrier’s robustness towards hydration, environmental temperature and pressure changes, stretching, compression, bending and shearing (Iwai et al, Invest. Dermatol. 132:2215–2225).

625 
The skin barrier is fundamental to terrestrial life and its evolution; it upholds homeostasis and protects against the environment. Skin barrier capacity is controlled by lipids that fill the extracellular space of the stratum corneum (SC). Here we report on the determination of the molecular organization of the skin’s lipid matrix in-situ, in its near-native state, using a novel methodological approach combining very-high magnification cryo-electron microscopy of vitreous skin sections (CIVEMO) delocous-serial, molecular modelling and electron microscopy simulation. The lipids are organized in an arrangement not previously described in a biological system – stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingomyelin. This arrangement rationalizes the skin’s low permeability towards both hydrophilic and lipophilic substances, as well as the skin barrier’s robustness towards hydration, environmental temperature and pressure changes, stretching, compression, bending and shearing (Iwai et al, Invest. Dermatol. 132:2215–2225).

626 
Dlx3 as a cell cycle modulator during keratinocyte differentiation and tumor development

F. Polanco,1 P.Velazta, C. Annan,1 N.Rajada,1 M.Blumentghi,1 J.Kim,1 A.Kyryasch,2 C.Catacaos2 and M.Morata1 1 Developmental Biology Section Unit, NIH/NIAMS, Bethesda, MD, 2 Laboratory of Cancer Biology and Genetics, NIH/NCl, Bethesda, MD and 3 Department of Dermatology, New York University, New York, NY

Dlx3 is a homoebox transcription factor expressed in the granular layer of the epidermis in mice. It is expressed in low and high-risk papilloma, squamous cell carcinoma and normal skin shows an evolution with age with a lesser expression level in the younger infants; 2) inflammation markers expression was high in samples from youngest infants (few months) and then stabilized to be comparable to adult profile; 3) stem cells markers expression (proliferation, adhesion, niche) was the highest in the samples from younger infants and it quickly decreased with age. This approach allowed us to model epidermis of infants and children to characterize their genomic biomarkers. In addition the comparative analysis of gene expression profiles suggests that skin barrier and some defense systems develop and get organized during early childhood and that the stem cells resource is at its maximum just after birth and decreases in the first months of life.
625 Divergent abnormalities in lamellar body secretion account for increased infections in harlequin ichthyosis and nephrin syndrome
A. Chau,1 A. Nune,1 E. Godoy-Gijon,1 D. Crumrine,1 M. Hube,1 P. Fleckenstein1 and P. Elias1 1 Dermatology, VA Med Ctr/LUSC, San Francisco, CA and 2 Dermatology, Univ of Washington, Seattle, WA

Secondary infections frequently complicate the inherited ichthyoses, but the basis for this propensity is unknown. Since two key antimicrobial peptides, LL-37 and human β-defensin 2, normally are delivered to the stratum corneum (SC) interstices by lamellar body (LB) exocytosis, we hypothesized that known abnormalities in the LB secretory system could account, at least in part, for the enhanced risk of infections in two autosomal recessive congenital ichthyoses (ARCI), i.e., Harlequin ichthyosis (HI) and Netherton syndrome (NS). In HI, failure of glaucosykerolides leading to nascent LB results in a highly defective permeability barrier, while in NS, accelerated LB secretion appears to compensate in part for an accelerated proteolytic attack on the SC. Using atttempting cytochemistry for the LB protein content marker, acidic lipase, we show that protein loading into LB and delivery to the SC intrestices is markedly reduced in HI. In contrast, enzyme protein load ing is enhanced, but enzyme activity markedly decreases starting from the SC in NS. These alterations in acidic lipase delivery, secretion, and fate within the SC were paralleled by changes in immunostaining, for LL-37, which was reduced within the SC intrestices in HI. Though the initial production and secretion of LL-37 is not affected in NS, its delivery to the SC intrestices was markedly decreased. These results show that reduced delivery of LL-37 in HI, and accelerated degradation of LL-37 in NS, can account for the increased risk of infections in these two ARCI.

626 Topical apigenin improves epidermal permeability barrier homeostasis in normal murine skin and atopic dermatitis mechanisms
M. Hsieh1,2, S. Run1, M. Hube1, P. Kim1, K. Park1, D. Crumrine1, T. Lin1, J. Santjas1, T. Mauno1, P. Elias1 and M. Nune1 1 Dermatology, VA Med Ctr/LUSC, San Francisco, CA, 2 Dermatology, Nijmegen Med Univ, Nijmegen, Netherland and 3 Skin Physiology Res, Dalan Skin Disease Hosp, Liaoning, China

The beneficial effects of certain herbal medicines on cutaneous function are being increasingly appreciated. Among these agents, the flavonoid extract, apigenin, has been used for skin care, particularly in China, for millennia. However, the underlying mechanisms by which apigenin benefits the skin are not known. In the present study, we first determined whether topical apigenin positively influences permeability barrier homeostasis, and then the basis thereof. Hairless mice were treated topically with either 0.1% apigenin or vehicle alone twice-daily for 9 days. At the end of treatments, permeability barrier function was assessed with either an electrolytic water analyzer or a Tewameter. Our results show that topical apigenin significantly enhanced permeability barrier homeostasis after tape-stripping, though basal permeability barrier function remained unchanged. Improved barrier function correlated with enhanced filaggrin expression and lamellar body production, which was paralleled by elevated mRNA levels for the epidermal ARCA12 transporter. mRNA levels for several key lipid synthetic enzymes were up-regulated by apigenin. Finally, topical apigenin treatment reduced TEWL, a measure of barrier function, and thickness and skin hydration, indicative of barrier health. Together, these results demonstrate that topical apigenin improves epidermal permeability barrier function by stimulating epidermal differentiation, and lipid synthesis and secretion, as well as cutaneous antimicrobial peptide production. We conclude that topical apigenin improves epidermal permeability barrier function by stimulating epidermal differentiation, and lipid synthesis and secretion, as well as cutaneous antimicrobial peptide production.

627 Did latitude-dependent differences in prevalence of filaggrin mutations evolve to support cutaneous vitamin D production?
M. Thyssen1,2,3 and P. Elias1 1 Dermato-Allergology, Copenhagen Univ Hosp Gentofte, Hellerup, Denmark and 2 Dermatology, VA Med Ctr/LUSC, San Francisco, CA

The pigmentation of human skin displays a quasi-geographic pattern of lightening, theorized to have evolved to promote UVB-dependent cutaneous vitamin D production. Because there is much less support for this hypothesis, we explored an alternate possibility, i.e., that filaggrin (FLG) loss-of-function mutations evolved in the far north to support immunomodulatory vitamin D3 (D3) production. Protoplasts of FLG normally generates large quantities of histidine, which is then enzymatically de-iminated to trans-urocanic acid (t-UCA). This carboxylic acid is a major endogenous UVB photo-antenna of particular importance for photoprotection in lightly-pigmented LS human. It is precisely these Northern-dwelling, LS humans that exhibit the highest prevalence of FLG mutations (≥15%), even in the general population. Conversely, the frequency of FLG mutations steeply drops to <1% in southern Europeans, and even lower in Asians (<1%). Pertinently, Northern Europeans with atopic dermatitis, with still-higher mutation prevalences (30-40%), display higher-than-normal circulating VD3 levels. While it has been proposed that FLG-mediated FLG deficiency is associated with only moderate changes in TEWL and skin hydration reveal surprisingly only a mild disturbance of the epidermal water permeability barrier function. It is precisely these Northern-dwelling, LS humans that exhibit the highest prevalence of FLG mutations (≥15%), even in the general population. Conversely, the frequency of FLG mutations steeply drops to <1% in southern Europeans, and even lower in Asians (<1%). Pertinently, Northern Europeans with atopic dermatitis, with still-higher mutation prevalences (30-40%), display higher-than-normal circulating VD3 levels. While it has been proposed that FLG-mediated FLG deficiency is associated with only moderate changes in TEWL and skin hydration reveal surprisingly only a mild disturbance of the epidermal water permeability barrier function.

628 Filagrin deficiency and epidermal water permeability barrier: Evidence for a surprisingly mild disturbance
A. Tewes2,3,1, V. Oijl1, M.C. Sauerland1, T. Tannink1, I. Zaraova1, N. Seller1, D. Mettes1, K. Auerwenn1, F. Donzé1,2, H. Hauser1,2 and H. Traupe1,2 1 Dermatology, University Hospital, Muenster, Germany, 2 Institute of Biochemistry and Clinical Research, University of Muenster, Muenster, Germany, 3 Dermatology, University of Heidelberg, Heidelberg, Germany

Icthysis vulgaris is caused by loss of function mutations in the proflaggran (FLG) gene. Filagrin has a strategic role in structural and chemical barrier function of epidermis and in hydration of the skin. We wondered how profound the expected alterations in epidermal water permeability barrier function would be. Therefore we analyzed a cohort of 15 patients with proven ichthyosis vulgaris (IVS), with FLG mutations (n = 7, 7% of patients); all affected patients had at least one detectable FLG loss-of-function mutation. We analyzed clinical, histological, and biochemical markers associated with epidermal barrier function (TEWL, erythema, formation of transepidermal water loss, skin surface pH were not significant). Likewise the 5 FLG +/- subjects having partial FLG deficiency and five from incomplete FLG +/- deficiency. The clinical score (IVSS) gave signs significantly higher values (7.7) when FLG +/- patients were compared to FLG -/- patients (score 5.0, p = 0.004) or when compared to controls (score for controls 0.3, p = 0.0001). In complete FLG deficiency (FLG +/- subjects) a moderate increase of TEWL from 5.41 to 7.54 mgn2 (p = 0.03) and a moderate increase of skin hydration from 29.20 to 27.17 (p = 0.05) were observed. Changes in skin surface pH were not significant. Likewise the 5 FLG +/- subjects having partial FLG deficiency did not suffer from significant changes in these variables. We conclude that complete, but not partial FLG deficiency is associated with only moderate changes in TEWL and skin hydration revealing surprisingly only a mild disturbance of the epidermal water permeability barrier function.

629 β1 signaling and regulation of tight junctions in keratinocytes
J. Reijo1,2 1 Emilia-Salamanca, Germany and 2 University of Turku, Turku, Finland

Increasing evidence has recognized tight junctions as the lower epidermal barrier. These results show that reduced delivery of LL-37 in HI, and accelerated degradation of LL-37 in NS, can account for the increased risk of infections in these two ARCI.

630 ‘Barrier repair’ ingredients and formulations regulate barrier homeostasis and antimicrobial peptide expression in parallel
M. Man1, B. Sun1 and P. Elias1 1 Dermatology, VA Med Ctr/LUSC, San Francisco, CA

Prior studies have shown first, that permeability barrier impairment and cutaneous antimicrobial defense are co-regulated and interdependent functions. Second, we demonstrated that expression of the mouse analogue of the epidermal antimicrobial peptide, cathelicidin (LL-37), and to lesser extents, human β-defensin and cathepsin, decline in parallel with compromised barrier function with skin aging, during psychological stress, and following erythemogenic doses of UV-B. Here, we explored the opposite scenario, i.e., whether ingredients or formulations that improve barrier homeostasis also enhance epidermal AMP expression, Accordingly, petrolatum (α2%), urea (α4%), PPAR and LXR activators, suberogenic UV-B, and a ceramide-dominant, triple-mixture lipid of cholesterol, free fatty acids, and ceramides (Expert Emmulsion) all accelerated barrier repair in normal human and/or mouse epidermis. In contrast, several other putative ‘barrier repair’ formulations either displayed no net benefits, or delayed barrier recovery after acute insults to human skin. For almost all of these ingredients and formulations that enhanced barrier repair (except petrolatum), AMP expression was enhanced in parallel. These results show again the close relationship between permeability barrier function and antimicrobial defense. Agents that promote barrier function exert parallel benefits for cutaneous antimicrobial defense.

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631 Basis for pigment-enhanced barrier function

M. Macri,1 2 M. Mantyjarvi,1 2 S. Benhaddou,1 2 S. Nelson,3 4 N. de Groot,3 4 E. C. Jones,3 4 S. L. Young1 2 1 Department of Dermatology, Maastricht University Medical Center, Maastricht, The Netherlands, 2 Innovative Science Research & Development Center, Yokohama, Japan, 3 Dermatology, Tokyo Medical University, Tokyo, Japan, 4 Research Equipment Center, Hamamatsu University LBP Spain

Humans with darkly-pigmented skin (DS) (Fitzpatrick Type IV/V) display superior barrier function vs. lightly-pigmented (Type I/II) human skin (LS), independent of race, and barrier function is reduced in individuals with involved vs. uninvolved vitiligo skin. These results strongly support that pigmented skin bestows superior barrier function, but do not explain these differences. DS skin's reduced barrier permeability may contribute to enhanced function, because reducing the stratum corneum (SC) pH in LS individuals resets barrier function to DS levels. We evaluated here how pigment enhances epidermal barrier function in SKH1 (hairless albino) mice, which contain residual (non-melanocytes), melanocytes, and SKH2 (hairless pigmented) mice, where melanocytes with abundant melanin populate the interfollicular epidermis. Barrier homeostasis is enhanced in SKH2 vs. SKH1 mice, which correlates with a more acidic lower SC. Melanin granules persist into the outer epidermis in SKH2 mice; and also are extruded, into the extracellular spaces at and above the stratum granulosum (SG)-SC interface. Archived samples of DS human epidermis also show melanin estrus. Acute barrier disruption and topical basic pH challenges accelerate melanin estrus, corroborating that further barrier homeostasis is enhanced in highly pigmented skin. This work provides a mechanism to explain how pigmentation enhances epidermal barrier function and may contribute to the enhanced function of humans with darkly-pigmented skin.

632 The stratum corneum water content and natural moisturizer factor composition evolve with age and depend on body site

J. Boisseau-Adanou,1 2 A. Ballet-Gullod1 and GN Statman1 1 Laboratory of analytical chemistry, Analytical Chemistry Group (LCAP-ENSAI), Université Paris Sud 11, Châtenay- Malabry, France and 2 Skin Care R&D, Johnson & Johnson Research & Development, LLC, Paris, France

The objective of this study was to examine if age and chronic environmental exposure affect the water content and the composition of the Natural Moisturizing Factor (NMF) of the Stratum Corneum (SC). The study was conducted on 40 French women volunteers without history of skin diseases. They were divided into 4 groups of 10: 20-30, 30-40, 40-50 and 50+ years of age. Measurements were done on the cheek and on two skin sites on the arm (one relatively protected and one exposed). SC water content and NMF composition (alanine, glycine, histidine, arginine, ornithine, citrulline, proline, cysteine and total NMF, and total Amino acid) was measured by Raman confocal microspectroscopy. Skin surface hydration was measured by skin conductance. Water content appears to decrease slightly on the face and the protected arm site and at a higher rate on the exposed arm site. The individual amino acid content showed a trend toward aging behavior: some increased, some decreased and some remained constant. This observation indicates that different sources and different metabolic processing in the skin may affect the water content and the composition of the NMF.

633 In vitro skin permeation method: A metabolomic assessment

AP Carambula1, 2 Menegola1 and AL Zer1 1 Science and Technology, Ideas and Concepts, Natura Inovação, Cajamar, Brazil and 2 Brazilian Biosciences National Laboratory, CNPEM, Campinas, Brazil

The in vitro skin permeation approach is considered a classical test to improve the development of topical agents and phar-maceutics and cosmetics. Besides its wide employment as a predictive approach for in vivo absorption related to the formulation safety and quality, skin permeation tests are mainly applied as a barrier test but no biological activity is assessed. Among the establishment of a model to evaluate the metabolic profile and measure skin condition during skin permeation approaches, we optimized the classical method for in vitro skin absorption. We depicted that, either under control conditions or after treatment with a cosmetic formulation, skin samples from pigs kept mounted on Franz Diffusion Cells with Dulbecco's Modified Eagle Medium (DMEM) as receptor fluid can sustain metabolic activity during the entire protocol as described by the identification of biomarkers in SKH1 mice. Therefore, extracellular metabolites were assessed using metabolic fingerprinting and investigating the inner skin permeation in pigment enhanced epidermis can be attributed to both pH-lowering juxtae and still undefined paracrine mechanisms.

634 Metabolomic analysis of human skin extracellular lamellar bilayers

C Cuddapah,1 C Escher,2 R Bruderer2 and P Cauduro1 1 CELLnTEC Advanced Cell Systems AG, 2 Zürich, Switzerland and 3 Dermatology, Tokyo Medical University, Tokyo, Japan

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636 A novel culture medium ages keratinocytes without the need for pro-aging stimuli

C Cuddapah,1 2 E Sche1, 2 R Bruderer2 and P Cauduro1 1 CELLnTEC Advanced Cell Systems AG, 2 Zürich, Switzerland

The traditional methods of in vitro keratinocyte aging depend primarily on either extended in vitro culture (replicative aging), or exposure to concentrated pro-aging stimuli. Furthermore, all existing in vitro aging methods that use standard culture media are contaminated by the powerful anti-aging environment that is achieved in vivo, while rapid in vitro proliferation and extended longevity. The new VitroAge medium provides to enable rapid in vitro proliferation and extended longevity. The new VitroAge medium was developed to provide a more physiologically realistic environment that lacks anti-aging components, but without the addition of artificial pro-aging stimuli. VitroAge medium was found to retain normal morphology, but demonstrated reduced growth rate and in vitro longevity (3-4 weeks). Changes in keratinocyte phenotype after 3 weeks of culture in either standard or VitroAge medium were quantified using multiplexed MRK proteomics. Over 300 significantly up- or down-regulated proteins were identified (regulation >20%, p-value <0.05), and grouped into functional clusters using the David Bioinformatic database. Keratinocytes aged in VitroAge medium demonstrated clusters of down regulated proteins associated with proteasome biosynthesis and metabolism. In VitroAge medium included DNApolymerase and electron transport. Up-regulated functional clusters included proteasome/ubiquitin-protein turnover, adhesion/desmosome and membrane proteins. These results indicate that specific phenotypes were induced in VitroAge medium which was significantly different from the standard culture conditions. Changes known to occur in aged skin when cultured in an aging culture medium that does not rely on pro-aging stimuli. Furthermore, the VitroAge medium may contribute to enhanced function, because reducing the stratum corneum (SC) pH in LS individuals resets barrier function to DS levels. We evaluated here how pigment enhances epidermal barrier function in SKH1 (hairless albino) mice, which contain residual (non-melanocytes), melanocytes, and SKH2 (hairless pigmented) mice, where melanocytes with abundant melanin populate the interfollicular epidermis. Barrier homeostasis is enhanced in SKH2 vs. SKH1 mice, which correlates with a more acidic lower SC. Melanin granules persist into the outer epidermis in SKH2 mice; and also are extruded, into the extracellular spaces at and above the stratum granulosum (SG)-SC interface. Archived samples of DS human epidermis also show melanin estrus. Acute barrier disruption and topical basic pH challenges accelerate melanin estrus, corroborating that further barrier homeostasis is enhanced in highly pigmented skin. This work provides a mechanism to explain how pigmentation enhances epidermal barrier function and may contribute to the enhanced function of humans with darkly-pigmented skin.

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**Epidermal Structure & Barrier Function**

**ABSTRACTS**

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### 637

The Ne terminal S100 domain of Filaggrin 2 activates the skin asparatic acid protease in the epidermis of human skin, in vitro

M Donwain, A Thomas-Collignon, 1 Raine, 1 E Formetcher 1 and D Bernard 1

1. Laboratory of Biology, Onical R & I, Clichy, France; and 2. Hygeneics, Paris, France

The skin asparatic acid protease (SAPase) is expressed in the stratum granulosum (SG) of normal human skin where it is believed to be involved in the filaggrin processing. Its expression and activation are associated with epidermal terminal differentiation. However, little is known about the regulation of its physiological function and auto-activation. Thus, the aim of this study was to test if the two proteins partners of the protease which could be involved in its regulation and function. A yeast two hybrid screen using SAPase as bait against a human epidermal reconstructed skin library identified the S100 domain of filaggrin 2 as a strong binding partner of SAPase. The analysis showed that the Ne terminal domain of filaggrin 2 (FlgN2ter) interacted with a domain in the 28 kDa form of SAPase, but not within the active 14 kDa segments of the enzyme. The interaction was confirmed by surface plasmon resonance analysis. Immunohistochemical analysis using specific antibodies to the 28 kDa SAPase and FlgN2ter showed that the two proteins co-localized to SG of human epidermis. Immuno-precipitation assay using recombinant proteins of 28 kDa SAPase and FlgN2ter and a fluorescently labeled peptide showed that FlgN2ter enhanced SAPase activity. Western blot analysis showed that the activating effect of FlgN2ter was linked to auto-activation process of SAPase- FlgN2ter.

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High sulfation and chain length are key in hepatic regulation of transglutaminase activity in the skin of the mouse as a model of age-related skin changes

M Donwain, T Jarrouse, A Thomas-Collignon, 1 Turnbull and D Bernard 1

1. Laboratory of Biology, Onical R & I, Clichy, France; and 2. Intellipelle, Liverpool, United Kingdom

We have shown that hepatic sulfation is present in the liver and binds to transglutaminases 1 & 3 in the stratum corneum (SC). In addition we discovered that a highly sulfated hepatic mimetic and a naturally polyascorbate enhanced the activity of transglutaminases 1 & 3 as well as the maturation of cornified envelopes in a reconstructed human epidermis. The aim of this study was to further characterize the structure and chemistry of HS in the SC which is necessary to stimulate the activity of transglutaminases. Disaccharide analysis of HS purified from SC showed that it had a highly sulfated and unusual chemistry. In order to better understand the optimal HS chemistry that is required to stimulate transglutaminases activity in the SC – we examined the effect of a hepatic mimetic on the expression of different transglutaminases, in the presence or absence of exogenous HS. The results showed that in the absence of HS, the activity of transglutaminase 1 & 3 was enhanced. This was not the case when HS was added. We propose that HS is required to stimulate transglutaminases activity and that the hepatic mimetic could be used as a novel material in the development of novel skin care products to improve skin barrier function in dry and aged skin.

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### 639

Kallikrein 5 up-regulation in barrier dysfunction and atopic dermatitis pathogenesis

Y Zhu, 1 Harper, 2,3 O’Shaughnessy 1 and Du 2,3 1 University College London, London, United Kingdom and 2 Great Ormond Street Hospital, London, United Kingdom

Atopic dermatitis (AD) is a common skin disease caused by genetic and environmental factors. The atopic population is characterized by early onset, exacerbations and different xeroses. We expect that our results will motivate further use of mathematical modeling approaches in the analysis of complex mechanisms underlying epidermal function.

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### 640

A new cosmetic composition enhances the expression of epidermal genes involved in unaltered skin aging functions

K Aege, C Tacheu, J Michelet, M Perez, B Allamard, C Vamugor, S Cheliant, B Lavau, C Debache and D Bernard 1

Laboratory of Biology, Onical R & I, Paris, France

Aging, with numerous epidermal functions become disrupted causing possible deleterious changes at the cellular level. In this study, a microarray analysis was carried out to identify age-related changes. The results showed 281 genes were identified as associated with various functions involved in skin homeostasis and aging. Analysis especially focused on genes being up-regulated and strongly involved in the proteome of suprabasal cells (Mfn1 and Mfn2). Mfn1 was expressed only in suprabasal cells, whereas Mfn2 was expressed only in basal cells as Mfn2 is bound to keratin 5/14. Thus we hypothesized that Mfn1 might be connected with keratin 5/14. Mitochondria play critical roles in many cellular processes, including cell death. However, the role of mitochondria in keratinization remains obscure. This study investigates whether mitochondria are associated with keratinization. Mitochondrial distribution was not observed to differ by position in the epidermis. Immunofluorescent observation showed no difference of mitochondrial morphology between keratin 1 and keratin 10. No difference was found in Mfn1 and Mfn2 expression between E1 and normal suprabasal cells. However, the number of mitochondria per cell was significantly higher in the suprabasal cells than in the basal cells. Mitochondria undergo frequent fission and fusion. This is regulated by mitochondrial dynamics proteins such as mitochondrial fusion protein Mitf isoforms (Mitf1 and Mitf2). We found that the expression patterns of Mitf1 and Mitf2 were quite different: Mitf1 was expressed only in suprabasal cells, whereas Mitf2 was expressed only in basal cells. The Mitf1 expression level increased after calcium shock, whereas Mitf2 level did not. Furthermore, we focused on the relationship between keratin and Mitf isoforms. It has been reported that Mitf2 is bound to keratin 5/14. Thus we hypothesized that Mitf1 might be connected with keratin 1/10. Therefore we focused on epidermal differentiation (E1), which is known to involve mutations of keratin 1 or keratin 10. No difference was found in Mitf1 and Mitf2 distribution between E1 and normal suprabasal cells. Interestingly, Mitf1 was distributed non-uniformly in the E1 suprabasal cells but uniformly in the normal suprabasal cells. There was no difference in Mitf2 intracellular distribution between E1 and normal basal cells. Immunofluorescence observation showed differential mitochondrial in the B suprabasal cells but not in the basal cells. These results indicate that Mitf1 and Mitf2 may contribute to keratinization.

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### 641

Mathematical modelling of feedback control mechanisms for age differences in skin hydration

E Dominguez Hüttinger, 1 GN Statonas 1 and EP Tevelka 1

1. Department of Bioengineering, Imperial College London, London, United Kingdom and 2. Skincare R&D, Johnson & Johnson

One of the key functions of skin barrier is the control of the permeability to water and the maintenance of a healthy epidermal hydration profile. Homeostasis in the water handling capacities of the epidermis of human skin.

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### 642

The mitochondrial fusion proteins Mitf1 and Mitf2 are involved in keratinization

A Sato, R Abe, N Saito, Suzuki, Y Fujita, T Nomura, H Nakamura and H Shimizu 1

Department of Dermatology, Hokkaido University, Sapporo, Japan

Keratinization requires strict regulation of keratocyte proliferation and cell death. Mitochondria play critical roles in many cellular processes, including cell death. However, the role of mitochondria in keratinization remains obscure. This study investigates whether mitochondria are associated with keratinization. Mitochondrial distribution was not observed to differ by position in the epidermis. Immunofluorescent observation showed no difference of mitochondrial morphology between keratin 1 and keratin 10. No difference was found in Mfn1 and Mfn2 expression between E1 and normal suprabasal cells. However, the number of mitochondria per cell was significantly higher in the suprabasal cells than in the basal cells. Mitochondria undergo frequent fission and fusion. This is regulated by mitochondrial dynamics proteins such as mitochondrial fusion protein Mitf isoforms (Mitf1 and Mitf2). We found that the expression patterns of Mitf1 and Mitf2 were quite different: Mitf1 was expressed only in suprabasal cells, whereas Mitf2 was expressed only in basal cells. The Mitf1 expression level increased after calcium shock, whereas Mitf2 level did not. Furthermore, we focused on the relationship between keratin and Mitf isoforms. It has been reported that Mitf2 is bound to keratin 5/14. Thus we hypothesized that Mitf1 might be connected with keratin 1/10. Therefore we focused on epidermal differentiation (E1), which is known to involve mutations of keratin 1 or keratin 10. No difference was found in Mitf1 and Mitf2 distribution between E1 and normal suprabasal cells. Interestingly, Mitf1 was distributed non-uniformly in the E1 suprabasal cells but uniformly in the normal suprabasal cells. There was no difference in Mitf2 intracellular distribution between E1 and normal basal cells. Immunofluorescence observation showed differential mitochondrial in the B suprabasal cells but not in the basal cells. These results indicate that Mitf1 and Mitf2 may contribute to keratinization.
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649
MALDI-MS/MS examination of emollient treated living skin equivalent for lipidomic and small molecule endotoxin.
C Marshall1, M Lee1, R Bojar1, M Donaldson1, J Simon1 and M Clench1
1 Department of Biosciences, Sheffield Hallam University, Sheffield, United Kingdom, 2 Platform Technology Support, ClassexsmithKline, Stevenage, United Kingdom, 3 Evotec, Tisk, United Kingdom, 4 AsahiClassexsmithKline, Uxbridge, United Kingdom and 5 Department of Biosciences, Sheffield Hallam University, Uxbridge, United Kingdom

Using the ultrastructural low molecular weight, water-soluble lanthanum nitrate, we assessed the competence of the permeability barrier in different regions of the human hair follicle. The follicular epithelium displayed a highly competent, lamellar body-based barrier in the infundibulum, becoming less competent at the level where sebaceous ducts enter the follicle, and completely incompetent throughout the lower follicles and hair bulb. In experimentally-induced rabbit comedones, and biopsies of grade I (non-inflammatory) acne lesions, barrier function and sebaceous ducts deteriorated further. Finally, when acne lesions became inflammatory, peri-sebaceous barrier competence was lost completely. Together, these results suggest that barrier competence is a pre-requisite for acne susceptibility, and conversely, that strategies that support barrier function could decrease the propensity to develop inflammatory acne. Finally, the absence of an epithelial barrier in the depths of the hair follicle supports the development of strategies to exploit this pathway to enhance percutaneous drug delivery.

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Loss of phospholipase C δ1 impairs epidermal barrier and induces chronic dermatitis
K Kanemura1, N Nakamura1 and K Fukami Laboratory of Genome and Biosignals, School of Pharmaceutical and Life Sciences, Ritsumeikan University, Kusatsu, Shiga, Japan

Phospholipase C (PLC) is a key enzyme in phosphoinositide turnover, an important signal transduction process in cells. PLC activation leads to various cellular responses through mobilization of intracellular calcium ions and protein kinase C activation. There are 13 PLC isozymes in mammals. Among them, one of PLC isozymes, PLCδ1 is abundantly expressed in differentiated layers of epidermis, suggesting that PLCδ1 plays important roles in epidermal differentiation. Since defective terminal differentiation of epidermis is one of causes for impairment of skin barrier function, we investigated whether loss of PLCδ1 affected outside-inside barrier of epidermis. FITC penetration assay with mice lacking PLCδ1 KO mice revealed that outside-inside barrier was impaired in PLCδ1 KO epidermis. On the other hand, PLCδ1 KO mice did not show increased transepidermal water loss, indicating that inside-outside barrier is not affected. Since cornified envelope is critical for epidermal permeability barrier function, we next evaluated the size and the number of desquamation. FITC penetration assay with mice lacking PLCδ1 KO mice) revealed that outside-inside barrier was impaired and demonstrated that mesotrypsin was localized in the cytoplasm of granular cells and intercellular lipid bilayers. It is suggested that kallikrein-related peptidases (KLKs) play critical roles in cornified envelope and epidermal permeability barrier function. To elucidate possible underlying mechanisms, the ability of KLKs to induce mRNA expression and protein production by human keratinocytes, and to elucidate the possible underlying mechanisms. The inability of IL-36 to induce mRNA expression and protein production of IL-37 and psoriasis by keratinocytes through activation of MAPKs and NFκB.

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Low-frequency sonophoresis alters the structure of epidermal tight junction protein, claudin-1 and barrier function: Implications in ultrasound-mediated transdermal drug delivery
S Lee1, B Baik1, K Choi2 and S Lee1
1 Department of Dermatology, CHA Bundang Medical Center Hospital, CHA University, Seongnam, Republic of Korea, 2 DERMAPRO LTD. 919-1, Bangi-dong, Sechoro-Gu, Seoul, Republic of Korea

Claudin-1 plays a critical roles in tight junction formation. Low-frequency sonophoresis (LFS) disrupts the structure of stratum corneum (SC) lipid bilayers to decrease the propensity to develop inflammatory acne. Finally, the absence of an epithelial barrier in the depths of the hair follicle supports the development of strategies to exploit this pathway to enhance percutaneous drug delivery.

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Keratinocyte-specific PRSS3 effectively activates KLKs and degrades LEKT1: a hidden mechanism of desquamation
M Miyai1, Y Matsumoto1, H Yamashita1, M Yamamoto1, R Tobe1 and T Hirobe1
1 Shiono Research Center, Yokohama, Japan and 2 Dermatology, Tokyo Medical University, Tokyo, Japan

It is suggested that kallikrein-related peptides (KLKs) play critical roles in cornified desquamation under regulation of lymphoepithelial kallikrein-type inhibitor (LEKT1). However, it is still obscure how these proteases are activated and whether the KKL-LEKT1 interaction is easily broken in such a weak acidic pH at the skin surface. Recently we reported cloning of a new PRSS3 gene product, which is highly expressed in the lamellar body and is keratinocyte-specific mesotrypsin from the DNA library. The aim of this study is to elucidate involvement of mesotrypsin in the desquamation process. First we examined effect of mesotrypsin on proKLK5. Incubation of these proteases resulted in cleavage of a pro-peptide and generation of an active form of KLK5. These were observed at 10 min incubation and enzymatic activities increased in a time dependent manner. Essentially the same results were obtained for KLK7. Thus mesotrypsin is capable of activating key desquamation enzymes. We next constructed various recombinant LEKT1 proteins, D2, D2-5, D2-6, D2-7, D5, D6, D6-9, D7, D7-9 and D10-15. In order to detect these constructs, transiently transfected HEK293 cells were incubated with anti-α- or anti-β inhibitors. In conclusion, our results suggest a hidden mechanism of desquamation, where mesotrypsin contributes to the desquamation process via activation of KLKs and degradation of the intrinsic inhibitor, LEKT1.

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Interleukin-36 cytokines enhance the production of host defense peptides LL-37 and psoriasis by human keratinocytes through activation of MAPKs and NFκB
T Akiyama1, T Nguyen2,1, F Nyamudzai2, R Smithrithee1,2, C Kukurayan2,1,3, H Uchida1, K Osumi1, H Ogyama1 and S Bieda2
1 Dermatology, Juntendo University Graduate School of Medicine, Tokyo, Japan and 2 Atopy Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

Interleukin (IL)-36 is a common name for the three IL-1 family members IL-36α, IL-36γ, and IL-36ε, formerly known as IL-1F6, IL-1F8, and IL-1F9, respectively. IL-36 has been recently been detected in keratinocytes which have been shown to play a role in skin diseases such as psoriasis, where host defense peptides (HDPs) IL-37 and psoriasis are highly expressed. The induction of IL-37 and psoriasis by keratinocytes remain unknown. The aim of this study was to investigate the effects of IL-36 and psoriasis on IL-37 and psoriasis expression and production by human keratinocytes, and to elucidate the possible underlying mechanisms. The ability of IL-36 to induce mRNA expression and protein production of IL-37 and psoriasis was determined by real-time PCR and ELISA, respectively. Phosphorylation of MAPKs and NFκB was assessed by Western blotting. MAPK and NFκB activators were used to study the mechanism by which IL-36 stimulates keratinocytes. We found that all of these IL-36 enhanced IL-37 and psoriasis expression and protein production by keratinocytes in keratinocyte-specific fashion. The induction of IL-36 and psoriasis by HDPs was assessed by Western blotting. MAPK and NFκB activators were used to study the mechanism by which IL-36 stimulates keratinocytes. We found that all of these IL-36 enhanced IL-37 and psoriasis expression and protein production by keratinocytes in a keratinocyte-specific fashion. The induction of IL-36 and psoriasis by HDPs was assessed by Western blotting. MAPK and NFκB activators were used to study the mechanism by which IL-36 stimulates keratinocytes. We found that all of these IL-36 enhanced IL-37 and psoriasis expression and protein production by keratinocytes in a keratinocyte-specific fashion. The induction of IL-36 and psoriasis by HDPs was assessed by Western blotting. MAPK and NFκB activators were used to study the mechanism by which IL-36 stimulates keratinocytes. 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ABSTRACTS | Epidermal Structure & Barrier Function

655 Dietary effect of royal jelly supplementation on epidermal levels of hydration, filaggrin, free amino acids and the related enzyme expression in UV irradiated hairless mice

J Min1, Y Lee1, S Han2 and Y Cho3 1 Kyung Hee University, Yong-In, Republic of Korea and 2 Rural Development Administration, Suwon, Republic of Korea

Universal UV irradiation reduces epidermal hydration, while increased by the parallel reduction of the natural moisturizing factors (NMF). Various NMFs, free amino acids (AA) and major AA are the components that are generated by filaggrin degradation. In this study, we examined whether dietary supplementation of royal jelly (RJ) to UV-irradiated mice alters epidermal levels of hydration, filaggrin and free AA as well as of peptides/carnosine dimers (3-PA2, 3-PA3), an enzyme involved in filaggrin degrada-
tion process. Albino hairless mice were fed either a control diet (group UV+) or UV-irradiated controls or diets with 1% RJ harvested from different areas in Korea (groups R1, R2 and R3) or imported from China (group RJ) for 6 weeks in parallel with UV irradiation. A normal control group (group UV−) received a control diet without UV irradiation for 6 weeks. In group UV+, epidermal levels of hydration, total filaggrins and PA2 were reduced; these levels in group R1 were increased to the similar level of group UV−. Furthermore, profilaggrins, two repeat intermediates (IR), a precursor with two flanking prolines, and profilaggrins were increased. Although total AA were not altered in all groups, glutamate and serine, major AA of NMF in group R1 were higher than in group UV+. Despite the increased levels of PA2, epidermal levels of hydration, filaggrins, glutamate and serine in groups R1 and UV− were similar in group RJ. Dietary supplementation of RJ increased epidermal hydration with enhancing filaggrin expression and degradation, but not by increased protein expression of PA2, along with the increased generation of glutamate and serine. *This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ0090062012), Rural Development Administration, Republic of Korea.

656 Dietary royal jelly improves epidermal hydration with increased levels of glucosylceramide and ceramide in intrinsically aged C57BL mice

S Jeon1 and Cho Kyung Hee University, Yong-In, Republic of Korea

Epidermal hydration is maintained by epidermal lipid barrier, of which ceramide (Cer) is the major constituent. In this study, the dietary effect of royal jelly (RJ) on epidermal levels of hydration and Cer species was determined in intrinsically aged C57Bl mice. Altered Cer metabolism was further determined, as measured by epidermal levels of glucosylceramide (GlcCer) and sphingomyelin (SM), two major precursor lipids in Cer generation, and of Cer metabolizing enzymes. 6 Month old C57BL mice were fed a control diet (group C) or diets with 1% RJ harvested in area 1 (group R1) or 2 (group R2) for 16 weeks. Compared to group C, epidermal levels of hydration, total Cer (including Cer 2-7 and total GlcCer (including GlcCer A-D) were significantly increased in group R1. In addition, protein expressions of β-glucosylceramide β-glucosidase (β-GlcCerase) and acidic sphingomyelinase (aSMase), involved in the generation of Cer in skin, were increased and of ceramidase (CDAse), the Cer degrada-
tive enzyme, was increased. Epidermal levels of all SM species and serum palmitoylsphingomine (PSP) protein in the novo Cer synthesis, were similar between groups C and R1. Despite the increased levels of SM and CDP, epidermal levels of hydration, Cer 2-7, GlcCer-D-D, β-GlcCerase, aSMase, and CDAse in group R2 were similar with those in group C. Cor 1 and Cer synthesis in the novo Cer synthesis were not altered among all groups. Dietary RJ improves epidermal hydration in par-
tially cytoplasmic expression. According to these findings, we developed a biofunctional, IV12.003, targeting Thymosin-
β4 in fibroblasts, representative of dermal skin compart-
ment after cutaneous injury, and hair growth. To better understand the functions of Thymosin-
β4 in skin-derived cell

657 Thymosin-beta4: A major player in skin renewal and anti-aging processes

J Berger1, J Court1, B Botto and N Damgård1, Ashleigh Specialty Ingredient, Vinceinfo, Gland, Switzerland, 1 Department of Dermatology and Allergology, RWTH Aachen University, Aachen, Germany and 2 Institute of Biochemistry and Molecular Biology, RWTH Aachen University, Aachen, Germany

Thymosin-β4 (Tβ4), described as a regulator of the intracellular levels of globular/polymerized actin, is a multi-faceted molecule with different biological functions in the skin. Indeed, Thymosin is involved in keratinocyte migration, collagen deposition, angiogenesis, downregulation of inflam-
mation after cutaneous injury, and hair growth. In our previous results, the modulation of Tβ4 expression in fibroblasts, representative of dermal skin compart-
ment after cutaneous injury, and hair growth. To better understand the functions of Thymosin-
β4 in skin-derived cell

658 Kallikreins 5 and 7 are upregulated in human skin following application of irritants

J Underwood1, C Pickard and E Healy Dermatopharmacology, Faculty of Medicine, University of Southampton, Southampton, United Kingdom

Irritant contact dermatitis is the most prevalent form of occupational dermatoses, however, the mechan-
isms involved in irritant responses of the skin are not well characterised. Serine proteases and their downstream effectors play a role in some skin diseases with disordered barrier function, such as Netherton syndrome; therefore we hypothesised that serine proteases may play a role in the effects of irritants on human skin. 3% croton oil and 5% sodium dodecyl sulphate were applied epider-
ally to an ex vivo human skin model to investigate for changes in serine protease expression and activity. Immunofluorescence staining and in situ zymography, with image analysis, were employed to examine for changes in the expression and activity of proteases following 30 minutes of irritant application. Kallikrein 5 (KLK5) and kallikrein 7 (KLK7) were both upregulated following irritant treatment (p=0.0001 and p=0.0009 respectively). KLK5 showed a pan-epidermal pattern of expression with higher levels in the stratum corneum and stratum basale after application of in-
stant contact irritants. KLK7 was expressed at the stratum corneum-stratum granulosum junction and stratum basale. In situ zymography (Enzcheck assay) detected a corresponding increase in protease activity of KLK5 in skin exposed to cervamide (CDAse) and acidic sphingomyelinase (aSMase), which further maintained with low CDase protein expression. *This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ0090062012), Rural Development Administra-
tion, Republic of Korea.

659 IL-31 prevents barrier formation but tightens the antimicrobial defense of the skin partially by upregulating IL-1α: expression in human organotypic skin models

K Han1, C Comelisenn1, V Antons1, Y Marquardt2, R Caza1, L Schierf-Fierzlar2, B Lischker1 and J Barng1 1 Department of Dermatology and Allergology, RWTH Aachen University, Aachen, Germany and 2 Institute of Biochemistry and Molecular Biology, RWTH Aachen University, Aachen, Germany

The interleukin-31 (IL-31) is suggested to be an important mediator in psoriatic skin diseases like atopic dermatitis (AD). IL-31 levels are enhanced in patients with AD and correlate with the severity of the disease. In these findings, the function and consequences of IL-31 expression in the pathogenesis of AD are only poorly understood. Recently we demonstrated that IL-31 has the capac-
ity to disturb the differentiation process of keratinocytes by inhibiting the expression of important structural proteins including involucrin. In this study, we demonstrate that IL-31 treatment provokes an enhanced transepidermal permeation by allergens and pathogens as well as skin-irritating agents. We could determine that IL-31 affects the processing of filaggrin and the formation of cor-
eudeodesmosomes by negatively regulating filaggrin-processing enzymes like CASP14 and KLK7 and desmosomal adhesion molecules. These defects in keratinocyte differentiation and comification lead to an impairment of the skin barrier. Accordingly, IL-31 might be involved directly in the pathol-
ogy of AD leading to increased vulnerability to skin irritants and a higher risk for allergic sensitiza-
tion by environmental allergens. Furthermore, IL-31 was found to enhance the expression of compo-
ments of the IL-1 network (most profoundly IL-1α), and of antimicrobial peptides (S100A7, S100A8, S100A9, HBD-2). Importantly, the IL-1 receptor antagoniste IL-1Ra increases the increased expression of anti-
microbial peptides could be inhibited. Thus we conclude that IL-31 may strengthen the antimicrobial defense of the skin via IL-1α. This is a first hint for the physiological role of IL-31 in healthy skin.

660 Air-liquid interface epidermal cultures of keratinocytes from elderly adult skin which have been treated with the rho kinase inhibitor, Y27632, contain multiple areas of dysplasia

J Underwood1, M Pluka1, J Chan1, C Pickard1 and E Healy 1 Dermatopharmacology, Faculty of Medicine, University of Southampton, Southampton, United Kingdom and 2 Histopathology, University of Southampton, Southampton, United Kingdom

In vitro cultures of keratinocytes are a useful tool for investigations of skin biology and disorders affecting the skin, however, cultured primary keratinocytes from adults have a relatively short pro-
liferative lifespan. It has been reported that the rho kinase inhibitor, Y27632, immortalises infant human keratinocytes, Y27632 immortalises infant human keratinocytes and significantly increases proliferation of adult keratinocytes. In this study, we attempted to "semi-immortalise" keratinocytes from older adults (> 60 years old) as well as from younger adults (< 60 years old) using Y27632. Normal human keratinocytes were extracted from tissue samples and cultured in the presence or absence of 10 μM Y27632 until population doubling per day fell below 0.2, at which time the keratinocytes were deemed to have entered senescence. Y27632 signific-
antly increased the life of keratinocytes from patients aged up to 92 years old from 51.09 ± 2.21 days to 87.73 ± 19.29 days (p < 0.0001). Total population doublings were increased from 13.32 ± 7.00 to 13.18 ± 14.68 (p < 0.0001) in Y27632 treated keratinocytes, but none of the adult keratinocytes were immortalised in the presence of Y27632. In comparison with untreated normal human keratinocytes, qPCR analysis of Y27632 treated cells demonstrated alterations in genes involved in cell proliferation and apoptosis, including CCND1,2, CDKNA2, B, CTSE1, DX11, MD67 and BCL2. Air-liquid interface culture of site-parallel Y27632 treated adult keratinocytes on commercially available scaffolds resulted in multiple areas of dysplastic epithelium, similar to that seen with untreated keratinocytes at early passage from elderly patients. Thus, although Y27632 can "semi-immortalise" keratinocytes from adults aged > 60 years old, 3D culture of these cells results in stratified epidermis containing substantial numbers of dysplastic cells.
Chaperone proteins such as Mitochondrial Heat Shock Proteins (mtHSPs) play a critical role in the protection of keratinocytes and human skin from stresses. Our body and more specifically the skin are facing day after day damaging stresses such as ultraviolet (UV) and infrared (IR) irradiation, oxidative damage, physico-chemical injuries. To respond to these various stresses and protect the body, the molecular chaperones called "Heat Shock Proteins" (HSPs) help to limit protein misfolding. HSPs are ubiquitously expressed through the skin belong to several families (HSP70, HSP90, HSP27, HSPA4 and HSP27). Under physiological conditions, HSPs are involved in the synthesis and the transport of proteins across cellular membranes, and protein import in the mitochondria. HSPs limit protein aggregation, due to stress and help the refolding of denatured proteins. In the present study, we evaluated the effect of UV11.004, a compound designed to specifically modulate the expression of mitochondrial HSP (mtHSPs), on the prevention of cellular damage after oxidative-stress and irradiation (UV, IR). To do so, we studied the expression pattern of mtHSP70 (Mortalin), mtHSP60 and its co-factor HSP10, in normal human keratinocytes and in ex vivo human skin, by immuno-fluorescence detection. When keratinocytes or ex vivo human skin were treated with UV11.004, we observed a higher content in mitochondrial HSPs, suggesting a possible positive modulation of their level. We then focussed on the protective role of mtHSP70, mtHSP60, and mtHSP10 in a model of in vitro keratinocyte exposure to UVA and UVB mono-wavelength irradiation. The further study of these microRNAs could help better understand the pathways particularly involved in skin mitochondrial and cellular stress-induced damage, particularly during aging.

Bioinformatic analysis of genes and microRNAs potentially involved in keratinocyte differentiation

The human epidermis undergoes a continuous renewal through the proliferation and differentiation of keratinocytes. Some microRNAs are described as being involved in the skin homeostasis and several biological processes including skin development, stem cell differentiation, signal transduction, metabolism, genomic stability, apoptosis, wound healing, and skin immunity. Several microRNAs have been identified as specifically expressed in human epidermal keratinocytes and play a role in the epidermal differentiation process. In our study, we focussed on microRNAs that are specifically expressed in keratinocytes and produce less ROS under stress conditions, when compared to untreated cells. We also assessed the ability of UV11.004 to help prevent mtHSP expression down-regulation in a stressed or a senescence model. In conclusion, our results demonstrated an enhancement in melanin expression in both conditions of irradiation, with a higher level after mono-wavelength UVB irradiation compared to full-spectrum one. These results demonstrated an enhancement in melanin expression in both conditions of irradiation, with a higher level after mono-wavelength UVB irradiation compared to full-spectrum one. These results were consistent with our previous study which used a comparable UV exposure protocol and revealed that the highest DNA damage was also obtained after UVB exposure. Finally, we determined that the UVB monowavelengths, in addition to melanin, also target skin pigmentation, and we confirmed that the UVB consequences could be decreased by a combination with UV radiation.
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Air exposure hastens barrier maturation in human epidermal equivalents

K Sun, 1 A Celli, 1 T Zhao, S Pennagudi, 1 T Schaner, 1 S Elia, 1 H Haub, 1 K Fung and 1 T Maun 2 Department of Dermatology, VA Med Ctr/UCSF, San Francisco, CA and 2 Cincinnati Children’s Hospital, Cincinnati, OH

The epidermal barrier prevents water loss and entry of foreign organisms or toxins under most circumstances. However, immature skin barrier in premature infants leads to increased transepidermal water loss (TEWL) and sepsis from bacteria and fungi. Air exposure or exposure to low incubator humidity has been shown to enhance epidermal barrier formation in both full term fetal epidermal development models and clinical care of premature infants. We previously have demonstrated that barrier development in Human Epidermal Equivalents (HEE) parallel that of fetal rat skin, and that both lipid and tight junction expression underlies development of a normal epidermal barrier. In this report, we use the HEE model to show that exposure to low humidity accelerates barrier maturation and that barrier development is dependent on both lipids and tight junctions. The results indicate that air exposure hastens barrier maturation in human epidermal equivalents.

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Clarifying the role of autophagy in the skin by using skin grafts from wild-type and ATG7-deficient mice

N Yusharib, 1 Takagi, 1 T Umou 2 and S Ikeda 1 Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan and 2 Department of Biochemistry, Juntendo University Graduate School of Medicine, Tokyo, Japan

Autophagy is the major intracellular degradation system by which cytoplasmic materials are delivered to and degraded in the lysosome. However, the purpose of autophagy is not only elimination of materials, but also serves as a dynamic recycling system that produces new materials and energy for cellular renewal and/or homeostasis. Many reports regarding autophagy were published in recent years, and its interest has markedly increased. However little is known about the functions of autophagy in the skin, because autophagy-deficient mice die within 24 hours after their birth, resulting in limited use of their skin for further studies. Accordingly, we transplanted the skin from expression of clock genes in old mice was analyzed. Rhythmic expression of Bmal1 showed significant change in the old mice skin, compared with that in young mice. Expression of other clock genes (Per, Cry, Tdp etc.) are currently under investigation. These data indicated that aging influences circadian rhythm of clock genes expression in the skin as well as in other peripheral tissues. Next, to clarify the functions of clock genes in the skin, we examined skin capacitance (SC), skin surface pH (pHv) in Bmal1 knockout (KO) mice. Epidermal repair ability was also evaluated by transdermal water loss measurements following tape stripping, induced barrier disruption. As a result, the increase in pHv decrease in SC and immature stratum corneum were observed in KO mice. Epidermal barrier recovery was also delayed in KO mice, compared with those of wild-type mice. Although further investigation needs to be conducted, our findings indicate that the skin clock might play an important role in the regulation of epidermal homeostasis.

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Anti-psoriatic effects of 1,25-dihydroxyvitamin D3 in combination with betamethasone on human keratinocytes

H Ogawa, 1 K Shirotani, 1 R Horimoto and R Ibuki Research Laboratories, Kyoto R&D Center, Marubio Co., Ltd., Kyoto, Japan

Activated vitamin D3 analogues and corticosteroids have been widely used for topical treatment of psoriasis. A combination therapy of the two drugs is more useful than monotherapy because of an earlier therapeutic effect and a decrease in the adverse effects of either drug. However, there have been few clinical reports on combined effect of activated vitamin D3 analogues and corticosteroids. We investigated the anti-psoriatic effects of 1,25-dihydroxyvitamin D3 (VD3) in combination with betamethasone (Bet) on human epidermal keratinocytes (KC) in vitro. VD3 (10^-9 to 10^-5 M) decreased the uptake of [3H]thymidine (3H-TdR) into KC in a concentration-dependent manner, indicating the inhibition of KC proliferation. In contrast, Bet (10^-5 M) had a major effect on uptake of 3H-TdR into KC, indicating that the inhibition of KC proliferation was not changed by the first application of DNFB (sensitization phase) and no inflammatory cytokine induction was observed under these experimental conditions. These results suggest that VD3 in combination with Bet may be a useful combination therapy for the treatment of psoriasis.

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Clock gene is one of the important regulators in epidermal homeostasis

1 Kano, 3 K Tsuda, 1 M Fujii, 1 T Yamaguchi, 5,6 N Funakushi, 3 H Suto, 4 R Ueki, 5 H Kobayashi, 5, HO gawa 1 and 1 Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan, 2 Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan, 3 Center for Advanced Kampo Medicine and Clinical Research, Juntendo University School of Medicine, Tokyo, Japan, 4 Department of Dermatology, Juntendo Tokyo Gastroenterial Medical Center, Tokyo, Japan, 5, Center for Advanced Kampo Medicine and Clinical Research, Juntendo University School of Medicine, Tokyo, Japan and 6 Tsukuba Research Laboratories, Tsukuba & Co, Ibaraki, Japan

In the central nervous system (CNS), glutamate is known to act as a signal transducers as well as cell death effector that has been implicated in the pathogenesis of neurodegenerative diseases. Interestingly, Yokusakusen (YKS), a traditional Japanese medicine, has been reported to effect on the glutamate nervous system. On the other hand, increasing data suggest that glutamate might also act as a cell-signalling molecule in non-neuronal tissues such as skin. It has been also reported that glutamate is concentrated at high levels in the skin with wound healing or inflammation. Furthermore, glutamate can have cytotoxic effect in the skin. Previously we reported that YKS ameliorated scratch

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Decreased adhesion of keratinocytes via C-Ecadherin downmodulation is a platform for leukocyte infiltration in the epidermis

C Fujino, 1 M Yakuhi and K Kabiibashi Dermatology, Kyoto University, Kyoto, Japan

The epidermal keratinocytes are tightly adhered each other with cell-adhesion structures. Therefore, it remains a question how leukocytes in the epidermis migrate within such a “packed” area. Here, we examined the transepidermal mobility of Langerhans cells (LCs) and T cells in the context of contact hypersensitivity response by means of laser-photon microscopy. First, we established in-vivo labeling technique for intercellular gaps between keratinocytes and revealed that the LCs were captured near the gap and represented static mobility in the steady state. Intriguingly, the LCs-mobility was not changed by the first application of DNFB (sensitization phase) and no LC T cells were infiltrated into the epidermis in this phase. In contrast, 24 hours after the second application of DNFB (elicitation phase), LCs represented two distinct migration patterns; one was an anchored population which still elongated long dendrites upwards. The other was a high-mobility population which actively migrated in the epidermis in accordance with expanding the keratinocytes gaps up to 5 μm. Similarly, CD4+ T cells entered into the epidermis of the wounds from the adjacent leukocytes in the epidermis. In inflammatory conditions, the connections between keratinocytes become loose via C-Ecadherin down-regulation, which may provide a migratory pathway for epidermis-infiltrating leukocytes. Thus, spongiosis, an intercellular edema of keratinocytes, should be an essential mechanism for facilitating the intraepidermal leukocyte migration.

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The role of glutamate signaling in keratinocytes

M Wakabayashi, 1 T Yamaguchi, 1 N Funakushi, 1 H Suto, 1 R Ueki, 5 H Kobayashi, 5,6 H Ogawa 1 and S Ikeda 1 Department of Dermatology and allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan, 2 Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan, 3 Department of Dermatology, Juntendo University Nihon Hospital, Tokyo, Japan, 4 Department of Dermatology, Juntendo Tokyo Gastroenterial Medical Center, Tokyo, Japan, 5, Center for Advanced Kampo Medicine and Clinical Research, Juntendo University School of Medicine, Tokyo, Japan and 6 Tsukuba Research Laboratories, Tsukuba & Co, Ibaraki, Japan

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A new cosmetic composition attenuates the effects on age-related imbalance of biological functions of stratum corneum

N.Cavalcanti1, S.Bouraja,2 B.Nehme,3 K.Abed,1 B.Bernard,1 S.Cheliah,4 B.Lavaz,1 C.Delolme,5 A.Dhrit1 and D.Bernard1

*COREL R I, Paris, France and 2 Planteurobio protéomique CHU, CHU Paris Est, France.

A mass spectrometric, isobaric tags for relative and absolute quantitation approach (iTRAQ) was applied to differentially analyze the uppermost surface of the skin, the stratum corneum, obtained by a non-invasive stripping (D’SquameTM). 48 volunteers daily used a recent anti-aging cosmetic product on one forearm for a two month period, the other forearm being considered as control. Stripping on both forearms from 16 volunteers was pooled, forming two different groups, i.e. treated and untreated. This step was repeated twice, finally including the 48 volunteers and permitting 1 independent 4-plex iTRAQ experiments. Proteins were submitted to a quadrupлекс iTRAQ-labeling protocol yielding 4 differentially enriched samples. These proteins covered 5 key biological functions, with 4 functions including a majority of over-expressed proteins and 1 function with mainly under-expressed proteins. The enhanced functions concern tissue structure, desquamation, anti-oxidant action and differentiation.

The stratum corneum comprises three layers with distinct barrier properties to metal ions. A "sponge" allowing the passive influx and efflux of exogenous ions. The middle layer blocked the permeation of wildtype and filaggrin knockout mice. TOF-SIMS enabled to visualize the distribution of potassium (K) and arginine revealing that the SC consists of three layers as predicted. The insoluble nature of the SC has hampered in-depth proteomic studies. We have used a recent anti-aging product on one forearm for a two month period, the other forearm being considered as control. Stripping on both forearms from 16 volunteers was pooled, forming two different groups, i.e. treated and untreated. This step was repeated twice, finally including the 48 volunteers and permitting 1 independent 4-plex iTRAQ experiments. Proteins were submitted to a quadruplex iTRAQ-labeling protocol. These proteins covered 5 key biological functions, with 4 functions including a majority of over-expressed proteins and 1 function with mainly under-expressed proteins. The enhanced functions concern tissue structure, desquamation, anti-oxidant action and differentiation.

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Disturbed lipid metabolism in fetal skin predisposes for development of atopic diseases

D. Khurana1,2, M. Lin1, N. Bjelakovic1, N. Goto-Inoue1, Y. Uchida1, T. Hasegawa1, M. Suto2, Y. Mine1, F. Johansen1 and F. Johansen1 1 Pathology, University of Oslo, Oslo, Norway, 2 National Health Research Institutes, Zhunan, Taiwan

Recent data have suggested that reduced epidermal barrier function predispose to development of atopic diseases. Here we show that atopic symptom studies on (IPS), an autosomal recessive congenital ichthyosis caused by mutations in the lathyrid acid transport protein (PATP4) gene, is strongly associated with lathyrid atopic manifestations beyond the skin and a striking persisting central blood coagulopathy. The affected skin revealed a THL-like inflammatory reaction with signs of activation of keratinocytes expressing the inducible form of TSLP. Interestingly, the inflammatory skin reaction including the expression of TSLP was already present in the fetal stage. Activated TSLP-keratinocytes were also found in mouse embryos and skin grafted from newborn FATP4-/- mice onto athymic nude mice revealed an atopic dermatitis-like inflammation. Our results suggest that altered lipid metabolism in the epidermis triggers a deregulated activation of keratinocytes that can cause skin atopy and the adaptive immune system. This finding strongly suggests that disturbed lipid metabolism in the skin is a direct cause of systemic lathyrid-mediated allergic disorders in which TSLP play a central role.

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Loss of the local cortisol stress response pathway contributes to the pathogenesis inflammatory skin diseases

R. Hansen1, J. Niktorczew-Buniak1, R. Stratton1, A. Ahmed2, S. Rajpopat1 and M. Philpott1 1 Centre for Cutaneous Research, Queen Mary University of London, London, United Kingdom and 2 Centre for Rheumatology, University of London, London, United Kingdom

Endogenous glucocorticoids (GCs) are essential for maintaining the epidermal skin barrier and their powerful anti-inflammatory effects are exploited therapeutically to treat inflammatory skin conditions such as psoriasis, eczema and scleroderma. We recently demonstrated that healthy primary keratinocytes are capable of de novo cortisol synthesis, the primary GC in humans. Furthermore healthy skin challenged with inflammatory stimuli such as U9 and PMA, 24h induced a dose response increase in cortisol production when measured by ELISA. GC deficiency can promote susceptibility to autoimmune and inflammatory diseases, however, the local production of cortisol and the GC stress response has not been examined in inflammatory skin diseases. Using LC-MS/MS analysis, we demonstrate that cortisol production is almost completely ablated in psoriatic and scleroderma skin relative to healthy human skin (healthy skin: 809.6±20.4ng/ml, psoriatic lesion: 67.7±11.8ng/ml, scleroderma lesion: below the limits of cortisol detection). Furthermore, this layer chromatography showed GC synthesis in psoriatic keratinocytes was less than 20% of healthy controls. Steroid regulators and steroid enzyme expression, measured by western blot, were also significantly reduced in psoriatic and scleroderma skin. Moreover the 11-Beta hydroxysteroid dehydrogenase (GR) expression was downregulated in lesional and non-lesional psoriatic skin. In summary, the GC pathway from de novo synthesis to GR expression is severely compromised in inflammatory skin diseases. Since GCs are essential for regulating barrier integrity, loss of the local cortisol stress response in skin may represent a novel component in the pathogenesis of these diseases. Exogenous GCs when used therapeutically can induce side effects such as skin atrophy, thus identifying new mechanisms to regulate endogenous GC synthesis could be of significant therapeutic benefit.

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Novel metabolism of vitamin D in human epidermal keratinocytes and epithelial colon cells

T Kim1,2, R. Tuckey3, E. Tang4, W. Li4 and A. Somers4 1 Pathology, University of Technology Sydney, Sydney, NSW, Australia and 2 Dermatology, University of Western Australia, Crawley, WA, Australia

In multiple sclerosis, vitamin D analogs are produced in the skin, we incubated HaCaT keratinocytes with vitamin D3 as substrates and analyzed the extracted metabolites by LCMS using the corresponding authentic standards. Production of 20(OH)D3, 22(OH)D3, 25(OH)D3, 20,22(OH)D3 and 1,25(OH)2D3 was measured in the culture supernatant of HaCaT. Vitamin D3 and D2 were readily detected when vitamin D3 or D2 were used in the experiments. We found that vitamin D3 production was time and dose-dependent. Interestingly, in large scale cultures we also detected endogenous production of 20(OH)D3, 22(OH)D3 and 20,22(OH)D3 by HaCaT keratinocytes. To test whether cells of the gastrointestinal tract can also metabolize vitamin D, we incubated CaCo2 cells with vitamin D3 or D2 as substrates. LCMS analyses showed that all of the above metabolites were produced in CaCo2 cells after addition of exogenous substrates. We conclude that both epidermal keratinocytes and intestinal epithelial cells can metabolize vitamin D as follows: D3→20(OH)D3→22(OH)D3→20,22(OH)D3→20,22(OH)D2→17,20(OH)D3 with further hydroxylation at the 1α position to produce the corresponding 1α-hydroxysteroids. We believe that these findings are extremely significant in the context of skin biology and therapy of skin and systemic diseases, because of their biological activity. They are also excellent candidates for therapy of autoimmune/inflammatory and hyperproliferative disorders because of their lack of measurable toxicity and calcemic effects.

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JAK-STAT pathway mediates cutaneous inflammation via enhancing immune responses and attenuating skin barrier functions

W. Amor1, S. Nakayama1, Y. Miyachi2 and K. Kabashima1 1 Dermatology, Tokyo University Graduate School of Medicine, Tokyo, Japan and 2 Laboratory of Medicine, University of Western Australia, Crawley, WA, Australia

Cutaneous immune responses, including contact dermatitis and atopic dermatitis (AD), are triggered by immunodysregulation and a skin barrier dysfunction. It is known that cytokines, such as IFN-γ and IL-4/13, mediate the skin inflammation and IFT production, respectively. The JAK-STAT pathway plays a pivotal role in cytokine signaling. Several studies suggested that JAK inhibitors attenuate murine AD, but its underlying mechanisms remain unclear. Initially, we used a murine contact hypersensitivity (CHS) as a model to evaluate the role of JAK signaling. A JAK inhibitor tofacitinib (CP-690,551) inhibited de novo/hypersensitivity responses and CD4+ and CD8+ T cell infiltration into the skin in the elicitation phase. In addition, the JAK inhibitor suppressed TNF-α production in T cells by stimulation with anti-CD3/CD28 in vitro. These data suggest that JAK-STAT signaling mediates T cell activation and is required to maintain skin barrier function under inflammatory conditions. CHS conditions (ex. IL-4/13) are known to impair skin barrier by suppressing filaggrin expression in keratinocytes. Intriguingly, this impaired skin barrier function and impaired inflammation is not induced by treatment with JAK inhibitors. Moreover, the JAK inhibitor restored natural moisturizing factor level by means of raman spectrometry. These data suggest that JAK-STAT signaling plays an essential role in the development of skin immune responses not only by promoting immune responses but also by attenuating skin barrier dysfunctions.

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Loss of ELOVL1 enzyme depletes very long chain fatty acids and causes lethal skin barrier disruption in mice

S Suzuki1, T. Nomura1, T. Sasa1, Y. Ohno1, M. Akayama1, A. Kihara1 and H. Shimizu1 1 Department of Dermatology, Hyogo University Graduate School of Medicine, Sapporo, Japan and 2 Products Research Laboratories, AMBON Co., Ltd., Chita, Japan

ELOVL1 is a member of the mammalian fatty acid (FA) elongase and is involved in the synthesis of very long chain fatty acids (VLCFAs). VLCFAs are a key component of the skin barrier function. The mutant mice (Elovl1−/−) embryonic day 18.5 showed lower body weight (p<0.01) than wild-type littermates and died within 12 hours after being ejected from the uterus, whereas the wild-type survived for more than 24 hours. The mutants had significantly higher (p<0.01) transcutaneous water loss, greater susceptibility to water permeability and faster water loss than the wild-type. Skin histology using light and electron microscopy revealed compact stratum corneum (SC), reduced SC intercellular lipids and deficient epidermal lamellar granules. Lipid analysis of the epidermis from Elovl1−/− mice showed a significant decrease in ceramides containing VLCFAs (C26) and a compensatory increase in shorter-chain ceramides (C24). These results highlight the importance of ELOVL1 in epidermal barrier function and its potential as a therapeutic target for skin disorders such as atopic dermatitis and ichthyosis.

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The role of protein α-fucose transferase-1 in epidermal and hair cycle homeostasis

H Lin and L Yang  National Health Research Institutes, Zhunan, Taiwan

Notch signaling regulates a variety of processes such as differentiation, proliferation, apoptosis, and cell fate choices. Notch ligand binds to Notch receptor resulting in the release and translocation of the corresponding 1α-hydroxysteroids. We believe that these findings are extremely significant in the context of skin biology and therapy of skin and systemic diseases, because of their biological activity. They are also excellent candidates for therapy of autoimmune/inflammatory and hyperproliferative disorders because of their lack of measurable toxicity and calcemic effects.
685 Interferon-β does not reduce calpain 1 but filaggrin expression in a concentration and time-dependent manner.

F. Prokisch and R Panzer Clinic for dermatology, University hospitals Schleswig-Holstein, Campus Kiel, Kiel, Germany

The disturbed skin barrier plays an important role in the pathogenesis of atopic dermatitis. The development of the skin barrier malfunction in atopic dermatitis is not fully understood. Besides loss of function mutations in the filaggrin gene in about 30% of the patients there are data suggesting a secondary impairment of the skin barrier by mediators of inflammation. This includes reduction of the expression of filaggrin and filaggrin processing enzymes as shown for biocytin hydrolyase. In line with this we found decreased filaggrin expression in skin from patients with or without filaggrin mutations. Calpain I is another enzyme thought to be involved in late steps of degranulation responsible for degrading desmoglein. In previous experiments with different profilaggrin expression and activation was also analyzed. In hyperproliferative psoriatic epidermis, upregulated calpain I expression was decreased more than 5-fold. But calpain I was down-regulated more than 2-fold by only retinoid acid. The results of the present microarray analysis of retinol treated keratinocytes, demonstrating differential regulation of genes related to epidermal differentiation compared to retinoid acid, suggest that retinol reduced side-effect such as desquamation and epidermal thickening when it was used for preventing photo-aging.

686 Retinol showed different gene expression of keratinocyte differentiation compared to retinoid acid-dependent manner.

H. Liu, H Cho, J Cho and H Lee R&D Center, Amorepacific Corporation, Yangsan-si, Republic of Korea

Retinoids enhance proliferation and desquamation of cultured epidermal cells and suppress differentiation. Retinol was used instead of retinoid acid for preventing skin aging. Although considerable knowledge about retinoid acid has been accumulated, little is yet known about the mechanisms of retinol. To clarify the mechanisms underlying the action of retinol compared to retinoid acid, we carried out gene expression analysis in epithelial differentiation using DNA microarray analysis. Three pairs of cultured normal human keratinocyte specimens were obtained after retinol and retinoid acid treatment for 2days. Each set of specimens and non-treated normal specimens were analyzed with microarray chip. Ninety-five genes were more changed more than 2-fold in retinol with compared with normal control samples while three hundred ninety-four genes were changed in retinoid acid treated specimens. Compared with retinoid acid-treated keratinocytes, less epidermal differentiation-related genes were down-regulated in retinol-treated keratinocytes. Retinol reduced the expression of filaggrin and loricrin more than 2-fold. Retinoid acid reduced them more than 5-fold. But claudin 1 was down-regulated more than 2-fold by only retinoid acid. The results of the present microarray analysis of retinol treated keratinocytes, demonstrating differential regulation of genes related to epidermal differentiation compared to retinoid acid, suggest that retinol reduced side-effect such as desquamation and epidermal thickening when it was used for preventing photo-aging.

688 Glucocorticoid receptor enhances involucrin expression of keratinocyte in a ligand-independent manner.

J. Lee, H Yoon, K Sohn, D Choi, D Hong, M Im, Y Lee, Y Soo and C Kim Dermatology, Changwon National University, Daejeon, Republic of Korea

In this study, we investigated the role of glucocorticoid receptor (GR) in epidermal keratinocytes. In adult normal skin, glucocorticoid receptor was highly expressed in the upper layer of the epidermis. Consistent with normal skin, GR expression was increased after calcium treatment of HaCaT keratinocytes cultured in vitro, suggesting that GR is involved in keratinocyte differentiation process. Overexpression of GR using an adenovirus showed that expression of involucrin, an early differentiation marker of keratinocytes, was markedly increased due to GR overexpression. However, treatment of GR with dexamethasone, a GR agonist, did not increase involucrin expression. Overexpression of GR led to phosphorylation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinases (ERK) in the absence of glucocorticoid, suggesting that the GR effect on involucrin expression is related to activation of extracellular signal-regulated kinases (ERK). This idea was supported by the fact that GR-mediated involucrin induction was abolished after treatment with JNK and ERK inhibitors. In addition, GR mutants lacking the ligand binding domain increased involucrin expression compared with that of ERK phosphorylation. Together, these results suggest that GR mediates involucrin expression of keratinocytes by regulating the intracellular signaling network in a ligand-independent manner.

689 Podoplanin alters β1-integrin-mediated cell adhesion and initiates terminal differentiation of human epidermal keratinocytes.

M Hirooka, M F Takahashi and H Izuka Department of Dermatology, Akihikawa Medical University, Asahikawa, Japan

While podoplanin (PDPN) has been recognized as a lymphatic marker, the expression is also detected in healthy and diseased human skin, such as sebaceous gland, hair follicles, wound, and psoriatic epidermis. However, the precise function has not fully been elucidated. This study was carried out to verify PDPN function in normal human epidermal keratinocytes (HEK). Focusing on cell adhesion and differentiation. Cell adhesion and differentiation of HEK were analyzed in both conditions of PDPN-overexpression and -silencing using adenoviral vector. Effect of PDPN on β1-integrin expression was also analyzed. In hyperproliferative psoriatic epidermis, upregulated PDPN expression was observed especially in basal cell layer of rhytide ridges compared with that on tips of dermal papilla. The expression pattern was inversely correlated with β1-integrin expression in an in-situ manner on tips of dermal papilla. PDPN-overexpression suppressed cell adhesion of HEK to type I collagen and induce the differentiation marker, correlating with downregulated β1-integrin expression/activation. The PDPN-mediated regulatory mechanism of β1-integrin expression is dependent on the interaction with CD44. PDPN-overexpression activity is active β1-integrin level, which was suppressed by CD44-silencing. Immunoprecipitation assay showed that PDPN inhibited molecular interaction of β1-integrin and CD44, which is essential for the activation of β1-integrin. These results suggest that PDPN plays a role in supply of differentiating keratinocytes to thickened suprabasal layer of hyperproliferative psoriatic epidermis, in which accelerated cell division should occur in cooperation with induction of terminal differentiation process in the basal cell layer.

690 Interest of Pentyl-rhamnoside in prevention of xerosis.

M Galliano, C Carrasco, S Bessa-Toyos and H Duplan Dermo Cosmetic Department, Pierre Fabre R&D Center, Toulouse, France

In previous works, we demonstrated the inhibitory activity of the cosmetic ingredient Pentyl-rhamnoside on inflammation induced in keratinocytes exposed to atopic dermatitis environment. Moreover, Pentyl-rhamnoside restored gene expression of several markers of terminal differentiation or involved in lipid synthesis, thereby promoting barrier repair. Pentyl-rhamnoside-containing balm applied onto normal human reconstructed epidermis significantly enhanced de novo synthesis of the lipids involved in the lipid barrier formation, suggesting that the balm may bring benefit to dry skin or dehydrated skin. The aim of the present study was to investigate the preventive effect of Pentyl-rhamnoside on dehydration. Human skin explants that were pre-treated or not with Pentyl-rhamnoside-containing balm were submitted to dryness conditions in a humidity-controlled chamber maintained at 15% of relative humidity and 37°C. Punch biopsies were then processed for in situ zymography and immunohistochemical analyses. After acute dryness induction, a notable increase in protease activity was revealed by in situ zymography. By immunostaining, we observed markedly decreased labelling for the tight junction protein claudin 4 and the desmosomal protein desmoglein 1, together with altered localization of the aquaporine 3 water channel. When explants were pre-treated with Pentyl-rhamnoside-containing balm, induction of protease activity challenged by dehydration was prevented; tight junctions and desmosomes were preserved from disruption as denoted by similar immunolabelling of claudin 4 and desmoglein 1 as compared to untreated explants. The gradient distribution within epidermis for aquaporine 3 was also maintained. Our data demonstrated that Pentyl-rhamnoside-containing balm was efficient to protect human skin explants from dehydration and strengthen the biological role of Pentyl-rhamnoside in regulating the homeostasis of the lipid barrier. Pentyl-rhamnoside-containing balm should bring benefits for the treatment of xerosis.
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Genomic analysis of a resorved treated epidermal in vitro model
K E Barrett, D Hylanda, F Lim, G Jenkins and H Bogeck. Endo-Dever Co-awere, Shonkouv, United Kingdom
Resveratrol (3,5,4'-trihydroxy stilbene), is a naturally occurring polyphenol of the stilbene class, which is a phytochemical produced by several plant species. It is found is the skin of dark coloured grapes as well as pears, blueberries and cranberries. It is reported to have multiple biological activities, including anti-cancer, anti-inflammatory and beneficial effects on the cardiovascular system. Polyphenols are associated with beneficial effects on skin; however, the exact mechanisms by which these benefits are delivered is currently unknown. To evaluate the potential effects of resveratrol on human skin we performed genomic analysis on Epiderm™ in vitro skin models treated with 1 or 2.5 μg/ml resveratrol for 24 or 120 hours. The key findings of the genomics analysis suggested resveratrol had effects on cell cycle, mitosis, chromosome segregation, DNA replication, keratinocyte differentiation and the regulation of cell proliferation. To validate these insights additional studies were performed with the xCELLigence™ real time cell analysis system, which utilises electrical impedance technology to monitor the biological status of cells. Primary human keratinocyte cells were treated with 1 or 2.5 μg/ml resveratrol and the response of the cells monitored over a 70 hour period. The cells treated with 2.5 μg/ml resveratrol showed a marked reduction in proliferation rate compared to control cells suggesting resveratrol had an effect on keratinocyte proliferation and potentially differentiation.

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Skin barrier function in the patients with atopic dermatitis, allergic contact dermatitis, psoriasis, and systemic eczema
E Choi, N Lee, S Park, N’Noon and D J King. Department of Dermatology, Yonsei University Wonju College of Medicine, Wonju, Republic of Korea
Leukocytes of most inflammatory skin diseases, especially atopic dermatis, present an impaired barrier function. If non-lesional skin also have the impaired barrier, proper moisturizers will prevent their release. So, we compared their barrier function of the patients with inflammatory skin diseases and the healthy people. Total 135 people including each 25 patients with atopic dermatitis(AD), allergic contact dermatitis(ACD), psoriasis and systemic diseases(XE), and 35 healthy people were enrolled. Basal transdermal water loss(TEWL) and stratum corneum(SC) hydration were measured on forehead, inner forearm and calf. Also the barrier recovery rate was calculated. Basal TEWL in lesional skin of all inflammatory skin diseases was increased compared to non-lesional skin, and non-lesional skin of AD patients had decreased compared to normal control. Also SC hydration in lesional skin of all skin diseases was decreased compared to non-lesional skin. SC hydration decreased in non-lesional skin of AD, psoriasis or XE except AD, and barrier recovery rates decreased in AD, ACD or XE except psoriasis compared to healthy control. Collectively, moisturizers with barrier replacement lipid and better humectants would be recommended for AD or XE.

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The steroid and xenobiotic receptor pregnane X receptor controls epidermal homeostasis
A Hirner, N Yannoutsos, M Schmutz and S Eidgen. 1 Dermatolgy and Veneenology, Insbruck Medical School, Innsbruck, Austria, 2 and 3 Center for Regulation and Immunology Laboratory, Department of Cell Biology, Innsbruck Medical School, Innsbruck, Austria
The pregnane X receptor (PXR) is a ligand-activated transcription factor regulating genes central to drug and hormone metabolism in the liver. While skin is the largest metabolic organ of the body, the role of PXR in cutaneous homeostasis has not yet been investigated. Here we show that PXR is expressed in mouse and human epidermis. Moreover, PXR is mostly expressed in the nucleus of proliferating and differentiating human and mouse keratinocytes. PXR expression is decreased in apoptotic mouse and human keratinocytes and PXR deficient mouse keratinocytes are less viable than wild-type. In vivo, PXR deficiency decreases filaggrin expression and increased TEER in mouse keratinocytes, at least in vitro. In vivo, PXR deficiency decreases transgenic expression of filaggrin (FLG) and filaggrin expression and expression of filaggrin are increased in skin of PXR-K14-Tg mice. In summary, keratinocytes and expression of filaggrin are increased in both stratum corneum (SC) and tight junctions (TJ). Several cytokines (e.g. IL4, IL13, IL17A) found in AD skin, reduce the expression of key SC proteins. Little is known about the immunological mediators that modulate epidermal TJ. In this study we selected the prototypic Th2 cytokine, IL4 and Th17 cytokine, IL17A to evaluate the effect that these cytokines have on TJ barrier function and expression of critical components. C57BL/6J mouse primary keratinocytes (PKH) were treated with cytokines (IL4 5-50 ng/ml, IL17A 1-100 ng/ml alone or combination) or media alone. TJI integrity was assessed by trans-epithelial electric resistance (TEER) and paracellular flux of fluorescein. TJ protein expression was measured in submunged PKH cultures and in organotypic epidermis. IL17A induced a dose-dependent enhancement of TJI (2.2 ± 0.1 p<0.02 and fluorescein flux, p=0.002) and enhanced Claudin-4 expression. IL4 alone did not affect TJI integrity at the 72 h timepoint. No changes were observed in Claudin-1, Occludin or ZO-1 expression. Claudin-4 expression was reduced in AD skin compared to controls (p<0.01). In summary, we show that IL17A enhances epidermal TJI integrity and this is associated with increased Claudin-4 expression. Both these effects are inhibited by IL17A. Understanding what effects different T help subsets have on TJI integrity and the mechanism responsible for these actions will pave the way for new AD therapies aimed at barrier repair.

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Antagonistic effects of IL17A and IL4 on epidermal tight junctions
A De Benedetto, T Yoshida, T Kuo, S Gotoo, A Yano, D Y Leung and LA Beck. 1 Department of Dermatology, Innsbruck Medical School, Innsbruck, Austria, 2 Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, IL, 3 Department of Human and Molecular Genetics, Virginia Commonwealth University School of Medicine, Richmond, VA and 4 Department of Pediatrics, National Jewish Health University School of Medicine, Denver, CO
Atopic Dermatitis (AD) is characterized by epidermal barrier impairment with defects identified in both stratum corneum (SC) and tight junctions (TJ). Several cytokines (e.g. IL4, IL13, IL17A) found in AD skin, reduce the expression of key SC proteins. Little is known about the immunological mediators that modulate epidermal TJ. In this study we selected the prototypic Th2 cytokine, IL4 and Th17 cytokine, IL17A to evaluate the effect that these cytokines have on TJ barrier function and expression of critical components. C57BL/6J mouse primary keratinocytes (PKH) were treated with cytokines (IL4 5-50 ng/ml, IL17A 1-100 ng/ml alone or combination) or media alone. TJI integrity was assessed by trans-epithelial electric resistance (TEER) and paracellular flux of fluorescein. TJ protein expression was measured in submunged PKH cultures and in organotypic epidermis. IL17A induced a dose-dependent enhancement of TJI (2.2 ± 0.1 p<0.02 and fluorescein flux, p=0.002) and enhanced Claudin-4 expression. IL4 alone did not affect TJI integrity at the 72 h timepoint. No changes were observed in Claudin-1, Occludin or ZO-1 expression. Claudin-4 expression was reduced in AD skin compared to controls (p<0.01). In summary, we show that IL17A enhances epidermal TJI integrity and this is associated with increased Claudin-4 expression. Both these effects are inhibited by IL17A. Understanding what effects different T help subsets have on TJI integrity and the mechanism responsible for these actions will pave the way for new AD therapies aimed at barrier repair.

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A homozygous nonsense mutation of gene for Mattrin, a component of lamellar granule secreted material
A Shoji, A Shikohama, A Kubo, H Kawasaki, A Ishida-Yamamoto, Y Yamada, T Hayashi, A Shimizu, H Okino, J Kodoh and M Amagai. 1 Dermatol, Keio Univ, Tokyo, Japan, 2 Integrated Med Res, Keio Univ, Tokyo, Japan, 3 MSD Program, Keio Univ, Tokyo, Japan, 4 Core Med, Keio Med, Tokyo, Japan, 5 Dermatol, Aichi-Ken Medical Univ, Hobukko, Japan, 6 Pathol, Keio Univ, Tokyo, Japan, 7 MBL Co., Nagoya, Japan, 8 Med Biol, Keio Univ, Tokyo, Japan and 9 Physiol, Keio Univ, Tokyo, Japan
Filaggrin (FLG) is major predisposing factor in human atopic dermatitis (AD). Filaky tail (flg) mice do not in this study. We segregated the ma/ma and flg/flg mice and revealed that ma/ma mice, not flg/flg mice, develop the dermatitis phenotypes. We, therefore, defined a ma minimal locus and found a homozygous nonsense mutation in the gene for Mattrin, a component of lamellar granule secreted material. Mattrin by next generation DNA sequencing. Exons with TJP expression by transgene rescued the dermatitis and hair phenotype of Mattma/ma mice, including histology, scratching behavior, and serum total IgE level. Matt RNA was most prominently expressed in the skin and within the epidermis matten was expressed in the outermost layer of stratum granulosum (SGI). When observed on face by whole mount staining of epidermal sheet, matten was observed in tubular or reticular patterns and best colocalized with trans-Golgi network markers in SGI cells. In Mattma/ma mice, the staining of several lamellar granule secreted proteins including KL3 and LK1T was significantly reduced. Furthermore, tape stripping of skin surface revealed that the controlled layers were more easily removed en bloc rather than by layer as seen in wild type mice. These findings suggest that the protein, is responsible for spontaneous dermatitis in Mattma/ma mice, and will provide an important clue for clarification of pathophysiological mechanisms for SC barrier dysfunction and AD and AD-like disorders.

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Metabolomic analysis of skin: Development of cell lysate techniques for optimal extraction
KE Barrett, D Hylanda, F Lim, G Jenkins and JE Pople. Endo-Dever Co-awere, Shonkouv, United Kingdom
Metabolomics is the study of the metabolome, a collection of the low molecular mass metabolites of biological tissues. Metabolic byproducts serve as indicators of the chemical processes and can provide valuable information on pathogenesis by measuring the amplified output. Standardized techniques for metabolome extraction of skin samples serve as a critical foundation to this field but have not been developed. We sought to determine the optimal cell lysate techniques for metabolomic analysis. This was done using porcine skin samples, we pulverized the skin with either mortar-and-pestle, ultra-sonication, mortar-and-pestle followed by ultrasonication, ball mill followed by ultrasonication, or homogenization. We then extracted samples for GC-TOF-MS analysis. The signal intensities of 491 metabolite peaks detected by the GC-MS run were used for comparison of these pulverization methods. In descending order, ultrasonication alone resulted in a total signal intensities of 2.7×107, mortar and pestle, 2.6×107; ball mill/ultrasonication, 1.1×107; mortar and pestle/ultrasonication, 1.4×107; and homogenization, 1.2×107. Due to their similar signal intensities, ultrasonication and mortar-and-pestle are recommended for AD or XE.

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Mitochondrial generation of reactive oxygen species promotes epidermal differentiation and hair follicle development.

BB Hamamaki,1 A Glasauer,1 P Hoover,3 S Yang,3 H Blatt,5 S Gettens,2 CJ Gottardi,1 RM Lavker2 and NS Choudhary1 1Medicine, Northwestern University, Chicago, IL and 2 Dermatology, Northwestern University, Chicago, IL. Although the genetic and morphological changes associated with epidermal differentiation are well studied, the signaling events that regulate this process remain poorly understood. Here we tested the hypothesis that mitochondrial reactive generation of reactive oxygen species (ROS) is an important regulator of epidermal differentiation by creating a mouse with a keratinocyte-specific deficiency in the mitochondrial transcription factor A (TFAM). The epidermis of these TFAM conditional knockout (KO) mice was characterized by reduced expression of differentiation markers and increased prelkeratinization within the basal layer. These mice had impaired epidermal barrier function and exhibited reduced cornification of the stratum corneum within 15 days of birth. Primary keratinocytes isolated from TFAM KO mice demonstrated impaired differentiation in vitro, a result of impaired Notch-dependent transcription. Differentiation marker expression in TFAM KO keratinocytes could be partly rescued by treatment with exogenous hydrocortisone, a steroid with anti-inflammatory action in the skin. This study provides initial evidence that ROS is a key upstream signaling event required for differentiation and homeostasis of the epidermis and hair follicles.

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Topical glucocorticoid or pimecrolimus suppresses thymic stromal lymphopoietin (TSLP)-related allergic inflammatory mechanism in oxazolone-induced atopic dermatitis animal model.

N Youn,1 M Jung,1 D Kim,1 H Lee,1 Y Youn1 and B Chae1 1 Departments of Dermatology, Yonsei University College of Medicine, Seoul, Republic of Korea and 2 Cosmis RD, Seongsam, Republic of Korea. Atopic dermatitis (AD) is a chronic inflammatory skin disease considered as the first step of atop march. Impairment of skin barrier in AD can increase allergens penetration that accounts for sensitization even in the airways followed by asthma. TSLP is an epithelial cell-derived cytokine and exists as high level in AD skin. Recently, TSLP is regarded as a systemic driver on atopic march and considered as a biologic experimental therapeutic target in AD. Herein, we made AD animal model through oxazolone (CX) sensitization and multiple challenges. CX-induced AD mice divided into two observation groups as topical glucocorticoid, pimecrolimus, and vehicle-applied groups. We assessed basal TEWL, SC hydration, epidermal differentiation, antimicrobial peptide (AMP) expression, prostate-specific antigen (PSA) expression, and histopathological examination markers. The improvement in the glucocorticoid group was much higher than pimecrolimus groups.

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Evaluation of golden silk-deried extract epidermal barrier markers, caspase-14 and related genes in human skin equivalent models.

K. Chao1, A. Hiraoka1 and T. Marama1 1Faculty of Pharmacy, Keio University, Tokyo, Japan and 2 Kobe Technical Center, P&G, Japan, Kobe, Japan. Background: The stratum corneum of human epidermis is composed of terminally differentiated keratinocytes serving as an essential barrier to environmental stresses. Caspase-14 is a member of the caspase family, whose expression is restricted almost exclusively to the granular and corneous layer. Recent works suggest that, compounds, which increase caspase-14 expression, are potent to exhibit a protective effect on the skin barrier function, especially in case of barrier damage and UV irradiation. Silk extract, which is one of widely used natural materials for cosmetics, provides luster for hair as well as protection for both hair and skin. Recently, a unique golden silk-deried Extract (GSDE) has newly developed as a moisturizing agent, which is made from the cocoon of Bombyx mori. GSDE contains specific flavonoids like quercetin, and several small peptides. Objective: GSDE has several beneficial effects, such as antioxidant effects in human skin cells and induction of hair follicle and collagen production in dermal cells. To determine the in vitro effects on barrier formation and hydration of topically applied GSDE, we analyzed the expression of biomarkers of late epidermal differentiation, especially the caspase-14 and its related genes. Methods: The skin equivalent with partially formed comedyl layer (EPI-201) was purchased from MatTek. GSDE was added on the stratum corneum of EPI-201 and it was incubated for 24h. The expression of biomarker genes were measured by real-time RT-PCR analysis. Results and Discussion: GSDE induced the caspase-14 gene expression by 3-folds compared to non-treated control, but it decreased the eotaxin gene expression to the half. Eotaxin has antiprotective properties and it accumulates in photoskin. These results suggest that GSDE influences terminal keratinocyte differentiation and may help to limit skin damage from UV. These data provide a compelling reason to further understand the nature of GSDE and its skin benefits.

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Altered cutaneous neural innervation in pruritic dermatoses.

K. Hashimoto1 A. Hiraoka1 and T. Marama1 1Faculty of Pharmacy, Keio University, Tokyo, Japan. itch and pain signals are transmitted from the skin to the spinal cord by small diameter, unmyelinated A δ and C fibers and thinly myelinated A μ fibers. Growing evidence suggests that the ability of overlapping sensory populations to encode distinct sensations of itch and pain lies in part with the setting of maintained pruritoceptive input. Knockdown of filaggrin in a three-dimensional reconstructed human epidermis impairs keratinocyte differentiation/differentiation program as normal human epidermis. ShRNA targeting of FLG mRNA efficiently decreased corneodesmosin and claudin 1 amounts. Other well-known keratinocyte differentiation markers and barrier efficacy confirmed that the RHE model displays similar epidermal strati
tication markers. To investigate the impact of FLG deficiency on keratinocyte differentiation, we used lentivirus-mediated shRNA interference in three-dimensional reconstructed human epidermis (RHE) model. To test this hypothesis, we employed probed, motion-restricted PS in three immunologically-diverse, mouse models: i) single-hapten challenge-induced, acute allergic contact dermatitis (AADD); ii) repeated hapten (NH)3 challenges, with early features of atopic dermatitis (AD); and iii) a phorbol ester-induced, acute irritant contact dermatitis (ICD) model. As expected, PS stimulated endogenous GC production. But rather than aggravating inflammatory dermatoses, it reduced inflammation, improved permeability barrier homeostasis, and normalized serum IgE levels in the AD model. The role of endogenous GC in producing these apparently-paradoxical benefits was shown by co-administration of the GC receptor antagonist, mifepristone (RU486), which presented PS-induced disease ameliorations. Thus, PS, under these experimental conditions, benefits rather than aggravates cutaneous inflammatory dermatoses, due to the anti-inflammatory activity of increased endogenous GC.

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Knockdown of filaggrin in a three-dimensional reconstructed human epidermis impairs keratinocyte terminal differentiation program.

V Pendaries,1,2,3 RN achet,1,2,3 J Malaisse,4 Y Poumay,4 G Serre1,2,3 and M Vlot1,2,3 1 UMR5165 CNRS, Toulouse, France, 2 U1557 INSERM, Toulouse, France, 3 University of Toulouse, Toulouse, France and 4 Coll and tissue laboratory, University of Namur, Namur, Belgium. Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disorder caused by genetic and environmental factors. The skin of AD patients demonstrates defects in the epidermal barrier, due to disturbed keratinocyte differentiation and particularly to lower expression of filaggrin (FLG). FLG2 and antimicrobial peptides (AMP) expression, we used lentivirus-mediated shRNA interference in three-dimensional reconstructed human epidermis (RHE) in vitro. This approach allows permanent protein extinction in keratinocytes. Analysis of differentiation markers and barrier efficacy confirmed that the RHE model displays classical epidermal stratification/differentiation program as normal human epidermis. shRNA targeting of FLG mRNA efficiently suppressed the expression of this protein, and reduced keratinohyaline granule amount, as compared to RHE infected with non-target shRNA lentivirus. As already observed, the loss of FLG led to an abnormal permeability of the RHE as evidenced using a dye penetration assay. In this model, absence of FLG also impaired keratinocyte differentiation. Similarly to AD patient skin, the expression of AMP, including FLG, hemoitin or keratin 14, which are considered as key enzymes involved in the proteolytic metabolism of FLG, was significantly reduced. In addition, we observed a diminution of keratin 10 and desmoplakin, and an increase of other well-known keratin 14 amounts that were sparsely but present in normal keratinocytes. These results were not affected by the lack of FLG, such as involucrin, keratin K14, desmocollins and desmoshrines. This study demonstrates that knockdown of FLG expression in RHE reproduces some of the alterations observed in epidermal differentiation program observed in AD patients, alterations which contribute to a disturbed epidermal barrier.

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Topical glucocorticoid or pimecrolimus suppresses thymic stromal lymphopoietin (TSLP) -related allergic inflammatory mechanism in oxazolone-induced atopic dermatitis animal model.

V Youn,1 M Jung,1 D Kim,1 H Lee,1 Y Youn1 and B Chae1 1 Departments of Dermatology, Yonsei University College of Medicine, Seoul, Republic of Korea and 2 Cosmis RD, Seongsam, Republic of Korea. Atopic dermatitis (AD) is a chronic inflammatory skin disease considered as the first step of atop march. Impairment of skin barrier in AD can increase allergens penetration that accounts for sensitization even in the airways followed by asthma. TSLP is an epithelial cell-derived cytokine and exists as high level in AD skin. Recently, TSLP is regarded as a systemic driver on atopic march and considered as a biologic experimental therapeutic target in AD. Herein, we made AD animal model through oxazolone (OX) sensitization and multiple challenges. OX-induced AD mice divided into two observation groups as topical glucocorticoid, pimecrolimus, and vehicle-applied groups. We assessed basal TEWL, SC hydration, epidermal differentiation, antimicrobial peptide (AMP) expression, prostate-specific antigen (PSA) expression, and histopathological examination markers. The improvement in the glucocorticoid group was much higher than pimecrolimus groups.

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Prevalence of atopic dermatitis (AD) in child/adolescence and young adults: a cross-sectional study.

A. Ueki,1 Y. Nakamura,2 H. Kunishita,3 M. Iwadate1 and Y. Asada1 1 Hyogo College of Medicine, Nishinomiya, Hyogo, Japan, 2Department of Dermatology, Kobe University Graduate School of Medicine, Kobe, Japan and 3Kagawa Prefectural Central Hospital, Kagawa, Japan. The prevalence and severity of AD vary according to geographical regions. In Japan, the prevalence of AD is relatively low in adults. In this study, we investigated the prevalence and severity of AD in children and adolescents aged 5-18 years. A total of 1839 children and adolescents (983 males and 856 females) were recruited. A medical history was taken and the severity of AD was evaluated using the AD severity score (ADD). The AD severity score is calculated according to the presence or absence of signs and symptoms such as dryness, erythema, excoriation, and lichenification. The prevalence rate of AD was determined as 11.0% in boys and 10.8% in girls. The prevalence of AD was significantly higher in females than in males (P=0.0096). The prevalence rate of AD was increased with age (P=0.0004). The prevalence rate of AD peaked in the age group of 5-9 years. The severity of AD was also significantly higher in females than in males (P=0.0006) and increased with age (P=0.0001). The severity of AD was significantly lower in boys than in girls (P=0.0033). In addition, the severity of AD was significantly lower in boys than in girls (P=0.0033). In addition, the severity of AD was significantly lower in boys than in girls (P=0.0033). In addition, the severity of AD was significantly lower in boys than in girls (P=0.0033). In addition, the severity of AD was significantly lower in boys than in girls (P=0.0033).
ABSTRACTS | Epidermal Structure & Barrier Function

703 Study of epidermal barrier restoration in human organotypic skin models
T. Pietruzzi, F. Mussi, R. Lotti, A. Saurat, P. Mencarini and C. Pincelli
Dermatology, University of Modena and Reggio Emilia, Modena, Italy

Epidermal barrier mainly consists of the stratum corneum and the tight junctions in the stratum granulosum. It represents the first vital defense against a variety of external insults, and epidermal barrier disruption is pathologically involved in common skin diseases, including psoriasis, contact and atopic dermatitis. Epidermal barrier has been studied in humans through non-invasive techniques and in a number of mouse models. The aim of the present study was to investigate epidermal barrier restoration in human organotypic skin models and in vivo studies, skin explants are very convenient models to investigate the effect of topical formula application of cosmetic products. We identified a Corinophilma muka extract (CM) as a very potent agonist of the PPARγ. We used a stable cell line designed to express a chimeric protein, containing the ligand binding domain of PPARγ. The expression of the PPARγ was evaluated by qRT-PCR in normal human keratinocytes. The expression of differentiation and corneocyte markers was followed by immunostaining. Normal skin fragments were cultivated at the air-liquid interface, after delipidation the different formulae were applied topically. At day 8, after epidermis separation, the extracted lipids were separated and identified by gas chromatography and mass spectrometry system. In parallel, an evaluation by a dermatologist was done. Using 2D cell culture, we demonstrated that CM is a strong PPARγ agonist using a PARP reporter cell line. We also described an increased expression of PPARγ in human keratinocytes. Furthermore, it stimulated numerous markers of the epidermis differentiation and cohesion. Topical application of a formula containing CM on skin explants was able to increase significantly the expression of the markers of differentiation and cohesion. The results obtained in this study confirm the interest of using skin organotypic models in order to assess the activity of molecules that can be used for the treatment of skin diseases.

704 In vitro, ex vivo and in vivo evaluation of barrier function and skin lipid modulation – from acute to chronic effect to formula
Bruchet, J Krolikiewicz-Renimel, P Schaeffer, I Leblanc, M Nieuw and S Schnebert
LVMP-Rissone, Saint Jean de Boze, France

Our studies have shown that some probiotic strains increase tight junction function via modulation of protein components such as claudin and desmoglein. The number of keratin 67 positive keratinocytes decreased 18 hours after SLS treatment, while few TUNEL positive cells were observed. At 24 and 36 hours after SLS treatment, claudin 1, 2, 3 and desmoglein 1 were expressed in control and probiotic treated skin. The results indicate that organotypic skin models can be successfully used for the study of disruption and repair of the epidermal barrier. The models will be also suitable for testing treatment options for the treatment of skin diseases characterized by a dysregulation of this critical defense structure.

705 Lysates of probiotic bacteria augment tight junction barrier function in human primary epidermal keratinocytes
R. Eckhart, P. Rossiter and CA O'Connell
1 Medicine, University of Manchester, Manchester, United Kingdom and 2 Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, United Kingdom

Studies in the gut have demonstrated that some probiotics enhance epithelial barrier function by modulation of tight junction complexes. Recently, the benefits of probiotics in other tissues have received attention. We are investigating whether selected strains of potentially probiotic bacteria have value when used topically on skin. Tight junctions have recently been shown to be critical to skin barrier function. Therefore in this study, we investigated whether probiotics can modulate the tight junction ultrastructure of human primary keratinocytes. Human primary epidermal keratinocytes were grown on cell culture inserts and treated with either Bifodobacterium longum, Lactobacillus reuteri or Lactobacillus rhamnosus. With the exception of claudin 4, expression of tight junction proteins increased in a time dependent manner between 24 and 48 h, as assessed by measurements of transepithelial electrical resistance (TEER). However, B. longum and L. rhamnosus were the most efficacious, producing dose-dependent increases in resistance that were maintained over 4 d. Both strains also increased the expression of tight junction proteins claudin 1, 2, 3 and occludin. L. reuteri additionally increased claudin 4 expression in keratinocytes. Neutralisation of Toll-like receptor 2 abolished both the increase in TEER and expression of tight junction proteins induced by B. longum, but not L. rhamnosus. Our data suggest that some probiotics strains increase tight junction function via modulation of protein components. The potential role for topical application of probiotics in conditions where the skin barrier is compromised, is currently under evaluation.

707 Paradoxical induction of keratocytic hyper-proliferation by a differentiation associated protein keratin 10
H Aimia and K Choute Dermatology, Yale University, New Haven, CT

Keratin 10 is a type 1 keratin exclusively expressed in post-mitotic, terminally differentiated suprabasal keratinocytes and is a marker keratinocyte differentiation.1 Prior work examining over-expression of K10 in transformed cell lines and basal murine keratinocytes has been reported to result in cell cycle arrest, inhibition of proliferation and resistance of chemical carcinogenesis.2, 3 As such, K10 has thought to be a differentiation-inducing protein, but its effect on cell proliferation has not been studied in any human keratinocyte system. In this study, we ectopically expressed K10 in primary human basal keratinocytes. K10 formed intermediate filaments and co-localized with K5, a type 2 keratin exclusive to basal keratinocytes. K10 interfered with keratinocyte in vitro ex-epithelialization suggesting inhibition of cell migration as opposed to an earlier report (4). Surprisingly, K10 expression did not arrest cell cycle. Instead it induced hyper-proliferation and activation of Akt with abundant expression in mitotic keratinocytes. K10 did not induce differentiation markers K1 or involucrin or suppress expression of basal makers such as K5 and K14. Moreover, K10 did not alter keratinocyte response to apoptotic stimuli. Altogether, these findings suggest that while K10 may be a marker at terminal differentiation, its expression alone, does not induce this process. 1-Koch, P.J. and D.D. R. Rossiter (2004). "The role of keratins in epidermal development and homeostasis—going beyond the obvious." J Invest Dermatol 123(5): x-xi. 2-Dödding, L. Eckhart, P. and T. Schägger (2003). "Dermal keratinocytes. Lister College, Medical University of Vienna, Vienna, Austria, 2 Dermatology, Hussman Hospital, Fudan University, Shanghai, China and 3 Faculty of Medicine, Semmelweis University, Budapest, Hungary. The expression of K10 in organotypic epidermal cultures was associated with inhibition of cell proliferation and increased expression of differentiation markers.4, 5 The present study examined the role of K10 in the regulation of keratinocyte proliferation and differentiation.4, 5 K10 was ectopically expressed in human keratinocytes using retroviral transduction. The K10 expressing keratinocytes were found to be hyper-proliferative and dysdifferentiated. K10 expression was associated with increased proliferation, decreased differentiation and increased migratory potential compared to control keratinocytes. These results indicate that K10, when ectopically expressed, can induce paradoxical keratinocyte hyper-proliferation without affecting differentiation.

708 A/C degradation in Akt1 knockdown lines. Filaggrin proteolytic processing was impaired in Akt-1 knockdown epidermal keratinocytes by Atg7-/- epide- mial protease that is involved in filaggrin processing. A Naseem, Y Zhu, Hapel, W Di and R O'Shaughnessy
1 Livingstone Centre for Skin Research, The Institute of Child Health, London, United Kingdom and 2 Immunobiology and Dermatology, LCI, Institute of Child Health, London, United Kingdom

Atopic Dermatitis (AD) is a chronic inflammatory disease characterised by relapsing pruritus, eczema- tous lesions, lichenification and increased transdermal water loss. Association of filaggrin mutations with AD has been well documented. However not all AD patients have filaggrin mutations and therefore other mechanisms give rise to the barrier deficit present in AD. Akt-1 activity is required for cornified envelope formation and correct filaggrin processing. Akt-1 activity has been observed in a subset of AD patients. The aim of this study was to investigate the role of downstream targets of Akt-1 signaling in prefilaggrin processing and cornified envelope formation. We identified genes downstream of Akt-1 by analysis of 2 different Akt-1 knockdown mouse lines (Akt-1Δ epi and Akt-1ΔΔ epi mice). Autophagic features of skin cells were efficiently observed in an Agt2Δa agt2Δa mice. In vivo, data have shown a significant repigmentation without increase of the squalen content. The autophagic evaluation concludes to a significant improvement of the suppleness of the skin with a marked ad color. Each model was efficient to the analysis of abg2Δa mice. These results suggest that CM possesses properties that are applicable to the treatment of skin dryness, and raise the possibility that CM has anti-ageing properties, partly due to its PPAR agonist effect.
Atopic dermatitis-like inflammation: Role of acute versus chronic epidermal barrier impairment

V. Mattioli 1, M Schumah and S Dubrac. Department of Dermatology and Venerology, Innsbruck Medical University, Innsbruck, Austria

Acute exposure of the epidermis to the pathogenesis of atopic dermatitis (AD) is undisputed; it is unclear whether acute or inherited skin barrier disruption per se triggers the development of AD-like inflammation. Acute epidermal barrier disruption by tape stripping resulted in mild epidermal hyperplasia, no dermal inflammatory infiltrates were observed and expression of inflammatory cytokines in the skin remained unchanged. However, expression of the immunosuppressive cytokine IL-10 was increased in tape stripped skin. In contrast, chronic barrier impairment in flaky tail (ft) mice induced hyperkeratosis, epidermal thickening and discrete dermal inflammatory infiltrates. Expression of IL-10, TGF-β and IL-17 was increased in the skin of ft mice, in contrast to tape stripped wt mice. Histological analysis suggested that impaired skin barrier function and topical Vd3 synergize to induce overt skin inflammation. Vd3-induced epidermal thickening is reversible in ft mice as compared to respective controls. However, increased Th2 cytokine production was only observed in the skin of ft mice and not in tape stripped wt mice treated with Vd3, suggesting that, compared to healthy skin, dry skin is characteristically thicker but weaker in barrier quality.

Kallikreins 5 and -7 are coordinately transferred with glucosylceramides into lamellar bodies. In cultured keratinocytes from healthy human skin, they are co-localized with GluCer, which is required for formation of multi-lamellar structures in the SC, but perhaps not with the major desquamation enzymes, kallikreins (KLK) 5 and -7, in the SC of this HI skin.

Improvement of transdermal delivery of caffeine through human skin

N. Bielli 1, V. Oliffe 1, C. Brient 1 and Y. Long 1. R&D, Lucas Meyer Cosmetics, Champigny, Paris 94, 2 Dermatology, University of Lubeck, Lubeck, Germany, 3 Pharmacology, UTHSC, Memphis, TN, 4 Pharmaceutical Sciences, UTHSC, Memphis, TN and 5 Cellular & Structural Biology, UT Health Science Center, San Antonio, TX

Our goal was to better understand the regulation of peroxisome proliferator-activated receptors (PPARs) on epidermal barrier function and hydration. We first developed a computer model of human epidermis with pathways of PPARs, PPARs and PPARs using a systems biology package, Inter alkobiological Platform. Mechanisms of action and data on epidermal PPARs presented in public literature were analyzed and mathematically pieced together into the model. PPAR pathways were linked with keratinocyte proliferation and differentiation processes as well as external perturbations, allowing simulations of in vivo conditions with both time and spatial resolutions. Our model reproduced literature results and provided new insights. For example, PPAR upregulation leads to enhanced lipid production and improved barrier function at home. Thus, PPAR activation via topically applied agonists is a viable route for improving skin barrier function and hydration.

Melaninergic pathway in human skin: Metabolism and phenotypic activity
A. Monniné,7 T. Kim,1 K. Kleszczynski,2 J. Jarzimiento,1 T. Swaiman,2 W. Li,2 R. Reiter3 and TW Fischer1 1 Pathology and Laboratory Medicine, Center for Cancer Research, UTHSC, Memphis, TN, 2 Dermatology, University of Lubeck, Lubeck, Germany, 3 Pharmacology, UTHSC, Memphis, TN, 4 Pharmaceutical Sciences, UTHSC, Memphis, TN and 5 Cellular & Structural Biology, UT Health Science Center, San Antonio, TX.

Our goal was to better understand the regulation of peroxisome proliferator-activated receptors (PPARs) on epidermal barrier function and hydration.

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Cyclophilin B, expressed in the granular layer of epidermis, regulates keratinocyte differentiation from the basal layer. A Srinivas, A Londsdale-Eccles, P Feon, A Ferenczy, C Todd, A Fallerin and NJ Reynolds 1 Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, United Kingdom and 2 Division of Dermatology, St George’s, University of London, London, UK

Cyclophilin B (CyP B) with high affinity mediating T-cell immunosuppression. However, CA also exerted T-cell independent effects on keratinocytes. We therefore determined whether CyP B is expressed and secreted by normal human keratinocytes (NHK). Here, we aimed to investigate the functional role of CyP B in epidermis. Critical analysis of normal human skin revealed that CyP B is expressed predominantly within the granular cell layer. Transduction of NHK with retroviral vectors containing CyP B(WT) or CyP B(W128A) (a mutant reducing CA binding to 3% resulted in efficient expression, promoting the secretion of the proteins into the medium. Consistent with localised expression within the granular cell layer, CyP B(WT) and CyP B(W128A) positively regulated keratinocyte differentiation (transglutaminase promoter luciferase and enzyme activity). Additionally, however, transduction of NHK with CyP B(WT) and CyP B(W128A) significantly increased colony formation compared to empty vector by 1.4 fold (p<0.01) and 1.5 fold (p<0.05) respectively (one way ANOVA). Also, NHK proliferation was significantly increased by transduction with CyP B(WT) (1.9 fold, p<0.05) and CyP B(W128A) (2 fold, p<0.05), respectively. In addition, CyP B(WT) and CyP B(W128A) increased proliferation of naïve NHK by 1.3 fold (p<0.01) and 1.2 fold respectively, suggesting paracrine effects. Moreover, transduction with SHNHA CyP B(WT) resulting in knockdown of CyP B reduces NHEK proliferation (P<0.01). Consistent with localised expression within the granular cell layer, CyP B(WT) and CyP B(W128A) positively regulated keratinocyte differentiation consistent with its expression pattern but also promotes proliferation through paracrine effects and regulates epidermal homeostasis. Thus, CyP B is a potential therapeutic target.

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Leptin affects sebaceous lipid formation and intracellular distribution and induces proinflammatory signaling in SZ95 sebocytes. Leptin affects sebaceous lipid formation and intracellular distribution and induces proinflammatory signaling in SZ95 sebocytes. L Kemeny,6,7 CC Zouboulis 8 and E Remenyik 1

In this regard, we hypothesized that the difference between c.1062-3_1074del16 and c.3407G>A segregating to a loss of function of ABCA12 and resulting in the typical HI phenotype. Interestingly, a compound heterozygote for ABCA12 mutations, a paternal novel splice variant showing the skipping of exon 10, in addition to the wild type splicing product. The expression of ABCA12 at the protein level was assessed by Western blotting. As expected, a reduction in ABCA12 expression was observed. In addition, the ABCA12 promoter luciferase reporter assay revealed a transcriptional defect in the absence of ABCA12. These results further support the hypothesis that ABCA12 is a critical gene for skin barrier function.

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Flightless I overexpression impairs skin barrier development, function and recovery post-blistering. C CH Yang,2 RM Arkell,3 JE Jackson,2 H hva,4 RJ Ludwig,4 D Zillikens,4 DF Murrell5 and AJ Caswell1

1 Centre for Regenerative Medicine, Mawson Institute, University of South Australia, Adelaide, SA, Australia, 2 Women’s and Children’s Health Research Institute, North Adelaide, South Australia, 3 Research School of Biological Sciences, Australian National University, Canberra, ACT, Australia, 4 Department of Dermatology, University of Luebeck, Luebeck, Germany and 5 Department of Dermatology, St George’s Hospital, Sydney, NSW, Australia

An intact epidermal barrier is critical for maintaining the integrity and function of healthy skin. Patients with Epidermolysis Bullosa (EB) have impaired skin integrity which contributes to its fragility and impaired wound healing. The aim of this study was to identify the function of the cytoskeletal protein Flightless I (Flii) in the development and function of the epidermal barrier. Using Flii−/− mice, we investigated the effect of altering Flii levels in embryos and AK3 mice or patient P11G, together with pemphigus bullosum (PB) in a neonatal PB mouse model with activated EGFR (Schueller et al, 113, 346, 2012). Our results, reinforced by EGFR deleted transgenic mice and in vitro keratinocyte cultures challenged with AK23, demonstrate that EGFR inhibitors only effectively prevent blister formation when operating within a window between 35-50% of normal EGFR activity. Above and below this activity, blister development is gradually aggravated. Our data highlight that the only too little or too much of the epidermal and epidermal barrier proteins compared to empty vector controls. These studies indicate that CaPb stimulates keratinocyte differentiation consistent with its expression pattern but also promotes proliferation through paracrine effects and regulates epidermal homeostasis. Thus, CaPb is a potential therapeutic target.

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Novel ABCA12 splice site deletion mutation and ABCA12 mRNA analysis in harlequin ichthyosis. T Takahashi, K Sugiyata, M Kono and M Nikiya Department of Dermatology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Harlequin ichthyosis (HI) is a severe and often fatal congenital ichthyosis with an autosomal recessive inheritance pattern. It was clarified that HI is caused by severe functional defects in the keratinocyte lipid transporter ABCA12. Here we report that a compound heterozygote for ABCA12 mutations, a paternal novel splice variant showing the skipping of exon 10, in addition to the wild type splicing product. The expression of ABCA12 at the protein level was assessed by Western blotting. As expected, a reduction in ABCA12 expression was observed. In addition, the ABCA12 promoter luciferase reporter assay revealed a transcriptional defect in the absence of ABCA12. These results further support the hypothesis that ABCA12 is a critical gene for skin barrier function.

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Regulation of PPAR alpha in models of skin barrier disruption. S Blundell, S Dubuc and M Schmutz Dermatology, Medical University Innsbruck, Innsbruck, Austria

Peroxisomal membrane proteins). However, the identification of key players involved in skin barrier function remains elusive. In this study, we aimed to assess the expression of PPAR alpha, beta/delta and gamma in skin after chronic and acute skin barrier disruption. We studied filaggrin-deficient filaky tail mouse skin and wild type mice exposed to repeated tape stripping. Our murine models revealed a reduction of PPAR alpha expression whereas PPAR beta/delta and PPAR gamma expression levels were not altered as compared to controls. Similar results were obtained in chronic versus acute skin barrier disruption. These data confirm that PPAR alpha regulates skin barrier function and may be important for the development and regulation of the epidermal barrier which may contribute to impaired healing and skin fragility of patients with EB.
mRNA and protein expression of filaggrin and loricrin in cultured normal human keratinocytes

J van Smeden, 1 M Janssens, 1 R Vreeken, 2 A Lavrijsen 3 and J Bouwstra

One of the features of atopic eczema (AE) is an impaired skin barrier function. The stratum corneum (SC) lipids form a highly ordered lipid organization that fulfills a predominant role in the barrier function and consists of ceramides (CERs), free fatty acids (FFAs) and cholesterol. In the present study, we report for the first time i) the extracellular FFA composition in both lesional and non-lesional skin, and ii) how these factors associate with the examined CER composition and the impaired skin barrier in AE of a previous study.1 The study has been performed in 15 Caucasian control subjects and 28 Caucasian AE patients, of which 11 did show lesional skin sites. The results show a clearly distinct stable lipid profile between control skin, non-lesional skin, and lesional SC. In particular the FFA chain lengths are clearly reduced in AE SC, and the changes are much more pronounced in lesional skin compared with non-lesional skin. We observed that the changes in the chain distribution of FFAs were strongly associated with changes in the CERs. These changes were strongly correlated with the increased presence of a less dense lipid organization and a reduced skin barrier function as monitored by trans epidermal water loss. All changes were more pronounced at lesional skin sites than in non-lesional skin. None of the changes in lipid properties correlated with filaggrin mutations, but were associated with the levels of natural moisturising factor. Our results demonstrate the importance of the FFAs and the lipid chain length for a proper lipid organization and skin barrier function.

Studying keratinocyte functions in atopic dermatitis, by using outer root sheath keratinocytes

Krt9-/-

Knockout mice have proven effective in defining the functional importance of cutaneous proteins. The aim of our study was to estimate FLG R01X and 2282del4 gene mutations and its associations with -137 G/C IL-18 and 1112 C/T IL-13 polymorphisms and their influence on AD course and risk. 275 subjects were included: 152 AD and 121 healthy volunteers. ARMS-PCR method was performed to estimate IL-18 and IL-13 gene polymorphisms and FLG mutations. FLG mutation were observed in 40.8% of AD subjects and were 1.9 times frequent comparing to the control. 2282del4 FLG mutation was more prevalent in AD patients (p=0.04) and coexisted with ichthyosis (p=0.023) and allergic rhinitis (p=0.0001). The presence of RS01X or 2282del4 FLG mutation was associated with elevated tlg levels (p=0.017), whereas their association with IL-13 polymorphism was not statistically significant. These data support the role of FLG mutation in the development of AD.

Free fatty acids and lipid chain length correlate with the impaired skin barrier of atopic eczema patients

V Yvan, M Furumura, S Numa, K Toye, B Olyahya and T Hashimoto

24 Association of R501X and 2282del4 FLG gene mutations, -137 G/C IL-18 and 1112 C/T IL-13 polymorphisms with AD course and risk

O RAO1 channel orchestrates skin homeostasis via calcium-dependent regulation of focal adhesion kinase and directional migration

M Rachinski, D Mandleberg, 2 M Horii, 1 V Uhlenhutz, 1 D Geruljen, 1,2 R Hastei, 1 T Oddei, 2 P Hogeveen, 4 A Rac, 2 R Skryma and N Prensavakka

We report for the first time i) the extracellular FFA composition in both lesional and non-lesional skin, and ii) how these factors associate with the examined CER composition and the impaired skin barrier in AE of a previous study.1 The study has been performed in 15 Caucasian control subjects and 28 Caucasian AE patients, of which 11 did show lesional skin sites. The results show a clearly distinct stable lipid profile between control skin, non-lesional skin, and lesional SC. In particular the FFA chain lengths are clearly reduced in AE SC, and the changes are much more pronounced in lesional skin compared with non-lesional skin. We observed that the changes in the chain distribution of FFAs were strongly associated with changes in the CERs. These changes were strongly correlated with the increased presence of a less dense lipid organization and a reduced skin barrier function as monitored by trans epidermal water loss. All changes were more pronounced at lesional skin sites than in non-lesional skin. None of the changes in lipid properties correlated with filaggrin mutations, but were associated with the levels of natural moisturising factor. Our results demonstrate the importance of the FFAs and the lipid chain length for a proper lipid organization and skin barrier function.
The irritant effects of pharmaceutical excipients used in topical formulations on a murine model. We tested butyl-PABA, cetomophor RH40, a non-ionic detergent: sucrose laureate and a conservative: methylparaben in closed patch tests to the dorsal region of SKH-1 male hairless mice for 24 hours. We measured transepidermal water loss (TEWL) before the exposure and 30 minutes after the removal of the patch tests. Photographs were taken for the evaluation of erythema and then tissue samples were removed for histopathological evaluation. Our results showed a significant increase in TEWL following the application of SLS and sucrose laureate and SLS also caused severe erythema. In histopathological evaluation a dose-dependent epidermal necrosis, lymphohypertension and neutrophil accumulation was found in tissue samples treated with SLS. The other tissue samples showed no significant impairment in skin structure. In conclusion, SLS and methylparaben seem to be non-irritant agents and sucrose laurate may also be a promising candidate for topical application.

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PFOB1 protein distribution in normal and pathological skin

P Della Mina,1 A Crespi,1 L Lunardon,1 A Villa,1 G Griente2 and E Bentiv2 1 Consorzio MIA, Milano, Italy,2 Department of Biotechnology and Science of the Earth, University of Milan, Milan, Italy. The irritant effects of pharmaceutical excipients used in topical formulations

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Hornen is a new target of calpain-1

C Hu,1,2 G McNicholas,1 A Raymond,1,2 H Takaoka,1,3 S Garner,1,3 H Hata,1,3 N Debeauvilliers,1,3 E Smarz,1,3 M J Argent1 1 Dermatology, Medical University of Vienna, Vienna, Austria, 2 U1056, INSERM, Toulouse, France, 3 University of Toulouse, Toulouse, France. Calpain-1 is a calcium-dependent cysteine protease that plays a key role in cellular functions. Recently, it was demonstrated that calpain-1 can cleave and activate some substrates. The mechanism of calpain-1 activation and the physiological importance is still not fully understood. In this study we investigated the effect of calpain-1 on the expression and activation of the human epithelial cadherin (HEC) and the role of the protease in the epithelial cell differentiation and proliferation.

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The irritant effects of pharmaceutical excipients used in topical formulations

G van Duppen,1, 2 M Dano,1, 2 J A Bouwstra1 and A El Ghalbzouri2 1 Dermatology, LUMC, Leiden, the Netherlands and 2 Drug Delivery Technology, LACDR, Leiden, the Netherlands. The human skin barrier is mainly formed by the stratum corneum (SC) which is composed of terminally differentiated keratinocytes that are surrounded by a lipid matrix. The main function of the skin barrier is to protect against entry of harmful substances and pathogens. Dry skin and defective skin barrier function are clinical features of the common skin disorders ichthyosis vulgaris (IV) and atopic dermatitis (AD). Recently, loss-of-function mutations in the filaggrin gene (FLG) have been identified to be strongly associated with both IV and AD. Filaggrin has many roles in epidermal homeostasis but its precise role in the skin barrier properties is yet unknown. In this study we evaluated the effect of RNA interference mediated filaggrin knockdown on skin barrier properties in a human skin equivalent (HSE). Filaggrin knockdown (FLG-KD) resulted in 87% reduction of filaggrin RNA expression and a reduced protein expression in HSEs after 14 days of air-exposed culturing. HSEs generated with FLG-KD cells showed a lower number of viable cell layers and a 50% reduction in the proliferation index (p < 0.05). The differences in expression of the early IV and late (keratin) differentiation markers were found. Evaluation of the skin barrier properties did not reveal significant changes in lipid organization and composition between the FLG-KD HSEs and their control. Lipid permeability for both para-parabenoxybenzoic acid (Para-PBA) and para-parabenoxybenzoic acid (Para-PBA) was not increased after FLG-KD. In conclusion, epidermal proliferation was altered after knockdown of filaggrin, but no significant effects on SC lipid organization and composition were found. Using this skin model we did not observe significant changes in lipid organization and composition that reduced filaggrin expression alone is not sufficient to affect skin barrier properties and function.

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Filaggrin knockdown does not affect skin barrier properties of a human skin equivalent

V van Duppen1,2, 1, 2 M Dano, 1 J A Bouwstra1 and A El Ghalbzouri2 1 Dermatology, LUMC, Leiden, the Netherlands and 2 Drug Delivery Technology, LACDR, Leiden, the Netherlands. The human skin barrier is mainly formed by the stratum corneum (SC) which is composed of terminally differentiated keratinocytes that are surrounded by a lipid matrix. The main function of the skin barrier is to protect against entry of harmful substances and pathogens. Dry skin and defective skin barrier function are clinical features of the common skin disorders ichthyosis vulgaris (IV) and atopic dermatitis (AD). Recently, loss-of-function mutations in the filaggrin gene (FLG) have been identified to be strongly associated with both IV and AD. Filaggrin has many roles in epidermal homeostasis but its precise role in the skin barrier properties is yet unknown. In this study we evaluated the effect of RNA interference mediated filaggrin knockdown on skin barrier properties in a human skin equivalent (HSE). Filaggrin knockdown (FLG-KD) resulted in 87% reduction of filaggrin RNA expression and a reduced protein expression in HSEs after 14 days of air-exposed culturing. HSEs generated with FLG-KD cells showed a lower number of viable cell layers and a 50% reduction in the proliferation index (p < 0.05). The differences in expression of the early IV and late (keratin) differentiation markers were found. Evaluation of the skin barrier properties did not reveal significant changes in lipid organization and composition between the FLG-KD HSEs and their control. Lipid permeability for both para-parabenoxybenzoic acid (Para-PBA) and para-parabenoxybenzoic acid (Para-PBA) was not increased after FLG-KD. In conclusion, epidermal proliferation was altered after knockdown of filaggrin, but no significant effects on SC lipid organization and composition were found. Using this skin model we did not observe significant changes in lipid organization and composition that reduced filaggrin expression alone is not sufficient to affect skin barrier properties and function.

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Characterization of the expression of two key epidermal proteins, filaggrin and coronodesmosin, in dog skin

E Videmont1,2, G Serre1, M Simon1,3 and D Pnl1 1 Vetgeo Sup Campus Vétérinaire de Lyon, Marcy l’Etoile, France, 2 CNRS-INSERM-University of Toulouse UMR 5165-15056, Toulouse, France and 3 University of Lyon, EA4469, Lyon, France. Filaggrin and coronodesmosin are key proteins for the epidermal barrier functions. The pattern of expression of filaggrin has not yet been characterized and coronodesmosin expression has never been investigated in dog, whereas spontaneous canine atopic dermatitis is one of the closest naturally occurring models for human dermatitis. We used a rabbit polyclonal antibody against human coronodesmosin and a monoclonal antibody against human filaggrin to investigate the expression of these two proteins in the epidermis of different dogs. These antibodies allowed the visualization of filaggrin and coronodesmosin in different skin diseases of dogs, including atopic dermatitis.

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The evolutionary origin of loricrin was associated with the transition to fully terrestrial life

E Videmont,1 M Herrmann,1 E Tschachler2 and L Edidin1 1 Dermatology, Medical University of Vienna, Vienna, Austria, 2 U1056, INSERM, Toulouse, France. The epidermal barrier of mammals is largely controlled by genes of the epidermal differentiation complex (EDC). To uncover the evolutionary history of this gene cluster, we compared genome sequences of multiple mammalian and non-mammalian species. A region homologous to the EDC was identified in all fully terrestrial tetrapods, i.e. mammals, reptiles and birds, but not in fish and amphibians. Homologs of the most abundant mammalian EDC protein, i.e. loricrin, were encoded by one gene in the green anole lizard and three related genes in the chicken. All of them had a glycine and serine-rich amino acid composition and a catenary-box terminal sequence motif similar to that of mammalian loricrin. RT-PCR confirmed the expression of loricrin mRNAs in reptilian and avian skin. We also generated antibodies against loricin of the lizard and performed immunohistochemistry. The reptilian loricin homolog was expressed specifically in the suprabasal epidermis during the regeneration phase of the skin shedding cycle. Thus, our data identify loricrin as a primitive avian and reptilian cornification protein that originated during the evolutionary water-to-land transition of tetrapods.
733 Fibroblasts contribute to the pathogenesis of atopic dermatitis: Impact of leukemia inhibitory factor

A Bertho,1 J Kühn,1 N Kurschi,2 A Schwarz,2 F Stöl,1 J Schwatz,2 H Weinck,2 R Füleier-Huhn2 and G Neunang1 1 Research and Development, Beiersdorf AG, Hamburg, Germany and 2 Department of Dermatology and Allergology, Christian-Albrechts University Kiel, Kiel, Germany

Atopic dermatitis (AD) is one of the most common skin disorders in industrial countries; its prevalence has increased in recent decades. The key symptoms of this chronic inflammatory, highly pruritic skin disease include xerosis, erythema, lichenification, pruritic papules and plaques, leading to a significant impairment in the quality of life. The pathogenesis of atopic dermatitis is multifactorial. Epidermal barrier defects, caused by an aberrant differentiation process of keratinocytes, as well as changes in the immune response patterns contribute to the development of AD, consequently4 attaining a crucial role to keratinocytes and immune cells. Due to the tightly regulated cross talk between fibroblasts and keratinocytes to maintain tissue function and structure, we studied whether fibroblasts may contribute to the pathogenesis of atopic dermatitis. Using organotypic skin models comprising keratinocytes from healthy and fibroblasts from atopic patients’ skin, we show that atopic fibroblasts influence the terminal differentiation process of keratinocytes. Additionally, healthy fibroblasts were able to balance the structural aberrations of the epidermis formed by atopic keratinocytes, proving that fibroblasts are an important player in the pathogenesis of atopic dermatitis.

734 Role of kindlin-2 in epidermal homeostasis

H-M Spiessl1,2,3,4,5,6,7,8,9,10,11 Sisson,1,2,3,4,5,6,7,8,9,10,11 Schmidt,1,2,3,4,5,6,7,8,9,10,11 Fitchett,10,11 Bruckner-Tuderman1 and C Has1,2,3,4,5,6,7,8,9,10,11,12,13 1 Dermatology, University, Freiburg, Germany and 2 Pharmacology, University, Freiburg, Germany

Kindlin-2 (K2) is an focal adhesion adapter involved in integrin activation. Animal models demonstrate the importance of K2 in development, and recent studies support in implication in multiple diseases. Because of the embryonic lethality of knock-out mice and lack of human disorders, its contribution to epidermal physiology has remained elusive. To address this we established skin equivalents using keratinocytes treated with K2-specific, or non-targeting shRNA and normal fibroblasts in collagen gels. K2-deficient epidermis was significantly thinner than control epidermis, with reduced dermal-epidermal and cell-cell adhesion. The mechanical stability of the epidermis was compromised, as demonstrated by numerous intrapapillary clefs, and the expression/distribution of proteins of the dermal-epidermal and cell-cell junctions was reduced. In contrast, differentiation markers were increased in the outermost layer. Co-immunoperoxidase demonstrated K2 in complexes with plakoglobin and β-catenin. Immunofluorescence staining of calcium-treated K2-deficient keratinocytes showed predominantly cytoplasmic distribution of β-catenin, plakoglobin and desmoplakin, in contrast to plasma membrane localization in control cells, suggesting that loss of K2 prevents the incorporation of these molecules in stable complexes at cell-cell junctions. In agreement, transient and stable knockdown of K2 in keratinocytes led to reduced mechanical stability of cell-cell junctions. These data suggest that K2 is a critical component of actin anchoring complexes contributing to epidermal homeostasis. Anomalies associated with K2 depletion were counteracted by activation of RhoA, a regulator of the actin stress fibers.
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**Effects of sleep quality on skin aging and function**

PA Oyetakin-White,1 B Koo,2 MS Matsui,3 D Yarosh,3 KD Cooper4 and ED Barone1,1

1 Dermatology, 2 TRI/Princeton, Princeton, NJ, 2 Rutgers University, Newark, NJ, 3 California Academy of Sciences, San Francisco, CA and 4 Skidmore College, Saratoga Springs, NY

In the current study, we assess the impact of sleep quality on skin aging and function. We demonstrate, using a novel ex vivo skin model, that sleep deprivation results in measurable changes in skin structure and function. Our findings have important implications for the prevention and treatment of skin aging.

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**Protection and repair of skin cell damage caused by exposure to extreme temperatures**

PA Oyetakin-White, B Koo, C Harwood and D Bergamaschi

London, London, United Kingdom

Extreme temperatures can cause significant damage to skin cells, leading to alterations in barrier function and increased susceptibility to infectious agents. The current study investigates the protective and reparative mechanisms of skin cells exposed to extreme temperatures. The results provide insights into the cellular responses to heat and cold stress.

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**The changes in stratum corneum lipid fluidity and packing induced by basic pH are dependent on exposure time**

EM Moore,1,2 DR Moore4 and G Menon1

1 TRI/Princeton, Princeton, NJ, 2 Rutgers University, Newark, NJ, 3 University of California San Francisco, San Francisco, CA and 4 Avon Products Inc. Global R&D, Suffern, NY

The current study investigates the effects of basic pH exposure on skin structure. We demonstrate that exposure to basic pH leads to changes in lipid fluidity and packing, which are dependent on exposure time.

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**IA5P is a novel autophagy inhibitor in keratinocytes**

A Chell,1 C Harwood and D Bergamaschi

Cotswold, Cheltenham, United Kingdom

IA5P is a novel autophagy inhibitor that has been shown to affect keratinocyte function. This study investigates the mechanism of its action on keratinocytes. The results provide insights into the regulation of autophagy and its potential therapeutic applications.

**743**

**Sensory nerves express interleukin-31 receptor A to mediate T helper cell-dependent pruritic and neuroimmune communication in atopic dermatitis**

E Cecchini,1 X Wang,1 T Miyata,1 T Savenick,1 A Antal1, C Kempe,1 M Helft1, G Kukova,1 T Rauh1, P Amosova,1 DR Dillon,1 B Shuster1, I Cremer1, B Haines1 and M Steinke1

1 Dept. of Dermatology and Surgery, University of California San Francisco, San Francisco, CA, 2 Dept. of Anatomy, University of California San Francisco, San Francisco, CA and 3 Dept. of Neurobiology, University of California Davis, Davis, CA, 4 Finnish Institute of Occupational Health, Helsinki, Finland and 5 Dept. of Dermatology, University Hospital Düsseldorf, Düsseldorf, Germany

Interleukin-31 (IL-31) is a cytokine that plays a key role in the pathogenesis of atopic dermatitis. This study investigates the expression and function of IL-31 receptor A (Il1r1) in sensory nerves. The results provide insights into the neuroimmune communication in atopic dermatitis.

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**SEBCAD defect in Darier disease causes ER stress leading to impaired trafficking and defective cell-to-cell adhesion strength**

M Savignac,1 M Simon,1 A Feile1, L Gabitoul1 and A Hoonemam7

1 Department of Genetics, INSERM U781, Université Paris V René Descartes, Necker Hospital, Paris, France and 2 INSERM, U1043, Toulouse, France

Darier disease (DD) is a genetic skin disorder characterized by defects in cell-to-cell adhesion and abnormal keratinization. The current study investigates the role of SEBCAD, a gene associated with DD, in the regulation of ER stress and cell adhesion. The results provide insights into the pathogenesis of DD.

References:


2. Oyetakin-White PA, Koo B, Matsui MS, Yarosh D, Cooper KD and Barone ED. Dermatology, 2 TRI/Princeton, Princeton, NJ, 2 Rutgers University, Newark, NJ, 3 California Academy of Sciences, San Francisco, CA and 4 Skidmore College, Saratoga Springs, NY.


Epidermal Structure & Barrier Function

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Non-invasive method to study pruritus in mice using in-vivo confocal microscopy

C. Kempen, A. Bioma, M. Langosz, F. Cevikbas, R. Marangoni, T. Buhl and M. Steinbohl Dept.s of Dermatology and Surgery, University of California San Francisco, San Francisco, CA

Pruritus is one of the major symptoms in dermatology worldwide, and its treatment is still a challenge. The study of mice has revealed that chronic itch is characterized by a barrier function that is impaired in atopic dermatitis patients. We have developed a novel in-vivo confocal microscopy method that allows for a non-invasive, rapid assessment of the barrier function in vivo. This method has been successfully employed in the past to assess the effect of different treatments on the barrier function in murine models of dermatitis.

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Atopic dermatitis confers enhanced skin responses to experimentally-induced tandem repeated irritation in vivo

Angela-Fischer,1 AK Hoek,1 D Dip,2 JT Fischer,1 D Zilliken1 and S Kezic2 1 Department of Dermatology, University of Lübeck, Lübeck, Germany, 2 Corinna Institute of Occupational Health, Amsterdam, Netherlands. 7 November 2013 3:00 PM-4:00 PM www.jidonline.org

The skin response to experimentally-induced in vivo tandem repeated irritation in atopic dermatitis patients has not been studied so far. We investigated the barrier function levels of primary cytokines and natural moisturising factor (NMF) after repeat single and tandem exposure to surfactants (0.5% SLS, and 5% water +1% SLS). As in atopic dermatitis patients and healthy controls genotyped for the prevalent European filaggrin gene (FIC) loss-of-function mutations. The irritation response was monitored by visual scoring, measurements of transdermal water loss (TEWL) and erythema up to 96h when tape strips for NMF and cytokine analysis were collected from the irritation-exposed and non-exposed (control) skin sites. Compared to the control group, repeated single exposure to AcA/AcA and SLS/SLS and tandem (AcA/AcA and SLS/SLS) irritation in the atopic patients resulted in greater visual score and erythema (+/− value). In atopic skin, the TEWL increase compared to baseline (baseline) was more pronounced at all exposed fields and assessment time points. Furthermore, TEWL after 96h exposure to AcA/AcA, SLS/SLS and AcA/AcA irritation in the atopic group were similarly evaluated at 4 and 96h. Scratching was increased exposure in normal skin, showing that infrared spectroscopic imaging of skin can directly resolve discrete skin layers to address many basic questions in dermatology. While applications of these imaging techniques have been focused on topical drug delivery there is a growing literature on various disease states that may be further relevant for development of chronic irritant contact dermatitis.

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Infrared imaging spectroscopy of ex vivo skin

DJ Moore1 and C. Holtz2 1. TRES/Princeton, Princeton, NJ and 2 Rutgers University, Newark, NJ

In recent years there has been a significant increase in the use of spectroscopic imaging methods to address many basic questions in dermatology. While applications of these imaging techniques have been focused on topical drug delivery there is a growing literature on various disease states that may be further relevant for development of chronic irritant contact dermatitis.

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Controversial effect of Matricaria chamomilla aqueous extract associated to an emollient as treatment for dermatitis induced in a murine model

Villaseñor,1 M González-Hernández,1 L Muñoz-Hernández,2 C Orta-Figurenza,1 M Ramírez-Anaya,1 R S. Reyes-Téllez1 and O Villanueva-Sánchez 1 Universidad de las Américas Puebla, Puebla, Mexico, 2 Benemérita Universidad Autónoma del Estado de Puebla, Puebla, Mexico, 3 Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán”, D.F., Mexico, 4 and 5 Hospital General de Puebla, Zona Norte, Puebla, Mexico

The aim of this quasi-experimental study was to evaluate the local effect of Matricaria chamomilla (mCh) lophofoliate aqueous extract associated to an emollient at 7% as treatment for dermatitis (D) induced in a murine model. D induction was performed with dichlorobenzene on 11 males, 7-week old BALB/c mice. Animals were divided into three groups: control (GC), control negative (GCN) and experimental (GE). All were treated for 4 weeks, except the GC. Liquid petrolatum was applied to the GCN and GE with hospitalised extract of C. Matricaria chamomilla. Data were gathered using histopathology, clinical and scratching observations and blood count. Skin biopsies were taken at 2 and 6 weeks and an evaluation of peripheral blood cells to correlate inflammatory cells with mCh. Scratching was measured exposure to the observation methodology of Kojohoshi et al. Histopathologically the GC improved by 75%, the GCN evolved unfavorably and the GE improved by 50%. Clinical improvement was positive (p<0.005) for all groups, revealing that mCh is a potent anti-inflammatory drug. The mCh aqueous extract showed no effect in the current study. There were no significant differences among white cells parameters after 4 weeks of treatment. The lophoholate aqueous extract associated to an emollient as treatment for D induced in this murine model. The lack of information about effectiveness of aqueous extract as treatment for D and the negative results of the present study highlights the necessity of more experimental studies to test the efficacy of this empirically used treatment.

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Constitutive autophagy as a terminal differentiation mechanism

O. Akandu1, K. Sully,1 A. Chiik,1 R. O. Vlahoughns,1 D. Bergmacher,1 R. C. Hunsdorfer,1 M. Pflood2 and C. Byrne1 1 Cutaneous Research, Blaze Institute, Raids and the London School of Medicine and Dentistry, London, United Kingdom 2 Institute of Child Health, University College London, London, United Kingdom

Epidermal keratinocytes move from a proliferating basal layer outwards to the granular layer where they terminally differentiate. During this process, the cytoskeleton becomes reorganized, forming a layer of flattened, flat-topped cells or stratum. This process of terminal differentiation involves a series of biochemical events that are essential for the maintenance of skin homeostasis. The terminal differentiation process involves autophagy, which is a cellular process that degrades cellular components in order to recycle them for use in other cellular processes. This process is essential for the maintenance of skin homeostasis and is involved in the development of various skin diseases. The aim of this study was to investigate the role of autophagy in the terminal differentiation process of epidermal keratinocytes.
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TRP channels govern the balance of keratinocyte proliferation and differentiation in vitro and in the context of skin disease

E A Lempicki,1 AM Nelson,1,2 SR Wilson,2,3 DM Owens,2 and DM Baustista1 1 Columbia University, New York, NY, 2 U C Berkeley, Berkeley, CA and 3 Bay Laboratory of Medicine, Houston, TX Transient receptor potential (TRP) channels regulate cell-cell signaling and differentiation in keratinocytes. However, their role in skin physiology remains poorly defined. Here, we investigated the functional role of TRP channels in keratinocytes and their potential implications in the development of skin disease.

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Neonatal and infant skin surface area expansion and correlation with the developing skin barrier and structure

M Mark Correa, P Khanna and RM Walters Johnson & Johnson Consumer Products Company, Skillman, NJ Infant skin is different structurally from adult skin, and these structural differences contribute to observed differences in functional and mechanical properties between infant and adult skin. Skin thickness increases with age from infants to adults, and correspondingly, the transepidermal water loss (TEWL) of infant skin has been shown to decrease with age over the first years of life. The objective of this study was to determine if the rapid body surface area expansion that occurs during the same period relates to the concomitant changes in the skin water barrier function. Caucasian and African American subjects aged 3 months old to 4 years old were recruited to participate in a clinical study involving non-invasive measurements of the skin. A subset of mothers was also recruited to complete a questionnaire assessing the various factors that may affect skin barrier function during infancy.

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Multifunctional molecules and the biomechanical function of human stratum corneum

K Kim and RH Thakur 1 Stanford University, Stanford, CA, and 2 Georgia Health Sciences University, Augusta, GA The stratum corneum (SC) is a layered, keratinized, non-living barrier that protects the underlying tissues from environmental stimuli and cell-cell signaling pathways. SC thickness increases with age in adults, and the relationship between SC thickness and environmental stimuli and cell signaling is not fully understood. Here, we review the role of SC molecules in biomechanical function and SC thickness, with a focus on the role of keratin intermediate filament proteins and extracellular matrix components.

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Protein kinase D1 negatively regulates epidermal keratinocyte differentiation

V Choobeh,1 E Olala,1,2,3 L Kaddour-Djebbar1,2,3 and WB Bollag1,2 1 Charlie Norwood VA Medical Center, Augusta, GA, 2 Georgia Health Sciences University, Augusta, GA, and 3 Columbia University, New York, NY Protein kinase D1 (PKD1) is a serine/threonine kinase that plays a vital role in various biological processes. In this study, we investigated the role of PKD1 in keratinocyte differentiation by utilizing a mouse model with reduced PKD1 expression. We observed that PKD1 knockdown resulted in increased expression of differentiation markers and decreased proliferation, suggesting a role for PKD1 in regulating keratinocyte differentiation.

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Roles for TRPA1 in epidermal homeostasis

M Park,1,2 JC Chee,1 Y Zhou,1,2,3 Y Lu,1,2,3 C Kaddour-Djebbar1,2,3 and WB Bollag1,2 1 Charlie Norwood VA Medical Center, Augusta, GA, 2 Georgia Health Sciences University, Augusta, GA, and 3 Columbia University, New York, NY TRPA1 is a transmembrane receptor that is activated by a variety of stimuli, including ischemia, hypoxia, and cold. Here, we investigated the role of TRPA1 in epidermal homeostasis by utilizing a mouse model with reduced TRPA1 expression. We observed that TRPA1 knockdown resulted in decreased expression of differentiation markers and increased proliferation, suggesting a role for TRPA1 in regulating keratinocyte differentiation.

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Effects of Aspergillus oryzae-challenged germinated soybean extract on skin changes induced by ovariecetomy in hairless mice

K Lee,1 H Jeong,1 S Boo,1 J Cheong,1 and S Lee1 1 National Skin Center, Singapore, Singapore Ovariecetomy (OVX) in female mice is a commonly used model for studying the effects of estrogen depletion on the skin. In this study, we investigated the effects of Aspergillus oryzae-challenged germinated soybean extract on skin changes induced by ovariecetomy in hairless mice. We observed that the extract significantly reduced skin thickness, decreased MMP-1 expression, and improved the mechanical properties of the skin.

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Klotho is downregulated in the skin of individuals with atopic dermatitis

Z Phromton,1 X Mo and M Tang National Skin Centre, Singapore, Singapore Klotho is a member of the Klotho protein family and has been suggested to play a role in the molecular mechanisms by which the FGF19 sub-family regulates bile acid synthesis, glucose metabolism, and as well as phosphate/vitamin D metabolism. We performed a study to evaluate the expression of Klotho in human skin and its potential role in the development of atopic dermatitis. Using immunofluorescence and immunohistochemistry, we analyzed Klotho expression in fresh skin biopsies and keratinocytes from lesional and non-lesional skin of AD patients, as well as healthy controls. Immunofluorescence analysis showed that Klotho expression was decreased in AD skin compared to healthy skin. These findings suggest a potential role for Klotho in the development of atopic dermatitis.