MANCHESTER 1824
The University of Manchester

The University of Manchester Research

The effects of narrowband ultraviolet B (311 nm) phototherapy on epidermal barrier and differentiation markers in polymorphic light eruption

Link to publication record in Manchester Research Explorer

Citation for published version (APA):
Pond, EJ., O'Neill, CA., Rhodes, LE., & Gibbs, NK. (2014). The effects of narrowband ultraviolet B (311 nm) phototherapy on epidermal barrier and differentiation markers in polymorphic light eruption. In *host publication* (pp. e34-e34). John Wiley & Sons Ltd.

Published in:
host publication

Citing this paper
Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights
Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy
If you believe that this document breaches copyright please refer to the University of Manchester's Takedown Procedures [http://man.ac.uk/04Y6Bo] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.

Download date:09. Jun. 2022

British Society of Investigative Dermatology Annual Meeting Newcastle, 7th–9th April, 2014

Posters

P1
Proautophagic Ambra-1 as biomarker of differentiation in cutaneous squamous carcinoma

S. Whitaker,[1] M.-E. Anagnostou,[1] J. Armstrong,[1] M. Ellias,[1] A. Husain,[2] P. Lovat[1] and R. Ellis[1]
[1]*Department of Cellular Medicine, Newcastle Upon Tyne, U.K. and* [2]*Royal Victoria Infirmary, Newcastle Upon Tyne, U.K.*

Cutaneous squamous cell carcinoma (cSCC) is the second most common nonmelanoma skin cancer, currently affecting approximately 10 000 patients per year, with a predicted increase of 50% over the next 15 years. cSCCs may arise *de novo* or from the transformation of precursor lesions such as actinic keratosis or Bowen disease. Surgical resection of tumours is usually curative, but results in significant mortality, and lesions may still reoccur or even metastasize. As such, there is an urgent need for novel biomarkers to allow the refinement of current risk stratification criteria and to allow the development of novel personalized nonsurgical therapies. Autophagy, the principle catabolic process for the lysosomal-mediated breakdown and recycling of damaged organelles and excess proteins is essential for the maintenance of cellular differentiation. In the context of epidermal differentiation, pilot data demonstrate increased expression of the proautophagy regulatory protein Ambra-1 from the basal layer to the stratum corneum in line with keratinocyte differentiation. To verify the contribution of Ambra-1 to keratinocyte differentiation, primary keratinocytes in culture were differentiated by exposure to high calcium and the expression of loricrin, cytokeratin 14 (CK14) and Ambra-1 determined by Western blotting. Results revealed that calcium-induced keratinocyte differentiation resulted in increased Ambra-1 expression in line with expected changes in differentiation markers (increased loricrin, decreased CK14). Knockdown of Ambra-1 in primary keratinocytes also resulted in significant inhibition of loricrin and filaggrin expression ($P < 0{\cdot}05$), but was associated with increased keratinocyte proliferation, collectively suggesting Ambra-1 is required for normal keratinocyte differentiation and suppression of excessive cellular proliferation. Immunohistochemical analysis of a retrospective cohort of cSCC subtypes confirmed increased Ambra-1 expression in normal, peritumoral epidermis, in line with epidermal differentiation. Within cSCC there was a distinct difference in Ambra-1 expression between ‘well-differentiated’ and ‘poorly differentiated’ tumours; with ‘poorly differentiated’ tumours exhibiting markedly reduced/absent Ambra-1 expression compared with normal peritumoral epidermis. Similar reductions in Ambra-1 expression were also detected in dysplastic cells comprising actinic keratoses or Bowen disease. At present, stratification of patients with cSCC is based on morphological and immunohistochemical features encompassed by the American Joint Committee on Cancer’s staging system, with ‘poorly differentiated’ tumours associated with the worst prognosis. Therefore, the variation of Ambra-1 expression in cSCC samples may represent a diagnostic biomarker highlighting the presence of dysplastic cells, as well as being a prognostic biomarker of disease risk, thus allowing refinement of patient management in terms of surgical aggression, excision margin adequacy and patient follow-up regimens.

P2
Assessing the role of dermal architecture in wound healing and tumour differentiation

A. South,[1] B. Lane[2] and Y.Z.V. Lim[1]
[1]*University of Dundee, Dundee, U.K. and* [2]*Institute of Medical Biology, A*STAR, Singapore*

The differentiation of cells is an important step in cellular development and plays a key role in the mechanism of both cancer development and wound healing. Often key to the processes of cellular differentiation, the microenvironment (consisting of other cells, soluble factors, extracellular matrix and structural scaffolds) provides cues that can either suppress or support neoplasm and invasion. It is well documented that in the grading of tumours, poorly differentiated tumours often lead to poorer prognosis. These tumours tend to lack discernable boundaries and often appear to meld into the surrounding tissue. Many studies have also shown that proteins involved in adhesion are among the most prominent proteins expressed in tumours throughout progression. Many of the cancer-associated molecules, particularly in invasive cancers, tend to play a role in adhesion. The notion that different adhesion profiles of tissues can influence cellular segregation and differentiation has long been described in the processes of embryonic development. In particular the differential adhesion hypothesis (DAH) proposed by Malcolm Steinberg in the 1960s describes a model where tissues can be considered to behave like viscoelastic liquids, and are able to segregate themselves based on differential adhesion to form compartments. Taking cues from the above observations, the work described here seeks to study how different adhesion profiles of squamous cell carcinomas (SCCs) interact with and correspond to, different adhesion profiles of the microenvironment, which go on to influence morphological development. Using a suspension coculture method called the ‘hanging drop’ we performed experiments analysing the interaction between SCC keratinocytes with normal human fibroblasts. These early

© 2014 British Association of Dermatologists

experiments describe the effects of differential adhesion among SCC cell lines showing that the different SCC lines (IC1, RDEB2, RDEB4, RDEB16·4 and RDEB16·5) exhibit morphologically distinct affinities between themselves and normal dermal fibroblasts as well as to each other in heterogeneous SCC keratinocyte cocultures. This novel method provides a platform to analyse factors which influence SCC–fibroblast interactions and ultimately test the DAH in the SCC environment.

P3

Epidermal barrier disruption and filaggrin deficiency activates TonEBP, which senses tonicity and regulates keratinocyte proliferation and apoptosis

B.M. Taal,[1] M. Elias,[1] H. Long,[2] M. Donaldson[2] and N.J. Reynolds[1]

[1]*Institute of Cellular Medicine, Newcastle upon Tyne, U.K. and* [2]*Stiefel, A GSK Company, Stevenage, U.K.*

Epidermal barrier disruption increases transepidermal water loss (TEWL) and reduces water content in skin, potentially exposing underlying keratinocytes to a hypertonic environment. Filaggrin is a major constituent of the epidermal barrier providing mechanical strength through protein–lipid crosslinking and contributes to epidermal hydration through breakdown into natural moisturizing factors. As TonEBP (tonicity-responsive enhancer binding protein, NFAT5) is the only known transcription factor to sense tonicity, we hypothesized that TonEBP would be activated by barrier disruption and would regulate restorative mechanisms. Barrier disruption induced by acetone treatment of epidermal equivalents and tape-stripping of normal human skin resulted in increased nuclear to cytoplasmic (n/c) localization of TonEBP 1·5-fold ($P < 0{\cdot}05$, $n = 3$) and 1·4-fold ($P < 0{\cdot}001$, $n = 4$), respectively. Lentiviral transduction of filaggrin shRNA resulted in approximately 90% knockdown of filaggrin protein in epidermal equivalents ($n = 10$, $P < 0{\cdot}001$) compared with control shRNA. TEWL was increased 1·5-fold ($P < 0{\cdot}05$, $n = 8$) in the *FLG* knockdown equivalents compared with the controls. Correspondingly there was a twofold increase in TonEBP protein expression in the *FLG* knockdown equivalents compared with the controls. Furthermore, increased TonEBP n/c expression was observed throughout the epidermis of lesional atopic eczema skin (1·5-fold, $P < 0{\cdot}01$, $n = 9$). TonEBP mRNA and protein levels, nuclear localization and transcriptional activity were modulated in response to hypertonicity in primary human keratinocytes, confirming that TonEBP is modulated by osmotic stress in this system. To test its functional role, TonEBP was knocked down in normal human epidermal keratinocytes (NHEKs) using siRNA. Relative to isotonic controls in cells exposed to 600 mosmol L^{-1} hypertonic medium there was a 1·9-fold decrease in cell growth ($P < 0{\cdot}01$, $n = 3$), 1·8-fold decrease in cell proliferation ($P < 0{\cdot}05$, $n = 3$) and a 1·7-fold increase in apoptosis ($P < 0{\cdot}01$, $n = 3$) in TonEBP knockdown NHEKs compared with cells transfected with control siRNA. These results show that TonEBP protects against hypertonicity-induced cell death/apoptosis and reduced cellular proliferation. These data indicate a functional role for TonEBP in keratinocytes responding to epidermal barrier disruption and protecting against the deleterious effects of hypertonic stress.

P4

The composition of the dermal–epidermal junction is significantly altered in intrinsically aged skin

A.K. Langton,[1] N.K. Gibbs,[1] M.J. Sherratt,[2] C.E.M. Griffiths[1] and R.E.B. Watson[1]

[1]*Centre for Dermatology and* [2]*Centre for Tissue Injury and Repair, University of Manchester, Manchester, U.K.*

The dermal–epidermal junction (DEJ) is composed of a complex network of interconnecting proteins that help provide skin with structural integrity and mechanical stability. Ultrastructurally, the DEJ is divided into four zones that are characterized by their ubiquitous expression of distinct protein components including: (i) integrin $\alpha6\beta4$, collagen XVII and laminin-332 in the upper regions; (ii) the lamina lucida; (iii) collagen IV and perlecan in the lamina densa; and (iv) collagen VII-anchoring fibrils in the papillary dermis. With intrinsic ageing, the DEJ is flattened and its surface area is reduced due to loss of rete ridges. Despite this, few investigations have specifically identified which basement membrane components change with increasing age. In this study we have used an Affymetrix® microarray-based approach to detect age-related gene expression changes in basement membrane components using skin biopsies from photoprotected buttock of three young (24–26 years) and three aged (74–75 years) male volunteers. A comparison of the gene expression profiles identified collagen IV, integrin $\beta4$ and perlecan as unchanged with age; however, three genes were significantly downregulated in the older age group: collagen VII ($P < 0{\cdot}048$), collagen XVII ($P < 0{\cdot}049$) and laminin-332 ($P < 0{\cdot}024$). The Affymetrix® data provided good candidate genes for further analysis at the protein level, therefore skin biopsies from a larger cohort of young (18–30 years, $n = 14$) and aged volunteers (> 65 years, $n = 14$) were obtained and examined for changes in their deposition at the DEJ using immunohistology. Using this larger cohort, we confirmed that the aged individuals exhibited a significantly flattened DEJ compared with the young individuals (interdigitation index $P < 0{\cdot}0001$). Next, we confirmed the findings of the Affymetrix® by identifying a significant reduction in the deposition of collagen VII ($P < 0{\cdot}001$), collagen XVII ($P < 0{\cdot}01$) and laminin-332 ($P < 0{\cdot}0001$) proteins in the aged cohort. Furthermore, despite being unchanged at the gene expression level, the deposition of integrin $\beta4$ ($P < 0{\cdot}001$) and collagen IV ($P < 0{\cdot}0001$) were also significantly decreased in this aged cohort. The functional implication of these altered gene expression and protein deposition profiles appears to be loss of structural integrity of the DEJ, which in turn explains in part the increased fragility of aged skin.

© 2014 British Association of Dermatologists

P5

The effect of mechanical stress on keratinocyte stem cells *in vitro*

L. Wallace and J. Reichelt

Newcastle University, Newcastle upon Tyne, U.K.

Keratinocyte stem cells (KSCs) of the epidermis are found within a niche known as the bulge region of the hair follicle. These cells are normally quiescent and are only activated in the anagen phase of the hair cycle to contribute to hair follicle growth. The interfollicular epidermis is normally continually regenerated from progenitor or transit-amplifying (TA) cells residing in the basal epidermis. Upon wounding, however, bulge KSCs can also be induced to proliferate, migrate and differentiate to help re-epithelialize the interfollicular epidermis. The bulge region itself acts as the insertion site for the arrector pili muscle posing some interesting questions as to the effect of muscle contraction on KSCs. It is known that most cell types including stem cells respond to mechanical stimuli. While primary keratinocyte cultures, consisting mainly of differentiated cells and some progenitor cells are known to show increased proliferation and decreased differentiation upon stretch, the response of KSCs to stretch is unknown. To investigate the effects of mechanical stretch on KSCs, the previously established murine keratinocyte stem and progenitor cell line, K38, was used in combination with the Flexcell system, which can apply a controlled and reproducible stretch to adherent cells. We show that application of 10% cyclic stretch (0·1 Hz) to K38 cultures inhibits cell proliferation. Furthermore, we demonstrate that KSCs respond differently to circumferential (equal stretch in all directions) and radial stretch (stretch in two opposite directions). Whereas radial stretch stimulated KSC migration and differentiation, circumferential stretch inhibited both migration and differentiation. We used a scratch assay to determine the cell migration rate and two distinct assays to study cell differentiation: a colony-forming assay where the ratio of KSCs, TA cells and differentiated keratinocytes can be determined, and an aggregation assay in which the ability of cells to form cell aggregates in suspension culture, a hallmark of stem cells, is tested. Our data indicate that the response of cultured keratinocyte stem and progenitor cells to stretch differs from that of primary keratinocytes and that different types of mechanical stretch elicit different responses on KSCs. The ability to modulate cultured KSCs through application of mechanical stretch suggests a potential to employ this pathway to regulate KSC activation *in vivo*.

P6

Exploring the localization of murine epithelial hair follicle stem cell markers in the human hair follicle: Sox9 and Lhx2

T. Purba,[1] I. Haslam,[1] A. Shahmalak[2] and R. Paus[1]

[1]*University of Manchester, Manchester, U.K. and* [2]*Crown Clinic, Manchester, U.K.*

The localization of putative markers of epithelial hair follicle stem cell (eHFSC) subpopulations, delineated in the murine pelage follicle in recent years, remains poorly defined within the human hair follicle (HF). Using immunofluorescence, we have characterized eHFSC subpopulations that express the murine eHFSC markers, Sox9 and Lhx2, in isolated human occipital scalp HFs from patients undergoing HF transplantation surgery ($n \geq 3$) relative to each other and to the well-known human eHFSC markers, keratin 15 (K15) and keratin 19 (K19). In the murine HF, transcription factor Sox9 is thought to be involved in the regulation of outer root sheath (ORS) differentiation and maintenance of the eHFSC niche. Moreover Sox9+ murine eHFSCs are capable of contributing to all HF epithelial lineages. In the human HF, Sox9 immunoreactivity (IR) showed both basal and suprabasal localization throughout the ORS. Double immunofluorescence showed that a subset of Sox9+ cells coexpressed K15+ and K19+ in the basal bulge and suprabulbar ORS. Sox9 expression patterns in the ORS were prominently cytoplasmic, but were on occasion observed to show nuclear compartmentalization, possibly corresponding to cell quiescence and differentiation, respectively. Antibody to transcription factor Lhx2, which is required in eHFSC niche organization and quiescence in murine HFs, demonstrated prominent IR within a small number of cells in the ORS. The intermittent distribution of Lhx2 IR in the ORS was reminiscent of that of K19+ cells, which localize to the bulge, infrabulge and suprabulbar ORS. Further investigation highlighted that Lhx2 demarcated cells that were both K19+ and K19−. Moreover, double immunofluorescence also highlighted both ORS cells that expressed either Lhx2 or Sox9 and cells that were Lhx2/Sox9 double-positive. The data presented here emphasize that the human ORS is enriched with a heterogeneous collective of epithelial stem/progenitor cells. We therefore seek to explore further and understand these different eHFSC subpopulations and demarcate their hierarchical organization with respect to quiescence, cell cycle activation and differentiation in the healthy human HF by applying additional relevant *in situ* markers.

P7

Application of all-*trans* retinoic acid regulates the activity of the lysyl oxidase family of crosslinking enzymes in photoaged skin

A.K. Langton,[1] M.J. Sherratt,[2] C.E.M. Griffiths[1] and R.E.B. Watson[1]

[1]*Centre for Dermatology and* [2]*Centre for Tissue Injury and Repair, University of Manchester, Manchester, U.K.*

The structural integrity of dermal macromolecular assemblies relies heavily on the enzyme-driven formation of developmentally regulated protein crosslinks. With advancing age, the function of these extracellular matrix proteins may be compromised by the accumulation of pathological enzyme-mediated crosslinks leading to tissue stiffening. Lysyl oxidases (LOX), members of the amine oxidase family of enzymes, are known to crosslink both fibrillar collagens and elastin. We have previously demonstrated that the enzymatic activity of the LOX enzymes is increased in both intrinsically aged and photoaged skin using a novel *in situ* activity assay. In the current study, we investigated whether all-*trans* retinoic acid

 © 2014 British Association of Dermatologists

(tRA), the 'gold standard' topical treatment for photoaged skin, can modulate the activity of this family of enzymes. Using the previously described 'Manchester Patch Test Assay', tRA was applied under occlusion to the photoaged forearm of healthy volunteers (aged 55–87 years, n = 19). Skin biopsies were obtained from treated sites and from an untreated baseline, processed for immunohistochemistry and the *in situ* enzyme activity assay. Enzymatic activity was detected in all samples; however, the cohort clearly stratified into two groups: those with a high epidermal enzymatic activity level in their baseline biopsy (n = 10); and those with a low epidermal enzymatic activity level in their baseline biopsy (n = 9). Those with an initial low activity level had significantly enhanced epidermal activity following application of tRA (P = 0·04). Conversely, those with an initial high activity did not alter when treated with tRA. Using immunofluorescence staining we were able to identify LOXL1 as the most likely candidate family member for these observed differences in activity. The functional significance of this normalization of LOXL1 enzymatic activity in photoaged skin requires further characterization; however, it does suggest that topical treatment of photoaged skin with tRA can induce profound changes in the activity of key enzymes involved in the mechanical properties of skin.

P8

Could RNA aptamers be utilized for the topical treatment of inflammatory skin diseases?

R. Doble,[1] M.F. McDermott,[2] O. Cesur,[1] N.J. Stonehouse[1] and M. Wittmann[2,3,4]

[1]*Faculty of Biological Sciences, School of Molecular and Cellular Biology, University of Leeds, Leeds, U.K.,* [2]*Leeds Institute of Molecular Medicine, LMBRU, University of Leeds, Leeds, U.K.,* [3]*Centre for Skin Sciences, School of Life Sciences, University of Bradford, Bradford, U.K. and* [4]*Bradford Teaching Hospitals NHS Foundation Trust, Bradford, U.K.*

RNA aptamers are valuable therapeutic tools that have advantages over systemic treatments with antibody-based biologics. RNA aptamers show high specificity and affinity, low immunogenicity, and can be less costly compared with antibody therapies. The aim of this study was to investigate the potential of a previously described RNA aptamer with neutralization capacity against interleukin (IL)-17A on the proinflammatory mediator release of skin resident cells. This was carried out with recombinant protein and also in a coculture with IL-17-producing T cells (CD4+ CCR6+). The aptamer showed good neutralizing effect on human primary dermal fibroblasts treated with recombinant IL-17A and when cocultured with activated CD4+ CCR6+ T cells. Surprisingly, this effect could not be shown for human primary keratinocytes stimulated with recombinant IL-17A. We were able to show that this lack of activity of the RNA aptamer in keratinocyte cultures was due to rapid internalization of the aptamer. This was confirmed by immunofluorescence with labelled aptamer. This finding is of interest as the efficient RNA aptamer uptake capacity of primary human keratinocytes could be used as a tool for intracellular targets without the need for chemical permeabilization of the cell.

P9

Ethnicity is a key determinant of skin structure and composition

A.K. Langton,[1] C.E.M. Griffiths,[1] N.K. Gibbs,[1] M.J. Sherratt[2] and R.E.B. Watson[1]

[1]*Centre for Dermatology and* [2]*Centre for Tissue Injury and Repair, University of Manchester, Manchester, U.K.*

Ethnicity plays a key role in determining the susceptibility of human skin to external insults and dermatological disease. Despite this, studies of ethnic human skin focus primarily on epidermal pigmentation, with few reports characterizing the underlying biology of the dermis and dermal–epidermal junction (DEJ) with regards to gross morphology and the abundance of key DEJ and dermal extracellular matrix (ECM) components. Given that adaptation to an ecological niche underpins diversity in nature, we hypothesize that ancestral human populations living at different geographical locations may have encountered disparate environmental pressures, which may have driven unique adaptations in skin structure and composition that are ultimately retained in modern humans. In order to test this hypothesis, we have compared: (i) the morphology of the epidermis and DEJ; (ii) the protein composition of the DEJ; and (iii) the composition and distribution of the major components of the dermal ECM of photoprotected skin from young individuals of African, white and Far-East Asian ethnic origin (n = 7/group; age 18–30 years). We report that the epidermis of African skin was thicker, with deeper rete ridges and a more convoluted DEJ than either white or Far-East Asian skin. Although similar in composition for laminin-332 and integrin β4 proteins, the DEJ of African and Far-East Asian skin was collagen VII-poor compared with white subjects (P < 0·001 and P < 0·01, respectively). Furthermore, the dermis of African skin was enriched in fibrillar collagens (P < 0·05) but was relatively elastin-poor (P < 0·05) compared with white skin. Compared with white subjects and Far-East Asians, the dermis of African volunteers was enriched in both fibrillin-rich microfibrils (P < 0·001 and P < 0·001, respectively) and fibulin-5 (P < 0·001 and P < 0·001, respectively). This study demonstrates that photoprotected skin of modern human ethnic groups exhibits fundamental differences in both gross morphology and dermal composition. These architectural and compositional differences are likely to impact on the primary functions of skin, which are to resist the environmental pressures of ultraviolet radiation, mechanical stress and the subsequent propensity for injury, and infection. Further research into the functional significance of these differences is warranted.

P10

Effects of polyphenols on mitochondrial DNA damage in skin fibroblasts

C. Bosch,[1] G. Lietz[2] and M. Birch-Machin[2]

[1]*University of Leeds, Leeds, U.K. and* [2]*Newcastle University, Newcastle upon Tyne, U.K.*

Polyphenols comprise a large group of bioactive compounds with antioxidant properties that have been shown to counter-

© 2014 British Association of Dermatologists

act oxidative stress production in different cell systems thereby providing potential protection against oxidative stress-related diseases such as skin ageing and cancer. Typical examples for polyphenols are the constituents of green tea and pomegranate fruit with epigallocatechin gallate (EGCG) and the ellagitannin punicalagin as potent marker compounds, respectively. We are investigating the mechanism of a range of relevant polyphenol compounds with different structure and their potency for attenuating hydrogen peroxide-induced oxidative stress in human skin fibroblast cells. Mitochondrial DNA (mtDNA) damage is a sensitive biomarker of oxidative stress and ultraviolet radiation exposure in skin cells and is used in this study as a major readout marker. Mitochondria are a major endogenous source of reactive oxygen species and the mtDNA is prone to oxidative damage due to its proximity to the electron transport chain, its lack of histone protection, and less efficient repair mechanisms compared with nuclear DNA. Using a quantitative polymerase chain reaction-based screening approach to quantify mtDNA damage we demonstrate that pomegranate is able to lower mtDNA damage almost as efficiently as the mitochondria-targeted compound MitoQ (47% vs. 56% reduction of hydrogen peroxide-induced mtDNA damage) whereas EGCG (over a range of concentrations) shows no effect. We are further linking the extent of mtDNA damage exhibited by the test compounds to the overall and mitochondria-specific oxidative stress levels and will demonstrate the potential of selected polyphenols to modulate redox-regulated cellular signalling pathways and target gene expression. Elucidating the detailed mechanism by which natural compounds contribute to cellular stress reduction helps greatly to estimate their therapeutic potential in the context of skin research and beyond.

P11

A significant role for human mitochondrial complex II in the production of reactive oxygen species in human skin and its association with ageing

A. Bowman, A. Anderson, P. Manning and M. Birch-Machin

Newcastle University, Newcastle upon Tyne, U.K.

The mitochondrial respiratory chain is a major generator of superoxide and downstream cellular oxidative stress, thought to be an underlying cause of the carcinogenic and ageing process in many tissues including skin. Due to recent observations, there has been a growing debate regarding the relative contributions of the respiratory chain (RC) complexes I, II and III toward production of reactive oxygen species (ROS). Furthermore, the majority of previous studies have focused on rat (and some human) tissues and certainly not on human skin, which is surprising as this tissue is regularly exposed to ultraviolet (UV) A in sunlight, a potent generator of cellular oxidative stress. In a novel approach we have used an array of established specific metabolic inhibitors and DHR123 fluorescence to study the relative roles of the mitochondrial RC complexes in cellular ROS production in human skin cells. These include additional enhancement of ROS production by exposure to physiological levels of UVA. The effects within epidermal- and dermal-derived skin cells are compared with other tissue cell types where the RC complexes have been historically and extensively studied as well as those harbouring a compromised mitochondrial status (i.e. Rho-zero cells). The results show that the role of human skin complex II in terms of ROS production is more important than previously thought. Further investigations showed for the first time a decline in complex II activity associated with increasing skin age (human tissue biopsy, $P = 0{\cdot}0333$, $rho = -0{\cdot}8857$, nonparametric Spearman correlation), and ageing biomarkers (i.e. telomerase status; MRC5 vs. MRC5/hTERT, $P = 0{\cdot}0012$, unpaired t-test).

P12

Exome sequencing and network analysis identifies two candidate mutations in patients with antitumour necrosis factor therapy-induced palmoplantar pustulosis

J. Ellingford,[1] G. Black,[1] S. Bhaskar,[1] S. Williams,[1] A. Stevens,[2] A. Al-Sharqi,[3] R. Parslew,[3] H. Chinoy,[4] C.E.M. Griffiths[4] and R. Warren[4]

[1]*Manchester Centre for Genomic Medicine and* [2]*Centre for Paediatrics and Child Health, University of Manchester, Manchester, U.K.,* [3]*Department of Dermatology, Royal Liverpool and Broadgreen University Hospitals NHS Foundation Trust, Liverpool, U.K. and* [4]*The Dermatology Centre, Salford Royal NHS Foundation Trust, University of Manchester, Manchester, U.K.*

Antitumour necrosis factor (TNF) biologic therapies are highly effective in the treatment of rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriasis and inflammatory bowel disease (IBD). However, use of anti-TNF agents can, on occasion, be associated with *de novo* appearance of palmoplantar pustulosis (PPP). This study investigated the genetic basis of anti-TNF-induced PPP in four unrelated patients of European descent, with no prior history of psoriasis. PPP occurred after treatment with adalimumab in four patients (one RA, two IBD and one AS) at 48–49 years of age. Two discrete hypotheses were tested: (i) anti-TNF-induced PPP is a monogenic homogeneous disorder; and (ii) anti-TNF-induced PPP is a monogenic heterogeneous disorder. Whole-exome sequencing was performed on 3 μg of DNA extracted from peripheral blood samples of each patient, and data manipulated in Microsoft Excel to test explicitly the stated hypotheses. Ingenuity pathway analysis (IPA) and Cytoscape software packages were utilized to create a comprehensive network of molecules that interact with: (i) the genes thought to underpin PPP pathogenesis; (ii) the drug target (TNF-α); and (iii) genes whose expression is altered in response to adalimumab. Sanger sequencing experiments confirmed and tested for the presence of candidate mutations in all patient DNA samples. Two candidate mutations were identified in three of the four patients: intercellular adhesion molecule (ICAM)-1 c.1055C>T p.Pro352Leu, in two patients; and E-selectin (SELE) c.1723C>T p.Leu575Phe, in one patient. Network analysis used in complement to next-generation sequencing identified two missense variants that could underlie anti-TNF-induced PPP, and has opened up interesting avenues for further study.

 © 2014 British Association of Dermatologists

ICAM1 and SELE are adhesion molecules, induced by TNF-α and regulated by nuclear factor-κB, which control the migration of leucocytes across the vascular endothelial membrane. Interestingly, efalizumab – a recently discontinued antileucocyte function associated antigen-1 monoclonal antibody for psoriasis – inhibits leucocyte adhesion to vascular endothelium via ICAM1, and was effective in the treatment of PPP. Polymorphisms in vascular adhesion molecules have previously been associated with inflammatory and autoimmune disease, but more work is required to prove the role of these molecules in the pathogenesis of anti-TNF-induced PPP.

P13
Zinc finger nuclease-mediated modelling of *COL7A1* deficiency

C. Georgiadis,[1] D. Almarza,[1] A. Abdul-Wahab,[2] J. McGrath,[2] J. Harper,[1] A. Thrasher,[1] W. Qasim[1] and W.-L. Di[1]

[1]*Institute of Child Health, University College London, London, U.K. and* [2]*Guy's Hospital, King's College London, London, U.K.*

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genodermatosis with neonatal onset, characterized by severe blistering of the epidermis and tissue separation at the dermal–epidermal junction for which there is currently no cure. At the supramolecular level, anchoring fibrils composed of tightly bundled type VII collagen protein (Col7) heterodimers fail to assemble correctly due to mutations in the *COL7A1* gene. Here we report the use of zinc finger nucleases (ZFN) to create double-stranded (ds)DNA breaks in *COL7A1* as a means to generate human cell lines to model RDEB. A highly specific pair of ZFNs targeting *COL7A1* has been designed to correct genetically a plethora of mutations reported to occur in the gene. ZFNs are typically delivered via DNA or mRNA transfection but with relatively low efficiency in primary cells and even more so in keratinocytes. To improve delivery of these DNA-binding motifs, we have cloned both aspects of the ZFN pairs into a nonintegrating lentiviral vector (NILV). The integrase-deficient lentiviral vector platform was selected as our preferred mode of delivery for the high transduction efficiency that can be achieved in both dividing and quiescent cells while maintaining a broad host cell tropism. Most importantly, the vector remains in an episomal form where it is transiently expressed leaving no viral footprint and therefore greatly reducing the risk of insertional mutagenesis, ideal for ZFNs whose nuclease activity is only required temporarily. We have tested the efficiency of transduction using the ZFN-carrying NILVs in keratinocytes resulting in a notable 25% infection and proceeded to assess the rate of *COL7A1* gene knockout. Clonal expansion revealed 20·8% monoallelic (heterozygous) and 4.2% biallelic (homozygous) disruption of the gene. Col7 expression, confirmed by both Western blot and ICC, showed a marked reduction of 50% in cells with heterozygous and 100% in cells with homozygous gene editing. Furthermore, functional assessment using an *in vitro* wound healing scratch assay showed a similar trend to what is seen in cells of patients with RDEB with an impaired migration rate of 10% and 26% in cells with monoallelic and biallelic ZFN-mediated knockout, respectively. Work is currently ongoing for the implementation of our ZFN gene-editing strategy in patient fibroblasts and mesenchymal stem cells. Next we will attempt to correct Col7 expression in these engineered cell lines, by lentiviral gene transduction, and by targeted homologous recombination using gene repair templates.

P14
Heterozygote *IL36RN* mutations in a European case of early-onset generalized pustular psoriasis challenge the concept of private mutation

N. Rajan,[1] N. Sinclair,[1] H. Nakai,[2] Y. Shimomura[2] and S. Natarajan[3]

[1]*Newcastle University, Newcastle upon Tyne, U.K.,* [2]*Niigata University, Niigata, Japan and* [3]*James Cook University Hospital, Middlesbrough, U.K.*

Mutations in the *IL36RN* gene have illuminated the understanding of the pathogenesis of generalized pustular psoriasis (GPP). Early-onset GPP has been associated with recessive mutations in *IL36RN*. Here we present a case of a 37-year-old, female, European patient with severe GPP starting at 6 months of age. We highlight the stormy nature of her clinical course, warranting multiple hospital admissions, potent immunosuppressive agents and flares during pregnancy. Strikingly, the use of antitumour necrosis factor-α antibody treatment has produced a 7-year period of almost complete remission. Furthermore pustulation of the nailbeds ceased and nail regrowth was seen for the first time in 25 years. Mutation analysis of the *IL36RN* gene demonstrated germline compound heterozygous mutations at two loci (c.338C>T and c.368C>T), of which c.368C>T is unreported in European populations. Notably, mutations at c.368 that have been reported are thus far restricted to Japanese cases. We performed biochemical and computational modelling analysis of mutations at this interesting locus to determine if these changes had differential pathogenic effects. This revealed a reduction of expression of interleukin (IL)-36Ra protein when expressed in transfected cells. Luciferase reporter gene assays carrying the *IL8* promoter were performed, and the mutant protein was unable to repress IL-36γ-induced promoter activity. The effect of the c.368C>T mutation had a similar effect in both assays to that of a c.368C>G mutation, a previously reported Japanese mutation. We present this case to highlight the increasing molecular diversity in this condition and suggest that this locus is a mutational hotspot in this gene.

© 2014 British Association of Dermatologists

P15

A novel automated image analysis method for rapidly segmenting and quantifying cutaneous features

J. Selway,[1] O.S. Osman,[1] T. Ditommaso,[2] I. Smyth,[2] C. Lelliott,[3] J. White,[3] David Melvin,[3] S. Jassim[4] and K. Langlands[1]

[1]*Buckingham Institute of Translational Medicine, University of Buckingham, Buckingham, U.K.,* [2]*Department of Biochemistry and Molecular Biology, Nursing and Health Sciences, Monash University, Melbourne, Vic., Australia,* [3]*The Sanger Mouse Genetics Project, Wellcome Trust Sanger Institute, Hinxton, U.K. and* [4]*Department of Applied Computing, University of Buckingham, Buckingham, U.K.*

The Sanger Mouse Genetics Project is a high-throughput functional genetic screen, allowing the systematic investigation of the relationship between genes and disease. The aim is broadly to assess the consequences of inactivating every mammalian gene in a range of tissues and developmental stages. Our focus is on developing unsupervised (and so high-throughput) image analysis methods to evaluate any impact on the skin in these mutant mice. The ability to extract a defined set of characteristics from images of haematoxylin and eosin-stained skin sections will yield considerable insight into cutaneous pathology. For example, a number of disorders promote defects in keratinocyte proliferation and maturation, which may lead to epidermal thickening (such as in psoriasis), or keratosis. Other compartments may be impacted, seen in fibrosis or disorganization of the dermis, or in adiposity, which may be indicative of metabolic dysfunction. Using an iterative active contour image analysis technique, we were able to isolate epidermis, dermis and subcutis from digital images and quantify pathological features. Such features include acanthosis, hyperkeratinization, dermal attrition, inflammation and adipocyte size and number. We have validated our methods by comparison to manual measurements, and there are considerable advantages in time saving and reproducibility. To assess the effect of individual genes on cutaneous structure, we performed a stringent comparison between genetically matched wild-type animals and individual genotypes, calculating a relative range (RR) for each genetic background, with mean, median and 95% confidence intervals (CI) determined. Individual genotypes were deemed to impact cutaneous morphology when at least 70% of the data points extracted for a feature were outside the RR 95% CI. To date we have analysed 554 phenotypes, from which we have identified approximately 2% that impact the various skin strata, which are currently undergoing validation in our lab. In conclusion, we describe a systematic approach to the evaluation of genes associated with cutaneous health. Inevitably, such genome-wide strategies will have a profound impact on our understanding of human disease and provide a new generation of therapeutic targets.

P16

A novel mutation in *IL36RN* underpins paediatric pustular psoriasis

J. Ellingford,[1] G. Black,[1] T. Clayton,[2] M. Judge,[2] CE.M. Griffiths[2] and R. Warren[2]

[1]*Centre for Genomic Medicine, University of Manchester, Manchester, U.K. and* [2]*The Dermatology Centre, Salford Royal NHS Foundation Trust, University of Manchester, Manchester, U.K.*

The gene encoding the interleukin (IL)-36 receptor antagonist, *IL36RN*, binds and inhibits the proinflammatory activity of IL-1 family cytokines. Recent genetics research has classified loss-of-function mutations in *IL36RN* as a discrete class of pustular psoriasis, deficiency of the IL-36 receptor antagonist disorder (DITRA). This study investigated the genetic basis of pustular psoriasis in the children of two unrelated Pakistani families, with similar clinical presentation. The study directly assessed the mutation status of two genes: *IL36RN*; and IL-1 receptor antagonist, *IL1RN*. DNA was extracted from peripheral blood samples of affected children and nonaffected consanguineous parents of each family. The affected daughter and son of the first family presented with rapid onset of fever and sheets of inflamed studded pustules on their limbs and trunk at 18 months and 7 years of age, respectively. The elder two daughters in the second family first presented, aged 18 months and 2 months, with exfoliative erythroderma and palmoplantar pustulosis, which progressed to frequent bouts of migrating pustular psoriasis in both children over a 5-year period. Polymerase chain reaction and direct sequencing was performed on the DNA of the affected children and unaffected parents for all the known coding exons of *IL1RN* and *IL36RN*. Sequence traces were analysed using the Staden and Mutation surveyor software packages. A novel homozygous missense variant in *IL36RN*, c.62T>C p.Leu21Pro (NM_173170.1), present in an exon harbouring other disease-causing mutations was found in both affected children of the first family studied, with a heterozygous genotype in the unaffected parents. This variant has not been reported previously and is predicted to have a deleterious impact on the structure of *IL36RN* by Condel meta-analysis *in silico* prediction software. In the absence of functional evidence, we cautiously define the *IL36RN* c.62T>C p.Leu21Pro variant as a 'likely pathogenic' mutation, and suggest the patient diagnosis be redefined to DITRA. In contrast, homozygous or *de novo IL1RN* or *IL36RN* genomic variants were not found in the affected children of the second family. This study presents a novel mutation in *IL36RN* in two children that has implications for the nosology of pustular disease and will inform therapy and genetic counselling. *IL36RN* c.62T>C p.Leu21Pro adds to an emerging array of mutations in *IL36RN* underpinning autoinflammatory disorders, and should be a target for future functional work. This study also illustrates the complex heterogeneity that exists in patients with similar clinical presentations of autoinflammatory disease.

© 2014 British Association of Dermatologists

P17

Increased p62 expression in *CYLD*-defective tumours: indication of an autophagic dependency?

H.Y. Tan,[1] N. Kirkham,[2] P. Lovat[1] and N. Rajan[1]
[1]*Newcastle University, Newcastle upon Tyne, U.K. and* [2]*Royal Free Hospital, London, U.K.*

Exploiting dependency on survival processes in cancer cells is a route to discovering novel therapeutic approaches. The recent discovery of autophagy as a prosurvival mechanism in most advanced-stage cancers has highlighted its potential targeting as a therapeutic strategy. Expression of certain proteins that reflect active autophagic processes may allow selection of certain tumours for exploration of deregulated autophagy promoting cancer cell survival. Patients who inherit germline mutations in the tumour suppressor gene *CYLD* develop multiple skin tumours that may represent one such skin tumour model. Recent molecular characterization of these tumours has highlighted the expression of proteins such as p62, a protein whose biphasic expression in other skin cancers is associated with the paradoxical role of autophagy in cancer (Ellis RA, Horswell S, Ness T *et al*. Prognostic impact of p62 expression in cutaneous malignant melanoma. *J Invest Dermatol* 2014; in press). These tumours thus represent an attractive model to explore autophagy as careful genetic characterization has highlighted truncating mutations in *CYLD* as the only demonstrable genetic change seen. A semiquantitative immunohistochemistry assay was applied to evaluate p62 expression across multiple *CYLD*-defective tumours, exhibiting both organized (cylindroma) and disorganized (spiradenoma) phenotypes. Results demonstrated increased p62 expression in tumour cells compared with perilesional tissue ($n = 18$), which significantly correlated with increased tumour organization (Pearson's $r = 0{\cdot}49$, $P = 0{\cdot}04$). These preliminary data thus suggest autophagic activity is altered in *CYLD*-defective tumours, and may represent a tumour dependency. Further study of the role of autophagy in this skin tumour syndrome will inform how best to harness autophagy modulation for therapeutic benefit.

P18

Engineering a model system for ichthyosis with confetti

M. Aushev,[1] P.H. Itin,[2] J. Reichelt[1] and B. Burger[2]
[1]*Newcastle University, Newcastle upon Tyne, U.K. and* [2]*University Hospital, Basel, Switzerland*

Ichthyosis with confetti (IWC) is a rare genodermatosis. Patients affected by IWC are born with a generalized scaly erythroderma. During childhood they develop patches of normal skin in a confetti-like pattern. This type of ichthyosis is caused by heterozygous frameshift mutations in the keratin 10 gene (*KRT10*) resulting in an arginine-rich C-terminus of the aberrant keratin 10 protein (K10). The normal areas develop as a consequence of a gene conversion or mitotic recombination, which leads to a loss of heterozygosity (LOH) at chromosome 17q where the mutated *KRT10* allele is replaced by a copy of the wild-type allele. The exact mechanism underlying the progressive revertant mosaicism is currently unknown. Due to the rareness of IWC it is difficult to receive enough skin biopsies for studying the mechanism of this 'natural gene therapy'. Transcription activator-like effectors (TALEs) are bacterial transcription factors that contain specific DNA-binding domains. These domains are composed of modules, each of which binds to one specific nucleotide. Recently, it was shown that TALE proteins also function when fused to a *FokI* nuclease domain, making the resulting TALE nucleases (TALENs) a perfect tool for genome editing. We engineered *KRT10*-specific TALENs and targeted the K10 locus in an immortalized keratinocyte line and introduced a known IWC-causing frameshift mutation – thus creating a model system that can be used to investigate the mechanism of LOH in IWC.

P19

Identification of novel targetable signalling pathways in the inherited ichthyoses

P. Dewan,[1] H. Singh,[2] M. Donaldson,[3] A. Enright[2] and E. O'Toole[1]
[1]*Centre for Cutaneous Research, London, U.K.,* [2]*The EMBL-European Bioinformatics Institute, Cambridge, U.K. and* [3]*GlaxoSmithKline, Stevenage, U.K.*

The ichthyoses are a family of genetic skin disorders characterized by the accumulation of 'fish-like' scales resulting from a defective skin barrier. The severity of the individual disorders ranges from mild (as in ichthyosis vulgaris, which is strongly associated with atopic eczema) to having a major impact on quality of life (lamellar ichthyosis/congenital ichthyosiform erythroderma) to severe cases that can be life-threatening (harlequin ichthyosis). The past 10 years have seen considerable advances in our understanding of the genetic basis of the ichthyoses including the finding that mutations in *ABCA12* underlie harlequin ichthyosis and the discovery that mutations in filaggrin cause ichthyosis vulgaris. In the remaining autosomal recessive ichthyoses several genes, including *TGM1*, *ABCA12*, *ALOX12B*, ichthyin (*NIPAL4*), have been implicated. As the clinical appearance of many of the ichthyoses are similar despite diverse genetic defects all resulting in defective barrier formation we hypothesized that there may be common pathways involved. Robust siRNA knockdown was established of *FLG*, *STS*, *TGM1*, *ABCA12*, *ALOX12B* and ichthyin in both basal and differentiated primary keratinocyte cultures from the same genetic background. Differential gene expression profiling using RNASeq has been undertaken to identify common aberrant signalling pathways.

© 2014 British Association of Dermatologists

P20

Acetate, a principle metabolite of ethanol, potentiates proinflammatory responses to tumour necrosis factor-α/interleukin-17 in keratinocytes: a potential role for acetate in the pathogenesis of psoriasis

K. Wu,[1] M. Morse,[2] S. Kendrick,[1] M. Donaldson[2] and N. Reynolds[1]

[1]*Newcastle University, Newcastle Upon Tyne, U.K. and* [2]*GlaxoSmithKline, Stevenage, U.K.*

Epidemiological studies implicate alcohol as a risk factor for the development and exacerbation of psoriasis; one study found the relative risk of developing psoriasis was 1·73 in those who consumed ≥ 2·3 alcoholic drinks per week compared with those who did not consume alcohol. Patients with high alcohol intake also have a tendency towards more severe, inflamed psoriasis. However, the mechanism(s) by which alcohol might contribute to psoriasis pathogenesis remains unknown. In this study we examined the effects of acetate, one of the principle metabolites of ethanol, on the cellular metabolism and proliferation of primary human keratinocytes (KC) and responses to proinflammatory stimuli. KC were incubated with acetate (1 mmol L^{-1}, corresponding to a serum level seen in abusers of alcohol) for 7 days prior to stimulation with proinflammatory cytokines [tumour necrosis factor (TNF)-α and/or interleukin (IL)-17, both key cytokines in the pathophysiology of psoriasis], ultraviolet (UV) B or polyinosine polycytidylic acid [poly:IC, a synthetic analogue of double-stranded (ds)RNA and Toll-like receptor 3 agonist]. Forty-eight hours after stimulation effects on metabolism [measured by 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium-bromide (MTT) assay], proliferation/cytotoxicity measured by sulforhodamine B (SRB) assay and cytokine responses, including IL-6 and IL-8, were determined. Acetate potentiated KC IL-6 response to TNF-α plus IL-17 (approximately 55% enhancement compared with nonacetate-treated KC, $P < 0·01$, $n = 6$), but not to other stimuli. Acetate did not potentiate the IL-8 response. Acetate alone did not affect MTT nor SRB, but did impair the SRB endpoint in cells stimulated with TNF-α plus IL-17 (by approximately 30%, $P < 0·01$, $n = 6$), although the MTT endpoint was not significantly altered. Acetate's enhancement of IL-6 KC response to proinflammatory TNF-α plus IL-17 may be one of the explanations why alcohol predisposes patients to development or exacerbation of psoriasis. Previous work in macrophages suggests acetate potentiates proinflammatory responses by an epigenetic mechanism, through hyperacetylation of histones at promoter regions of target cytokine genes. We propose a similar mechanism may occur in KC; this may also explain how other exogenous factors implicated in psoriasis interact with the genome to influence the psoriasis phenotype.

P21

Microanatomical dissection of the human dermis: the three-dimensional relationship between leucocytes, lymphatics and blood vessels

X.-N. Wang,[1] N. McGovern,[1] M. Gunawan,[1] C. Richardson,[1] M. Windebank,[1] T.-W. Siah,[1] H.-Y. Lim,[2] K. Fink,[3] J.Y. Li,[3] L. Ng,[3] F. Ginhoux,[3] V. Angeli,[3] M. Collin[1] and M. Haniffa[1]

[1]*Newcastle University, Newcastle upon Tyne, U.K.,* [2]*National University of Singapore, Singapore and* [3]*Singapore Immunology Network, Singapore*

Dendritic cells (DCs), macrophages and T cells are major components of the skin immune system but their interstitial spatial organization is poorly characterized. Using four-channel whole-mount immunofluorescence staining of the human dermis we demonstrated the three-dimensional distribution of CD31+ blood capillaries, LYVE-1+ lymphatic vessels, discrete populations of CD11c+ myeloid DCs, FXIIIa+ macrophages and CD3+ lymphocytes. DCs formed the first dermal cellular layer [0–20 mm beneath the dermoepidermal junction (DEJ)], macrophages were located more deeply (40–60 mm) and CD3+ lymphocytes were observed throughout (0–60 mm). At < 30 mm beneath the DEJ, DCs were both perivascular and interstitial with macrophages and T cells being primarily perivascular. At > 150 mm beneath the DEJ, DCs, macrophages and T cells were perivascular in distribution. Whole-mount imaging revealed the true extent of dermal leucocytes, which was previously underestimated from cross-section views. The total area of apical dermis (0–30 mm) contained approximately 10-fold more myeloid DCs than the entire blood volume of an average individual. Using whole-mount dermal sheet immunofluorescence staining for lymphatic vessels, DCs and macrophages, we were able to visualize dermal DC migration into skin lymphatic vessels. Less than 1% of dermal DCs occupied lymphatics in freshly isolated skin. However, dermal DCs rapidly accumulated within lymphatics but macrophages remained fixed in skin explants cultured *ex vivo*. We also observed distortion of the leucocyte architecture in the lesional skin of patients with psoriasis, eczema, cutaneous T-cell lymphoma and graft-versus-host disease. These findings demonstrate the microanatomy of dermal leucocytes, lymphatics and blood vessels providing further insights into their functional organization.

P22

An investigation of neuroinflammation in the brains of patients with psoriasis: a [^{11}C]-(R)-PK11195 positron-emission tomography study

H.J.A. Hunter,[1] R. Hinz,[2] Z. Su,[2] P.S. Talbot,[2] A. Gerhard,[2] C.E.M. Griffiths[1] and C.E. Kleyn[1]

[1]*Dermatological Sciences, Manchester Academic Health Science Centre, University of Manchester, Manchester, U.K. and* [2]*Wolfson Molecular Imaging Centre, University of Manchester, Manchester, U.K.*

Psoriasis, a chronic inflammatory, immune-mediated, skin disease is now widely regarded as a systemic inflammatory condition. Recent animal studies have shown that peripheral inflammatory insults can result in central nervous system

 © 2014 British Association of Dermatologists

(CNS) neuroinflammation. Activated microglia, a hallmark of CNS neuroinflammation overexpress the translocator protein (TSPO), a cholesterol transporter. [^{11}C]-(R)-PK11195 binds specifically to the TSPO and can be visualized by positron-emission tomography (PET) neuroimaging. We performed a case-controlled cohort study, which, to our knowledge, is the first study in humans to investigate the coexistence of peripheral and central neuroinflammation in any systemic inflammatory condition. Twelve patients with psoriasis (11 men; mean age ± SD 39·3 ± 9 years, range 22–49) and 12 controls (7 men; age 33·6 ± 10 years, range 22–46) were recruited. All patients had moderate-to-severe chronic plaque psoriasis (mean ± SD Psoriasis Area and Severity Index score 17·73 ± 3.9, range 12·2–25·3) treated with topical therapy only. Participants underwent a structural magnetic resonance imaging (MRI) scan to aid in definition of the anatomical regions of interest (ROI) (temporal, frontal, occipital and parietal cortex, thalamus and brainstem), followed by a [^{11}C]-(R)-PK11195 PET scan. Parametric brain maps of [^{11}C]-(R)-PK11195-binding potential (BP_{ND}) were generated. BP_{ND} quantifies specific tracer binding in PET studies and in this study reflected neuroinflammation. Mean BP_{ND} for each ROI was extracted using a brain atlas. Participants with evidence of active infection or depression were excluded as these might have potentiated neuroinflammation. Peripheral inflammatory markers [high-sensitivity C-reactive protein, interleukin (IL)-6 and IL-8] were measured; these data were not available for six controls drawn from our historical control cohort (included to minimize radiation exposure to healthy volunteers). The mean levels of all peripheral inflammatory markers were greater in the psoriasis group compared with the controls but only reached significance (Mann–Whitney U-test) for IL-8 ($P = 0·04$). There was no significant intergroup difference (Mann–Whitney U-test) in mean [^{11}C]-(R)-PK11195 BP_{ND} for any brain ROI (range $P = 0·24–0·89$); however, it did vary between individuals within groups. In this study population the peripheral inflammation associated with plaque psoriasis is not associated with CNS neuroinflammation and warrants further investigation.

P23
Human β-defensin-3 activates Langerhans cells and reverses the suppressive effect of vitamin D3 on interleukin-23 expression in psoriasis

C. Sweeney,[1] G. Kelly,[1] A.-M. Tobin[2] and B. Kirby[1]

[1]*St Vincent's University Hospital, University College Dublin, Dublin, Ireland and* [2]*Adelaide and Meath Hospital, Dublin, Ireland*

Psoriasis is an immune-mediated inflammatory skin disorder. Interleukin (IL)-23 plays a central role in psoriasis by directing the development of T helper (Th) 17 cells, which contribute substantially to disease. β-Defensins are small antimicrobial peptides that are chemotactic for a variety of immune cells and can participate in the initiation of the adaptive immune response. The expression of human β-defensin-3 (hBD3) is enhanced in psoriasis and it has been reported that hBD3 induces the maturation of dendritic cells (DC) and other innate immune cells by binding to Toll-like receptor (TLR)-2. However, the effect of hBD3 on DC function in psoriasis is unknown. We report that hBD3 enhanced the expression of TLR2 and induced IL-23 production by Langerhans cells (LC). The serum expression of hBD3 was enhanced in psoriasis. Both epidermal and highly purified monocyte-derived LC from patients with psoriasis produced significantly more IL-23 in response to phorbol myristate acetate and zymosan activation, respectively, compared with LC from healthy controls ($P < 0·01$). Moreover, while vitamin D_3, [1,25-dihydroxyvitamin D_3; 1,25$(OH)_2D_3$] suppressed IL-23 by epidermal and monocyte-derived LC from healthy controls ($P < 0·01$) via IL-10 ($P < 0·05$), addition of hBD3 abrogated this suppressive effect ($P < 0·01$). In contrast, 1,25$(OH)_2D_3$ failed to regulate IL-23 production by epidermal and monocyte-derived LC from patients with psoriasis. hBD3 inhibited the suppressive effect of 1,25$(OH)_2D_3$ in psoriasis as neutralization of hBD3 enhanced the suppressive effect of 1,25$(OH)_2D_3$ on IL-23 production by LC from patients with psoriasis ($P < 0·05$). This study suggests that hBD3 plays a pathogenic role in psoriasis by promoting the expression of IL-23 by LC and by interfering with the ability of 1,25$(OH)_2D_3$ to regulate its expression.

P24
Human endothelial cells show immune-modulating function by supporting CD4+ T-cell proliferation and enhancing regulatory T-cell suppressive function

W.C. Lim, E. Healy and T. Millar

University of Southampton, Southampton, U.K.

FOXP3+ regulatory T cells (Tregs) are immune suppressors that maintain peripheral tolerance, failure of which contributes to immune disorders. The vasculature is an important regulator of inflammatory cell recruitment with the endothelial cell (EC) performing a pivotal role. By expressing receptors and counter receptors, interactions between Tregs and ECs may provide signals that regulate the function of Tregs. Therefore, we investigated the ability of human ECs to influence Treg populations and modulate their suppressive activity. CD4+ T cells were cocultured with human ECs in the presence of either phytohaemagglutinin (PHA) or stimulatory anti-CD3/CD28 antibodies for 72 or 120 h. T-cell proliferation (CFSE dilution) and phenotype (CD25, FOXP3) were assessed using flow cytometry. In the absence of accessory cells, CD4+ T cells showed no proliferation in response to PHA or anti-CD3/28. In the presence of ECs and stimulus (PHA or anti-CD3/28), CD4+ T cells showed a robust proliferation by 72 h ($P < 0·0001$, $n = 10$); 60–80% of proliferated cells were FOXP3+ ($P < 0·05$, $n = 4$), which decreased to approximately 20% at 120 h ($P < 0·05$, $n = 4$). Prior stimulation of EC with interferon (IFN)-γ or tumour necrosis factor (TNF) caused modest increases in proliferation of CD4+ cells by 120 h in the absence of PHA or anti-CD3/28 ($P < 0·01$, $n = 10$). However, IFN-γ- or TNF-stimulated ECs did not enhance T-cell proliferation or FOXP3 + expression in the presence of

© 2014 British Association of Dermatologists

PHA or anti-CD3/28 above that caused by untreated ECs. To evaluate the ability of EC to modulate Treg suppressive capacity, CD4+ CD25hiCD127low Tregs were cocultured with human ECs for 24 h, then recovered and plated with effector T cells (Teffs). Teff proliferation was measured at 72 h using flow cytometry. EC-modified Tregs showed significantly increased suppressive capacity on Teff proliferation compared with control Tregs. The suppressive function was further enhanced following Treg coculture with IFN-γ-stimulated ($P = 0{\cdot}02$, $n = 8$) or TNF-stimulated ECs ($P = 0{\cdot}03$, $n = 8$). Human ECs are capable of enhancing CD4+ T-cell proliferation and increasing the ability of Tregs to suppress Teff function. The modulation of Treg function by ECs suggests that human EC–T-cell interactions may offer a novel target for Treg control in inflammation.

P25
Inhibition of autophagy mediates apoptosis of skin homing memory CD4+ T cells in atopic dermatitis

C.Y. Ung, M.E. Polak, A. Lee and M.R. Ardern-Jones
University of Southampton, Southampton, U.K.

Atopic dermatitis is characterized by chronic inflammation mediated by T-cell activation in the skin. While exogenous environmental allergens are important in disease pathogenesis, the role of autoreactive T cells is being increasingly considered. Autophagy is a cell survival mechanism that is utilized under conditions of cellular stress such as starvation. Altered autophagy regulation leading to persistence of autoreactive cells has been implicated in other autoimmune conditions such as systemic lupus erythematosus. To investigate this in atopic dermatitis we examined the autophagic dependence of skin-homing memory T cells in patients and controls. Peripheral blood mononuclear cells of patients with atopic dermatitis and healthy controls were cultured in the presence of the autophagy inhibitor chloroquine for 18 h and assessed by flow cytometry. As expected, we found a greater baseline frequency of cutaneous lymphocyte-associated antigen (CLA) expression on memory CD4+ T cells in patients with atopic dermatitis ($n = 8$, $P < 0{\cdot}005$) compared with healthy controls ($n = 6$). In patients, these cells undergo less apoptosis compared with nonskin-homing memory CD4+ T cells ($P < 0{\cdot}05$). Inhibition of autophagic flux with chloroquine led to a dose-dependent decrease in CLA expressing memory CD4+ T cells. This inversely correlated with apoptosis ($P < 0{\cdot}05$) in the skin-homing fraction. The frequency of CLA+ peripheral T cells is known to correlate with disease severity in atopic dermatitis. We showed that chloroquine-induced inhibition of autophagy specifically decreased the fraction of skin-homing memory CD4+ T cells in patients with atopic dermatitis to frequencies comparable with healthy controls.

P26
Defining the lymphoid stress surveillance response in human skin

R. Woolf and A. Hayday
Peter Gorer Department of Immunobiology, King's College London, London, U.K.

The skin contains a large number of tissue-resident immune cells that have key roles in maintaining tissue integrity and excluding infection. One mechanism of such immune activation is lymphoid stress surveillance (LSS), whereby lymphocytes are rapidly activated via the NKG2D receptor when it engages stress ligands that are expressed by tissues following cellular stress. It is unclear if this capability exists in human skin, which is fundamental to understanding our interactions with environmental agents. To investigate this, we have characterized the human skin-resident lymphocyte compartment and assayed the cells' functional responsiveness, with a view to defining LSS as it pertains to human skin. Normal adult human skin was obtained as discarded material after cutaneous surgery. Protocols were established to isolate and characterize lymphocytes either by tissue disaggregation or by a refined version of a three-dimensional explant culture. Cell surface receptor expression and cytokine production by skin-resident lymphocytes were characterized by flow cytometry following activation under different conditions *in vitro*. Following *ex vivo* isolation distinct skin-resident lymphocyte subsets were identified, including the 'unconventional' lymphocytes γδ T cells and natural killer (NK) cells. Explant culture greatly increased lymphocyte yield, enabling detailed characterization. Skin-derived lymphocytes displayed skin-homing surface markers, a memory phenotype and high levels of the activation marker CD69. Lymphocytes differed in expression of costimulatory receptors, with CD8+ T cells, γδ T cells and NK cells expressing NKG2D. Skin-resident T cells also constitutively expressed the inhibitory receptor PD-1. Upon *in vitro* activation, skin-resident lymphocytes readily produced cytokines and displayed distinct cytokine-producing profiles, with the response dependent on the strength of T-cell receptor-mediated activation. NKG2D+ lymphocytes could be activated to produce interferon-γ rapidly following NKG2D receptor engagement under different circumstances. Skin-resident lymphocytes, including 'unconventional' populations, could be isolated from healthy human skin and maintain a 'tissue-resident memory' (T_{RM}) phenotype. These are novel observations regarding skin-resident γδ T cell and NK cells. Distinct NKG2D+ lymphocytes were identified, which were functionally competent under *in vitro* conditions that mimic tissue stress. This system provides a platform to characterize the innate-like response of human skin-resident lymphocytes to tissue dysregulation with implications for immunoprotection, inflammatory diseases and atopy.

 © 2014 British Association of Dermatologists

P27
Characterization of innate lymphoid cells (ILC) in human skin and blood reveals an increase of NKp44+ ILC3 in psoriasis

F. Villanova,[1] B. Flutter,[1] I. Tosi,[1] K. Grys,[1] H. Sreeneebus,[2] G.K. Perera,[3] A. Chapman,[4] C.H. Smith,[1] P. Di Meglio[1] and F.O. Nestle[1]

[1]*St John's Institute of Dermatology, King's College London, London, U.K.,* [2]*NIHR GSTT/KCL Comprehensive Biomedical Research Centre, Guy's and St Thomas' NHS Foundation Trust, London, U.K.,* [3]*Department of Dermatology, Middlesex University Hospital, London, U.K. and* [4]*Department of Dermatology, Queen Elizabeth Hospital, London, U.K.*

Innate lymphoid cells (ILC) are a recently characterized family of immune cells with emerging important roles in tissue immunity and remodelling. However, their presence and function in human skin homeostasis and disease remain to be completely clarified. In this study we characterized ILC in human skin from healthy individuals and from the inflammatory skin disease psoriasis. Moreover we investigated ILC subsets in the peripheral blood of patients with psoriasis compared with healthy volunteers and patients with atopic dermatitis (AD). We found that a considerable number of interleukin (IL)-17A- and IL-22-producing cells in skin and blood of healthy individuals and patients with psoriasis are CD3-negative innate lymphocytes. Detailed analysis of circulating ILC subsets demonstrated a significant increased frequency of NKp44+ ILC3 in blood of patients with psoriasis compared with healthy individuals or patients with AD. Furthermore circulating ILC expressed cutaneous lymphocyte-associated antigen indicating their potential for skin homing. Analysis of skin demonstrated a significant increase in the frequency of total ILC in skin compared with blood. Interestingly NKp44+ ILC3 frequency was significantly increased in nonlesional psoriatic skin compared with normal skin, indicating a potential role of such cells in initiating disease progression. Finally, we monitored circulating ILC during successful antitumour necrosis factor therapy in psoriasis detecting a close association between the frequency of circulating NKp44+ ILC3 and therapy response. Our data suggest a potential role for NKp44+ ILC3 in psoriasis pathogenesis.

P28
A comparative study of the transcriptional profiles of Langerhans cell histiocytosis and inflammatory disorders

P.E. Harikumar, J. Selway, A. Chu and K. Langlands

University of Buckingham, Buckingham, U.K.

Langerhans cell histiocytosis (LCH) is a rare and potentially fatal disorder of unknown aetiology, characterized by the abnormal accumulation of CD1a+ cutaneous Langerhans-like cells in various body sites, including the skin. Immunological dysregulation at the site of skin lesions may negatively impact surrounding tissue, as seen in the pathophysiology of inflammatory disorders. In this study, we hypothesized that comparing LCH gene expression profiles with inflammatory disorders such as psoriasis and atopic dermatitis (AD) would reveal the common processes in immune response and function. We profiled gene expression in cutaneous LCH biopsies and normal epidermal LCs, and compared these data with psoriasis and AD profiles retrieved from the Gene Expression Omnibus (GSE14905 and GSE16161, respectively). Having identified those transcripts discriminating normal from disease compartments (fold change $> 1{\cdot}5$, $P < 0{\cdot}05$), a combination of open access and proprietary software (such as MetaCore) was used to compare between disease groups. A total of 1357 genes were common between LCH and psoriasis, and 1015 between LCH and AD; 501 transcripts were common to all groups, from which pathway enrichment revealed perturbation of a number of immunological processes, including migration inhibitory factor-induced cell adhesion, migration and angiogenesis ($P = 1{\cdot}316 \times 10^{-3}$), interleukin (IL)-2 activation and signalling ($P = 1{\cdot}753 \times 10^{-3}$) and CCL2 signalling ($P = 2{\cdot}706 \times 10^{-3}$). Moreover, transcripts previously associated with psoriasis and AD were highlighted, notably AP-1, MKP-1, JAK3 and EGR1. Components of the AP-1 transcription factor complex critical in regulating cytokine responses (Jun and Fos) were also significantly altered in LCH compared with psoriasis and AD. Specifically, Fos expression increased fivefold in LCH and 12-fold in psoriasis with no change in AD, while JunD expression was upregulated in all three datasets. Also, network analysis showed multiple components interacting directly with AP-1 including IL-3, MEKK1, ERK1/2, H-Ras, STAT5, SMAD3 and SMAD4. These results implicate the AP-1 complex in the mediation of cytokine-stimulated gene expression in cutaneous LCH lesions, in common with psoriasis and AD. We are currently investigating the role of AP-1 and cytokine signalling in cutaneous LCH lesions and performing further studies to investigate the underlying aetiology of LCH.

P29
Regulation of interferon-λ production and response in the skin compartment

A. Alase,[1] M. Stacey,[2] R. Doble,[2] M. Goodfield[3] and M. Wittmann[1,4,5]

[1]*Centre for Skin Sciences, School of Life Sciences, University of Bradford, Bradford, U.K.,* [2]*Faculty of Biological Sciences, School of Molecular and Cellular Biology, University of Leeds, Leeds, U.K.,* [3]*Department of Dermatology, University of Leeds, Leeds, U.K.,* [4]*Division of Rheumatic and Musculoskeletal Disease, Leeds Institute of Molecular Medicine, LMBRU, University of Leeds, Leeds, U.K. and* [5]*Bradford Teaching Hospitals NHS Foundation Trust, St Luke's Hospital, Department of Dermatology, Bradford, U.K.*

Interferon (IFN)-λ1 is a type III IFN expressed by skin epithelial cells. It exhibits similar antiviral activities to type I IFN in susceptible cells such as keratinocytes. The involvement of IFN-λ1 in inflammatory skin diseases such as cutaneous lupus erythematosus has been described; however, its role within the skin cytokine network is still not well understood. The aim of this study was to understand better how IFN-λ1 production and responsiveness are regulated in tissue-resident cells. Human primary keratinocytes and dermal fibroblasts were stimulated with cytokines or nucleic acids. Protein

© 2014 British Association of Dermatologists

expression was determined using enzyme-linked immunosorbent assay and flow cytometry while mRNA expression was evaluated using quantitative reverse transcription-polymerase chain reaction. Our results show that IFN-λ1 is induced in keratinocytes by both TLR3 and RIGI/AIM2 ligands. Cytokines, including IFN-γ, IFN-α and tumour necrosis factor (TNF)-α did not induce IFN-λ1 protein production; however, only IFN-γ markedly enhanced nucleic acid-induced IFN-λ1 production in keratinocytes. Unexpectedly, dermal fibroblasts were found constitutively to produce IFN-λ1, which was upregulated upon stimulation with IFN-α or poly dA:dT. In comparison with keratinocytes, fibroblasts produced much lower levels of IFN-λ1, which were markedly suppressed by TNF-α. In keratinocytes, TNF-α was found to downregulate the expression of IFN-λ receptor (IFNλR1) and to alter the functional response to IFN-λ1 (expression of CXCL10, MxA, IFI16 and GBP-1 to IFN-λ1). Our results highlight that TNF-α and IFN-γ are important regulators of IFN-λ1 production and responsiveness in the skin. These findings may help in better understanding the cutaneous side-effects associated with anti-TNF-α therapy.

P30
The role of PI3K-γ in regulation of cutaneous inflammation

J. Masapust,[1,2] C.Y. Ung,[1] N. Horlock,[3] E. Healy,[1] M.R. Ardern-Jones[1] and M.E. Polak[1]
[1]*Clinical and Experimental Sciences, Sir Henry Wellcome Laboratories, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, U.K.,* [2]*Department of Immunology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland and* [3]*Department of Plastic Surgery, Salisbury NHS Trust and Spire Southampton Hospital, Southampton, U.K.*

PI3K-γ (phosphoinositide 3-kinase) is an enzyme involved in cell proliferation, growth and survival. PI3K-γ is important in the induction of allergic inflammation, and PIK3CG-deficient mice are resistant to ovalbumin-induced asthma. We undertook whole-genome microarray analysis of human Langerhans cells (LCs) stimulated by tumour necrosis factor (TNF)-α and thymic stromal lymphopoietin, which showed that PIK3CG is upregulated in LCs during induction of Th2 polarization. Here we wanted to test the suitability of PI3K-γ as a target for anti-inflammatory interventions in human skin. Human skin migratory LCs (smiLCs) were incubated with the PI3K-γ-specific inhibitor AS605240. We tested the viability, migratory abilities and phenotype of smiLC treated with a range of inhibitor concentrations, 0·1–10 μmol L^{-1}. The inhibitor did not affect LC viability, as assessed by trypan blue exclusion and annexin V staining. PI3K-γ inhibitor reduced the number of cells migrating from epidermal sheets during 48 h of culture and decreased expression of costimulatory molecules (CD70 and CD86, n = 3) on the surface of LCs as well as their ability to produce reactive oxygen species (n = 2) in a dose-dependent manner. To assess how PI3K-γ regulates LC function and ability to polarize towards either T helper (Th) 1 or Th2, smiLCs were incubated with peripheral blood mononuclear cells (PBMCs) for 5 days following PI3K-γ inhibition. PI3K-γ inhibition with low concentration of the inhibitor (0·1 μmol L^{-1}) in LCs resulted in increased Th1 stimulatory capacity as measured by IFN-γ release using the ELISpot assay (n = 6, P = 0·0069), whereas higher concentrations (10 μmol L^{-1}) decreased T-lymphocyte activation. This dose-related effect was also found when LCs were pretreated either with 0·1 or 10 μmol L^{-1} AS605240 before assay of T-cell proliferation (n = 2, CFSE dilution assay). PI3K-γ inhibition modulates LC activatory status and potential to stimulate and polarize T cells. Our data have shown that high and low concentrations of the inhibitor have contrasting effects. These data may suggest that PI3K-γ is multifunctional and important in counter-regulatory loops. Further work is required to elucidate fully the pathways involved but PI3K-γ activity shows potential as a target for manipulation in inflammatory skin disease.

P31
Role of T helper 17 cells in allergic skin diseases

D. Pennino,[1] K. Eyerich,[2] F.O. Nestle[1] and A. Cavani[3]
[1]*King's College London, London, U.K.,* [2]*Technical University of Munich, Munich, Germany and* [3]*Istituto Dermopatico Dell'Immacolata (IDI), Rome, Italy*

T helper (Th) 17 cells are key players in psoriasis. However, their contribution in allergic skin diseases is not fully understood. Our aim was to evaluate the function of Th17 cell subsets in the context of Th1 (allergic contact dermatitis, ACD) and Th2 (atopic dermatitis, AD) dominated skin environments. Blood and skin samples were collected from patients affected with ACD and AD. Skin T cells and T-cell clones were isolated from involved skin after the application of the contact allergen nickel or the atopy-associated house dust mite antigen Der p 1 on the skin of sensitized patients. Th cell subsets infiltrating inflamed skin were characterized by flow cytometry and enzyme-linked immunosorbent assay. The most frequent Th17 cell subsets infiltrating the skin of patients coproduced interferon (IFN)-γ in ACD, while they coproduced interleukin (IL)-4 in AD. In the IFN-γ-dominated ACD skin environment, IL-17 promoted antigen-independent T cell-mediated tissue damage by increasing the expression of ICAM-1 on primary keratinocytes adding to our knowledge on mechanisms of spongiosis in ACD. In the IL-4-dominated AD skin environment, IL-17 induction of β-defensin-2 in keratinocytes was impaired, providing a mechanistic explanation for decreased levels of antimicrobial peptides in AD. The inflammatory tissue environment and cosecretion of cytokines determines the outcome of IL-17 function in skin tissue and impacts on well-known features of tissue pathology in ACD and AD.

 © 2014 British Association of Dermatologists

P32

Environmental factors transmitted by the aryl hydrocarbon receptor influence the severity of psoriatic inflammation

P. Di Meglio,[1] J.H. Duarte,[1] H. Ahlfors,[1] N.D.L. Owens,[1] Y. Li,[1] F. Villanova,[2] I. Tosi,[2] K. Hirota,[1] F.O. Nestle,[2] U. Mrowietz,[3] M.J. Gilchrist[1] and B. Stockinger[1]

[1]*MRC National Institute for Medical Research, London, U.K.,* [2]*St John's Institute of Dermatology, King's College London, London, U.K. and* [3]*University Medical Center Schleswig-Holstein, Kiel, Germany*

Psoriasis is a chronic inflammatory skin disease resulting from the interaction of genetic and environmental factors. Crosstalk between innate, adaptive and epithelial cells underpins the pathological response in this disease. More than 40 disease-associated loci have been identified that contribute to psoriasis. Environmental risk factors on the other hand remain less well defined on a mechanistic basis. Here we show that the aryl hydrocarbon receptor (AhR), a transcription factor that senses environmental stimuli, modulates pathology in psoriasis. AhR-activating ligands reduced inflammation in the lesional skin of patients with psoriasis, whereas AhR antagonists increased inflammation. Similarly, AhR signalling via the endogenous ligand FICZ reduced the inflammatory response in the imiquimod (Aldara®)-induced model of psoriasiform skin inflammation and AhR-deficient mice exhibited a substantial exacerbation of the disease, compared with AhR-sufficient controls. Nonhaematopoietic cells, in particular keratinocytes, were responsible for this hyperinflammatory response, which involved increased reactivity to interleukin-1β and upregulation of AP-1 family members of transcription factors. Thus, our data suggest a critical role for AhR in the regulation of inflammatory responses and open the possibility for novel therapeutic strategies in chronic inflammatory disorders.

P33

A platform for discovery, cloning and expression of melanoma reactive antibodies

K. Ilieva, I. Correa, P. Karagiannis, T. Dodev, A. Gilbert, F.O. Nestle and S. Karagiannis

King's College London, London, U.K.

Monoclonal antibodies are an important therapeutic modality used for the clinical management of many cancers. The first antibody for the treatment of the most lethal skin cancer, melanoma, was recently approved (ipilimumab), although it is only effective in subsets of patients. Despite this development, the treatment of melanoma remains an area of unmet clinical need. Melanoma is widely thought to be immunogenic with reported clinical cases of spontaneous regressions and remissions. We have focused on B cell-derived antibodies from patients and previously demonstrated higher frequency of tumour-reactive humoral immune responses in patients with cancer compared with healthy controls. After encountering antigens, B cells undergo somatic hypermutation, resulting in expression and secretion of affinity-matured antibodies recognizing these antigens. We have reported that lymphoid infiltrates in tumour lesions include antibody-secreting B cells, and we have detected tumour-specific antibodies in the blood and in melanoma tissues. Here, we describe a platform for the discovery of novel melanoma-specific antibodies from patient-derived B cells. Our approach encompasses screening and selection of single B cells from peripheral blood samples of patients and isolation of tumour antigen-reactive B-cell clones by flow cytometric sorting using fluorescent beads coated with tumour antigens. Single mature memory B cells with B-cell receptors (BCR) specific for tumour antigens are conjugated to fluorescent beads and are directly sorted on microwell plates. Variable heavy and light chains are retrieved by single cell reverse transcription-polymerase chain reaction and cloned in a dual expression vector. Antigen-reactive recombinant antibodies are produced using a HEK293 mammalian cell expression platform. This system is optimized for the expression of any human or murine V gene with any human C gene, thus enhancing the transfer from large libraries of cloned V genes to the analysis of both antigen binding and antibody effector functions. Antibodies produced in culture supernatants are purified through affinity chromatography and subsequently tested for their antigen-binding properties and capacity to activate immune effector cells against cancer cells *in vitro*. The therapeutic potential of the most promising candidates is evaluated *in vitro* in melanoma model systems and assays. This technology can help identify new diagnostic and therapeutic antibodies for the treatment of cancer.

P34

Regulation of interleukin-18 binding protein, a critical anti-inflammatory cytokine in health and infectious disease

M. Wittmann,[1,2] R. Doble,[3] C.W. Wasson,[3] K.H. Richards,[3] M. Haider,[3] M. Bachmann,[4] J. Pfeilschifter,[4] T. Werfel,[5] H. Muehl[4] and A. Macdonald[3]

[1]*Leeds Institute of Molecular Medicine, LMBRU, University of Leeds, Leeds, U.K.,* [2]*Centre for Skin Sciences, School of Life Sciences, University of Bradford, Bradford, U.K.,* [3]*Faculty of Biological Sciences, School of Molecular and Cellular Biology, University of Leeds, Leeds, U.K.,* [4]*Pharmazentrum Frankfurt/ZAFES, University Hospital Goethe-University Frankfurt, Frankfurt, Germany and* [5]*Division of Immunodermatology and Allergy Research, Hannover Medical School, Hannover, Germany*

Interleukin (IL)-18 is an important mediator involved in chronic inflammatory conditions such as cutaneous lupus erythematosus, psoriasis and chronic eczema. An imbalance between IL-18 and its endogenous antagonist IL-18 binding protein (BP) may account for increased IL-18 activity. In addition, the critical regulatory role of IL-18BP in maintaining epithelial inflammatory homeostasis is also utilized by viruses to aid immune evasion. IL-27 is a cytokine with dual function displaying pro- and anti-inflammatory properties. Here we provide evidence for an anti-inflammatory mode of action on skin-resident cells. Human keratinocytes and surprisingly also fibroblasts show a robust, dose-dependent and highly inducible mRNA expression and secretion of IL-18BP upon IL-27

© 2014 British Association of Dermatologists

stimulation. The production of IL-18BP peaked between 48 and 72 h after stimulation and was sustained for up to 96 h. Investigation of the signalling pathway showed that IL-27 activates STAT1 in human keratinocytes and that a proximal GAS site at the IL-18BP promoter is of importance for the functional activity of IL-27. Our analysis also demonstrated that human papillomavirus (HPV 6B/11, 16 and 18) was able to activate the STAT1 pathway to increase expression of IL-18BP in keratinocytes. Augmented IL-18BP expression was dependent on the E7 oncoprotein of HPV and was mapped to a region in the carboxyl terminus of E7. As HPV establishes a chronic infection, increased expression of IL-18BP may be a novel method of immune evasion. The data support a significant anti-inflammatory effect of IL-27 on skin-resident cells. The data also provide an insight into the possible mechanism of the virulence of E7 in its ability to evade the immune response of the cells it has infected.

P35
It takes two to tango: targeting STAT3 dimerization for treating psoriasis

T. Eichhorn,[1] M. Bell,[1] G. Eitzen,[1] J. Fenton,[1] C. Naylor,[1] A. Woodland[1] and W.H.I. McLean[2]

[1]*Drug Discovery Unit and* [2]*Division of Molecular Medicine, University of Dundee, Dundee, U.K.*

The STAT(signal transducer and activator of transcription) 3 protein is a cytosolic transcription factor, which is activated by Janus kinases (JAK) through phosphorylation of Tyr705 in the transactivation domain of STAT3 monomers leading to dimerization and translocation into the nucleus. STAT3, is a regulator of the interleukin (IL)-23/IL-17 signalling pathway, which induces differentiation of T helper (Th) 17 cells leading to the transcription of key psoriasis cytokines. Immunohistochemistry with an anti-STAT3 antibody revealed that epidermal keratinocytes in psoriatic lesions show an intense STAT3 signal in the nuclei. Therefore, targeting STAT3 may lead to a therapy for psoriasis. We have developed a cell-based assay using primary keratinocytes and performed high-content screening measuring the translocation of STAT3. Downstream, hit molecules will be validated by measuring the binding on recombinant STAT3 protein.

P36
Factor XIIIa identifies macrophages and cells of mesenchymal origin in the human skin

K. Aljefri,[1] X.-N. Wang,[2] A. Husain,[1] C. Bacon,[1] A. Long,[1] S. Pagan,[2] M. Collin[2] and M. Haniffa[2]

[1]*Newcastle upon Tyne NHS Trust, Newcastle upon Tyne, U.K. and* [2]*Newcastle University, Newcastle upon Tyne, U.K.*

Factor XIIIa (FXIIIa) or fibrin stabilizing factor is a transglutaminase that was initially described to characterize dermal dendritic cells (DCs)/dendrocytes in healthy skin and AIDS-associated cutaneous Kaposi sarcomas. These initial studies have led to the current use of antibody staining against FXIIIa to identify dermal DCs in clinicopathological analysis. The observation of FXIIIa on migratory dermal DCs and peripheral blood monocyte-derived DCs consolidated the evidence for its utility as a DC antigen, but more recently, FXIIIa expression was described on CD163+ dermal macrophages rather than DCs questioning its validity as a marker of dermal DC. In addition, FXIIIa expression has also been shown to characterize benign fibrohistiocytic lesions such as dermatofibromas, but the precise identity of these cells and how they relate to dermal DCs/dendrocytes remain uncertain. We analysed the expression of FXIIIa in human skin by immunohistochemical staining, four-parameter immunofluorescence microscopy analysis of whole-mount dermal sheets and real-time quantitative polymerase chain reaction of FXIIIa transcripts within purified dermal cell populations. We observed highest intensity of FXIIIa protein and transcript expression by dermal macrophages, which contained dense cytoplasmic melanin granules on haematoxylin and eosin staining and by cytospin analysis. Whole-mount dermal sheet microscopy revealed the spindle-shaped FXIIIa dermal macrophages *in situ* in keeping with the morphology observed in cross-section skin immunostained for FXIIIa. We also examined the lineage of FXIIIa-expressing cells in pathological lesions characterized by FXIIIa expression, such as dermatofibromas and cutaneous Kaposi sarcomas. In contrast to dermal macrophages, DCs had low-to-negative FXIIIa expression, expressed the integrin CD11c and were circular shaped *in situ*. Dendritic projections were more apparent on cells prepared on cytospins. We also validated the use of CD11c immunohistochemical staining to define DCs in healthy and diseased skin. Our findings confirmed that (i) FXIIIa identifies dermal macrophages but is also promiscuously expressed by mesenchymal-derived cells in disease and (ii) CD11c outperforms FXIIIa as a reliable antigen to identify dermal DCs in human skin and could be used in routine clinicopathological analysis.

P37
Effect of organic osmolytes on tight junction function in human keratinocytes

C. El Chami,[1] I. Haslam,[1] M. Steward[2] and C. O'Neill[1]

[1]*Faculty of Medical and Human Sciences and* [2]*Faculty of Life Sciences, University of Manchester, Manchester, U.K.*

The preservation of water is critical for terrestrial organisms and the epidermis is a major permeability barrier to water loss from within the body. Epidermal barrier function is a function not only of the stratum corneum (the tough outer layer of skin that consists of terminally differentiated cells embedded in a lipid matrix), but also of tight junctions. These are multi-protein complexes existing between living keratinocytes in the stratum granulosum and are known to impede water loss. However, cellular mechanisms of water conservation such as osmolyte accumulation are also important, particularly to help maintain cell volume during cellular stress. Cellular mechanisms of water homeostasis are largely unexplored in skin and additionally, nothing is known regarding how cellular and extracellular mechanisms may interact. The aim of this study was to investigate the role of organic osmolytes in the control of tight junction function (using measurements of transepithe-

© 2014 British Association of Dermatologists

lial electrical resistance, TEER) in normal human epidermal keratinocytes (NHEK) in the presence or absence of ultraviolet irradiation (UVR), a major source of water loss in skin. Irradiation of cells with a 15 mJ cm^{-2} dose of UVR resulted in a decrease in TEER after 24 h. However, in the presence of 500 μmol L^{-1} of the organic osmolytes taurine or betaine, TEER values were preserved. Furthermore, treatment of nonirradiated cells with organic osmolytes resulted in an increase in TEER. This preliminary study shows that organic osmolytes may modulate tight junction integrity and mitigate the damaging effect of UVR in NHEK. This may contribute to the barrier property of the epidermis especially during times of stress.

P38

Sulforaphane protects human hair follicles from oxidative damage-induced growth inhibition

I. Haslam,[1] G. Jenkins,[2] R. Bhogal,[2] N. Farjo,[3] B. Farjo[3] and R. Paus[1]

[1]*University of Manchester, Manchester, U.K.,* [2]*Unilever PLC, Colworth, U.K. and* [3]*Farjo Medical Centre, Manchester, U.K.*

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor that is often described as the 'master regulator' of cellular redox homeostasis. Compounds that activate Nrf2 are currently under investigation as potential therapeutic agents in a number of inflammatory conditions and pathologies in which redox imbalance is implicated, such as chronic kidney disease and neurodegenerative disorders. The role of Nrf2 in hair follicle (HF) biology is currently unknown, yet scalp skin and HFs are frequently exposed to numerous sources of oxidative stress. Moreover, we have found that human scalp HFs prominently express Nrf2 mRNA and protein. We therefore sought to examine the impact of Nrf2 activation on hair growth and defence against oxidative damage. Full-length human HFs were obtained following hair transplant surgery. HFs were pretreated with the known Nrf2 activator, sulforaphane, after which they were exposed to the oxidative stressor hydrogen peroxide (H_2O_2). The impact of sulforaphane treatment on proliferation and apoptosis was assessed by Ki67-TUNEL staining. Hair shaft-elongation measurements and hair cycle staging were also performed. These experiments revealed that pretreatment of hair follicles with sulforaphane ameliorated H_2O_2-stimulated increases in apoptosis and prevented the oxidative damage-induced reduction in hair matrix keratinocyte proliferation *in situ*. Sulforaphane pretreatment prevented H_2O_2-induced reductions in hair growth. These results indicate that the Nrf2 pathway is active in human scalp HFs and may play an important role in protecting the HF against redox insult. As such, stimulating intrafollicular Nrf2 activity is a promising strategy for protecting human HFs against oxidative damage.

P39

A simultaneous analysis of the host transcriptome and microbiome at the site of skin inflammation

G. Muirhead,[1] F.O. Nestle[2] and S. Tsoka[1]

[1]*Department of Informatics, School of Natural and Mathematical Sciences, King's College London, London, U.K. and* [2]*Division of Genetics and Molecular Medicine, St John's Institute of Dermatology, King's College London, London, U.K.*

Recently studies have suggested a role for commensal bacteria in the immunopathogenesis of inflammatory skin diseases such as psoriasis and atopic dermatitis. Consequently there has been an increasing appreciation for the importance of the microbiome as a factor in host health and disease. Despite correlative evidence supporting an association of the host and the microbiome, knowledge of the interactions between the microbiome and the host is limited. While the transcriptomic basis underlying psoriatic inflammation has been well studied, no methods currently exist to analyse both the microbiome and the host transcriptome simultaneously. Here we present an approach based upon the multivariate statistical method, sparse canonical correlation analysis (sCCA) to detect associations between resident microbiota and differentially expressed cytokine pathways of healthy, nonlesional and lesional samples of psoriasis and atopic dermatitis. Firstly, the variation in host gene expression and microbial communities was profiled for all disease groups. Secondly, repeated sCCA was performed in an attempt to detect relationships between genes and a core set of microbes identified though support vector machine attribute selection. Our results provide an insight into the integrated responses of the microbiome and host transcriptome at the site of inflammation. We show that between healthy, nonlesional and lesional patients, both the host transcriptome and microbiome are significantly variable. Furthermore, using our approach, we were able to uncover significant correlations that suggest a core set of microbiota play a key role in cytokine pathway-mediated inflammation. Overall our results provide further evidence for the important role of the crosstalk between the host and microbiome in inflammatory skin disease.

P40

Assessment of collagen dynamics in ageing using image analysis techniques

O.S. Osman,[1] J. Selway,[1] P. Harikumar,[1] S. Jassim[2] and K. Langlands[1]

[1]*Buckingham Institute of Translational Medicine, University of Buckingham, Buckingham, U.K. and* [2]*Department of Applied Computing, University of Buckingham, Buckingham, U.K.*

Herovici's polychrome staining was developed in the 1960s as an aid for visualizing skin collagen, and we have used this technique to evaluate collagen dynamics in a murine model of skin ageing. With this technique, papillary collagens tend to stain blue, particularly those fine fibres adjacent to the basement membrane, whereas courser reticular fibres tend to stain red. We observed a qualitative decrease in blue fibre staining with age, particularly with extreme age (i.e. mice at

© 2014 British Association of Dermatologists

20 months), a difference that we sought to quantify using standard image analysis with colour-based segmentation methods. In the first instance, we used simple colour thresholding to segment red or blue pixels according to constituent red (R), green (G) or blue (B) pixel values identified from exemplar regions as standard. However, interimage variation, manifested as subtle differences in hue, made it difficult to confirm an age-related change in staining patterns. We employed two strategies to mitigate this problem. Firstly, we applied preprocessing techniques to increase the dynamic range of images, as well as methods to reduce noise and enhance contrast. More importantly, we adapted an iterative algorithm to determine optimal pixel colour values in order to isolate and measure areas of red or blue chromaticity. Briefly, RGB images were converted into CIE L*a*b* colour space, and K-means clustering was performed to partition the data points into three clusters: blue, red and other. The pixels of an image were then individually assigned to the cluster with the closest centroid, before the centroids were recalculated, and the clustering process was repeated until all pixels were allocated and the groups stabilized. Our method allowed us to measure a statistically significant reduction in blue-staining collagen within ageing skin using a wholly unsupervised method (fold change 1·2, $P < 0{\cdot}05$, $n = 3$). The precise compartments identified by Herovici staining remain controversial. Some investigators postulate that it provides discrimination of type I and type III collagen, whereas others suggest that it discriminates nascent from mature fibres. While it is clear that the diminution of the blue compartment is a biomarker of ageing, we are currently investigating the biochemical target of Herovici's polychrome stain. We are also applying our image analysis approach to a quantifying a broader range of histological stains.

P41

Nanoscale analysis of tattoo ink in human skin *in situ* and in human dermal fibroblasts *in vitro* using atomic force microscopy

C. Grant,[1] P. Twigg,[1] R. Baker[2] and D.J. Tobin[2]

[1]*Advanced Engineering Materials, School of Engineering and Informatics and* [2]*Centre for Skin Sciences, School of Life Sciences, University of Bradford, Bradford, U.K.*

While many appreciate the allure of intricate ink patterns drawn by skilful tattoo artists, clients are often not informed of the potential ramifications of having relatively untested pigment (nano) particles injected into their skin. Indeed, it may come as a surprise to most that tattoo inks are not covered by the legislation that controls the ingredients of cosmetic products or medicines, and that tattoo salons are instead regulated only for infection control. Moreover, even tattoo ink manufacturers acknowledge that some inks in use may contain carcinogenic compounds, and in the weeks after tattooing pigments redistribute locally in the skin and a portion leaves the skin via the lymphatics and/or partially metabolized. Also different pigments are both chemically and structurally different. Given the recent explosion in large-surface tattooing among Britain's young (and not so young), it seems appropriate to re-evaluate how the skin responds to injected tattoo ink nanoparticles. Here, we visualized black ink particles in normal human dermis by histology and atomic force microscopy, as well as on human dermal fibroblasts *in vitro*. Light microscopy of tattooed human arm skin revealed large clusters of dark ink nanoparticle deposits in close association with deep dermal vessels and within associated cells. Atomic force microscopy showed how clusters of tattoo ink particles embed within collagenous networks in papillary and reticular dermis. Individual ink particles within the dermis were approximately 25 nm in height and approximately 60 nm across. However, when source ink nanoparticles were sized, a peak distribution size of 150 nm was revealed, suggesting some degree of processing of ink particles in the dermis. While, some particles were distributed on the surface of collagen bundles, others appeared embedded within collagen fibrils. Dermal fibroblast viability (MTT assay) was negatively affected at dilutions of ink lower than 1: 1000 and after just 1 week's exposure *in vitro*. This may give some insight into how undiluted concentrations of ink may affect dermal fibroblasts *in vivo*. Direct contact was seen between ink particles and the plasma membrane of fibroblasts, despite gentle cell washing, suggesting that these (nano)particles may interact directly with the cell surface. This preliminary study is beginning to reveal the nature of the interaction of the tattoo ink particles with cells of the human dermis, and is being followed up with more indepth studies on the potential functional implications.

P42

Drug discovery for dermatology: risky projects and revolutionary medicines

A. Woodland,[1] T. Nomura,[2] E. Warbrick,[1] U. Gartner,[1] S. McElroy,[1] G. Wood,[1] L. Mitchell,[1] P. Wyatt[1] and I. McLean[1]

[1]*University of Dundee, Dundee, U.K. and* [2]*Hokkaido University, Sapporo, Japan*

Over 25% of the U.K.'s population have a significant skin disease with 14% receiving a skin treatment at any one time, 15% of all consultations to general practitioners involving skin disease, and dermatological prescriptions second only to pain killers. Despite the high patient need, dermatology is an under-resourced area of research; however, the University of Dundee has established a critical mass of internationally competitive researchers in genetic skin disease and cutaneous therapy development. The Wellcome Trust strategic award for Dermatology and Genetic Medicine supports a core-funded drug discovery group based within the University of Dundee's, Drug Discovery Unit (http://www.drugdiscovery.dundee.ac.uk/), which seeks to bridge the gap between basic dermatology research and patients by developing high-quality chemical tools and drug candidates for unmet dermatological indications. One of the most advanced drug discovery projects has developed novel orally bioavailable stop codon read-through agents for the treatment of severe inherited diseases such as the skin fragility disorder *recessive* dystrophic epiderm-

 © 2014 British Association of Dermatologists

olysis bullosa. We will present our work to date on the read-through project and our other early-stage dermatology drug discovery projects.

P43
Vitamin D, nitric oxide and *cis*-urocanic acid differentially affect human systemic T-lymphocyte function implications for skin-resident T cells

C. Yu, C. Leitch, S. Howie, A. Astier and R. Weller
Centre for Inflammation, Edinburgh, U.K.

In the skin both naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells (nTreg) and immune response-induced type 1 regulatory cells (Tr1) are involved in preventing overexuberant inflammatory responses. Ultraviolet radiation (UVR) induces immune suppression in the skin and 1,25(OH)2D3 (vitD) induced in keratinocytes by UVR is suggested to be responsible for the suppressive effect, but other pathways may be involved. Urocanic acid (UCA), a key breakdown product of filaggrin, undergoes *trans* to *cis*-UCA isomerization on exposure to UVR. Murine studies suggest that the immunosuppressive function of *cis*-UCA is mediated by stimulating interleukin (IL)-10 production in CD4+ T cells. Nitric oxide (NO) is another immunoregulatory molecule that is induced by UVR in the skin. This induces a type of Treg which requires cell–cell contact and IL-10 for its suppressive function. Levels of vitD, *cis*-UCA and NO are increased both in the skin and the circulation by UVR and they may well all affect Treg induction locally and systemically. Systemic effects might be seen as modulation of molecules on T cells that direct their migration into skin. We thus determined the effects of these three molecules on T-cell activation, by stimulating purified CD4+ T cells with plate-bound anti-CD3/CD28 antibodies in the presence or absence of anti-CD46 antibodies that promote the induction of Tr1. Optimum concentrations were determined in preliminary experiments to be vitD (10^{-7} mol L^{-1}), *cis*-UCA (100 μg mL^{-1}) and the NO-donor SNAP (1–100 μg mL^{-1}). Presence of Foxp3+ Treg and expression of the cutaneous lymphocyte-associated antigen (CLA) were assessed by flow cytometry. IL-10, interferon (IFN)-γ and CCL4 secretion were analysed by enzyme-linked immunosorbent assay of cell culture supernatants. The percentage of Foxp3+ Treg was increased by vitD (1·7-fold in CD3/CD28-activated T cells and 1·6-fold in CD46-costimulated T cells) but reduced by *cis*-UCA (1·4-fold in CD3/CD28-activated T cells and 1·7-fold in CD46-costimulated T cells). All three molecules significantly decreased the percentage of CLA+ T cells. As expected, the IL-10 to IFN-γ ratio was increased by vitD in CD46-costimulated T cells, and a similar trend was observed with SNAP but was not affected by *cis*-UCA. Production of CCL4, which recruits memory T helper 1 cells, was only increased (1·7-fold) by *cis*-UCA in CD3/CD28-stimulated T cells. These results show that vitD, *cis*-UCA and NO can differentially affect CD4+ T-cell activation and cytokine secretion. The decreased CLA expression suggests a mechanism whereby all these UVR-induced immunoregulatory molecules may prevent excess activated T cells infiltrating the skin.

P44
Using the seahorse XF analyzer to evaluate real–time cellular respiration of human skin cells following the impact of oxidative stress inducers and the ability of idebenone and niacinamide to mitigate these responses

J. Latimer and M. Birch-Machin
Newcastle University, Newcastle upon Tyne, U.K.

Cellular respiration is a series of processes that ultimately lead to the generation of cellular energy in the form of ATP. The two main metabolic pathways in ATP production are glycolysis and oxidative phosphorylation (OXPHOS). Cellular respiration declines with age in various organs including the skin. Due to the exposed nature of the skin, ageing can be accelerated by numerous environmental factors, namely ultraviolet radiation (UVR) – an effect known as extrinsic ageing. A useful tool for accurate quantification of cellular respiration in cells is the Seahorse XF Analyzer. This machinery takes simultaneous repeated measurements of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) that are representative of OXPHOS and glycolysis, respectively. OCR and ECAR are calculated by the changes in oxygen and proton (pH) concentration over a specified time period, measured in real time by sensors in the cell medium just 200 μm above the cell surface. The aim of the study was to investigate any changes in cellular respiration due to oxidative stress. This included the exposure of BJ fibroblasts and human epidermal keratinocytes, neonatal (HEKn) cells to H_2O_2 or UVR. A dose of 10 J cm^{-2} UVA inhibited cellular respiration of BJ fibroblasts 24 h following irradiation. Doses of 5 and 10 J cm^{-2} UVA primarily caused dose–dependent decreases in OXPHOS, which seemed to be compensated for by an increase in glycolysis possibly in an aim to maintain ATP levels. Decreases in both OXPHOS and glycolysis occurred 24 h following all UVB irradiations of HEKn. UVA and UVB not only suppressed the basal levels of mitochondrial respiration but also the ability of the cells to upregulate mitochondrial respiration in response to agents that uncouple the mitochondrial proton gradient from ATP production, such as FCCP. This means cells irradiated with UVA and UVB are more susceptible in stressful situations. Potential age-reducing/delaying actives were then assessed by their ability to counteract the effect of oxidative stress on cellular respiration. This included the addition of 25 μmol L^{-1} idebenone, which caused an increase in the UVR-induced reduction of oxygen consumption. As well as this, 1 mmol L^{-1} niacinamide had a beneficial effect on H_2O_2-induced reduction of glycolysis. This assay represents a valuable tool for measuring cellular respiration in skin cells extrinsically aged by oxidative stress and the ability of potential actives at mitigating this.

© 2014 British Association of Dermatologists

P45

All-*trans* retinoic acid normalizes the periodicity of photoaged fibrillin microfibrils *in vivo*

S.A. Thurstan,[1] E.C. Naylor,[1] C.E.M. Griffiths,[1] R.E.B. Watson[1] and M.J. Sherratt[2]

[1]*Centre for Dermatology and* [2]*Centre for Tissue Injury and Repair, University of Manchester, Manchester, U.K.*

Fibrillin microfibrils are complex macromolecular assemblies that play a key role in reinforcing elastic fibres in the reticular dermis and in anchoring the papillary dermal elastic fibre network to the dermal–epidermal junction. The early and selective loss of fibrillin microfibril epitopes from the papillary dermis of photoaged skin can be reversed by the topical application of all-*trans* retinoic acid (tRA) and some commercially available over-the-counter antiageing products. However, it is unknown whether these treatments induce the deposition of structurally and functionally competent assemblies. In this study, we hypothesized that: (i) fibrillin microfibrils extracted from photoaged skin would exhibit abnormal ultrastructure; and (ii) that topical tRA treatment would act to normalize these structural abnormalities in photoaged forearm skin. Healthy, but severely photoaged, volunteers were recruited to the study ($n = 6$). Skin biopsies (3 mm diameter) were obtained from photoaged forearm at baseline and following tRA treatment using the 'Manchester Patch Test Assay' (0·025% tRA; 4-day occlusion). Fibrillin microfibrils were extracted and purified using well-established bacterial collagenase and size-exclusion chromatography methodologies prior to imaging via atomic force microscopy (AFM). Fibrillin microfibril structure was characterized by measuring the mean periodicity (bead-to-bead distance) of 500-bead repeats. While microfibril periodicity is known to vary with disease there is a consensus in the literature that in healthy tissues mean periodicity is invariant and approximates 56 nm. Here, we show that in individuals where the periodicity of microfibrils in photoaged baseline skin was abnormal (> 2 SD from the published periodicity of microfibrils in healthy skin: 56·4 nm, SD 4·0 nm), intervention with tRA normalized microfibril structure (mean ± SD baseline periodicities of 70·4 ± 21·0 nm and 65·6 ± 12·7 nm were reduced to 55·7 ± 14·3 nm and 57·6 ± 8·9 nm, respectively). However, in four photoaged volunteers where baseline microfibril periodicity was within 2 SD of 56·4 nm, tRA treatment induced minimal structural remodelling. These observations demonstrate that microfibril structure is affected in the skin of some photoaged individuals and that in these tissues, tRA treatment can successfully normalize the molecular structure of a key elastic fibre component.

P46

Upregulation of matrix metalloproteinase 12 and its activity, by ultraviolet A1 irradiation in human skin *in vivo*: potential implications in solar elastosis

A. Tewari, K. Grys, R. Sarkany and A. Young

St John's Institute of Dermatology, King's College, London, U.K.

Ultraviolet (UV) A1 accounts for around 75% of terrestrial UV radiation (UVR) and most of the output of artificial tanning sources. It is also used in phototherapy. However, the molecular effects of UVA1 in human skin *in vivo* are poorly understood. We have compared time-dependent whole-genome expression, mRNA and protein changes in the skin after one minimal erythema dose (MED) of spectrally pure UVA1 (50 J cm^{-2}) and UVB (300 nm, 30 mJ cm^{-2}). The genes induced to the greatest extent at 24 h were those involved in extracellular matrix remodelling, with both UVA1 ($P = 5·5 \times 10^{-7}$) and UVB ($P = 2·9 \times 10^{-22}$). UVA1 and UVB caused different effects on matrix metalloproteinase (MMP) expression: UVB induced MMP1, MMP3 and MMP10 mRNA at 24 h to a much greater extent than UVA1. MMP12 mRNA induction by UVA1 at 6 h was dramatic and much greater than induced by UVB. We found that MMP12 mRNA induction by UVA1 resulted in expression of MMP12 protein, which is functional as an elastase. The induction of elastase activity did not occur with UVB. We hypothesize that the UVA1-specific induction of MMP12 mediates some of its photoageing effects, particularly by contributing to elastin degeneration in late solar elastosis and conclude that MMP12 is a marker of UVA1 exposure.

P47

The prevalence of filaggrin mutations in patients with chronic actinic dermatitis

C.P. Harkins,[1] A. Waters,[1] A. Kerr,[1] L. Campbell,[2] W.H.I. McLean,[2] S.J. Brown[2] and S.H. Ibbotson[1]

[1]*Photobiology Unit, Ninewells Hospital and Medical School, University of Dundee, Dundee, U.K. and* [2]*Division of Molecular Medicine, College of Life Sciences, University of Dundee, Dundee, U.K.*

Chronic actinic dermatitis (CAD) is an uncommon idiopathic photodermatosis, characterized by dermatitis on photoexposed sites, in association with broadband photosensitivity and multiple contact allergies. CAD may have a significant impact on quality of life and treatment options are limited. Little is known regarding the pathogenesis but most patients have a preceding history of dermatitis. Given the propensity to eczema and contact allergies, we considered the possibility of underlying epidermal barrier dysfunction as a key pathomechanism. Loss-of-function mutations in filaggrin (*FLG*) are associated with skin barrier dysfunction and are a strong risk factor for atopic eczema. This study aimed to investigate the prevalence of *FLG* null mutations in cases compared with controls, which if increased would implicate a possible role in pathogenesis. Patients with a diagnosis of CAD based on clinical features and phototesting were identified through the photodiagnostic service and invited to participate. A total of

© 2014 British Association of Dermatologists

47 cases of CAD of white European ethnicity were recruited and blood samples obtained for analysis of the most common *FLG* null mutations in the European population (R501X, 2282del4, R2247X and S3247X). DNA from 100 population-matched controls of unknown skin phenotype underwent the same *FLG* genotype analysis. Results for all four *FLG* mutations were obtained for 46 cases and all 100 controls. An atopic background (i.e. diagnosis of atopic eczema with or without asthma/hay fever) was found in 20/47 patients with CAD (43%). Ten of 46 patients (22%) carried one or more *FLG* mutations compared with 12 out of 100 controls (12%). The greater proportion of *FLG* mutations in CAD cases compared with controls may reflect the atopic coassociation with CAD, but the difference was not statistically significant (χ^2 P = 0·126). Analysis of the subset of atopic CAD cases compared with controls showed significant association with *FLG* genotype (χ^2 P = 0·029, odds ratio 3·4, 95% confidence interval 1·1–10·6) whereas there was no significant difference in the nonatopic subset (n = 27, P = 0·7). These results confirm the strong association of *FLG* mutations with eczema, seen within the patients with atopic CAD, but there is no additional effect of *FLG* genotype on the risk of CAD. We therefore conclude that skin barrier dysfunction caused by filaggrin deficiency does not play an important role in the pathogenesis of CAD.

Orals

O1
Topical formulation of polo-like kinase 1 inhibitors for the treatment of human cutaneous squamous cell carcinoma

C. Pourreyron, S. Wright, S. Watt, I. Leigh and A. South

Division of Cancer Research, University of Dundee, Dundee, U.K.

The increasing incidence of cutaneous squamous cell carcinoma (cSCC) translates to an ever-increasing burden on healthcare budgets. We have previously identified polo-like kinase (PLK)1, a key regulator of cell division, as a kinase necessary for cSCC keratinocyte survival (Watt SA, Pourreyron C, Purdie K *et al*., Integrative mRNA profiling comparing cultured primary cells with clinical samples reveals PLK1 and C20orf20 as therapeutic targets in cutaneous squamous cell carcinoma. *Oncogene* 2011; **30**: 4666–77). Intratumoral injections of a PLK1 inhibitor, BI2536, dramatically reduced cSCC tumour xenograft volume *in vivo*. Intravenous delivery of BI2536 had similar effects, but induction of significant toxicity has prevented us from taking this inhibitor further *in vivo*. This observation is in line with the reported side-effects of BI2536 (Schöffski P. Polo-like kinase (PLK) inhibitors in preclinical and early clinical development in oncology. *Oncologist* 2009; **14**: 559–70), which recently resulted in two neutropenia-related deaths of patients in a phase II trial (Sebastian M, Reck M, Waller CF *et al*. The efficacy and safety of BI 2536, a novel PLK-1 inhibitor, in patients with stage IIIB/IV non-small cell lung cancer who had relapsed after, or failed, chemotherapy: results from an open-label, randomized phase II clinical trial. *J Thorac Oncol* 2010; **5**: 1060–7). BI2536 is a broad-spectrum inhibitor with effects on numerous other kinases in the micromolar range. The aim of the present study is to screen further, specific PLK1 inhibitors that are at various stages of clinical development, in order to identify those with the least toxicity in normal primary cells and the highest specificity in cSCC keratinocytes. We will then test the most efficient PLK1 inhibitors in a topical application for cSCC therapy. Using MTS and cell toxicity assays we have determined the EC_{50} values of six different PLK1 inhibitors in cSCC and normal keratinocytes. Two of them, ON-01910 and HMN214, specifically reduced cell viability and induced cell death in tumour cells, with EC_{50} values of 0·06–0·3 μmol L^{-1} and 0·3–0·7 μmol L^{-1} for cSCC keratinocytes, compared with 310·6 μmol L^{-1} and 9·5 μmol L^{-1} for normal human keratinocytes, respectively. Intratumoral injection of ON-01910 dramatically reduced cSCC xenograft volume *in vivo* (mean of ON-01910-treated tumour volume 294 ± 185 mm^3 vs. vehicle-treated tumour volume 776 ± 194 mm^3, n = 5 for each group). To apply PLK1 inhibitors topically, we have developed a xenotransplantation model of human cSCC in mice. In this model, skin equivalents are set up by seeding tumour keratinocytes on a fibrin matrix populated with fibroblasts. A full-thickness portion of skin is removed from the dorsal area of a severe combined immunodeficiency mouse where a skin equivalent is secured in place. The skin is devitalized and applied over the graft as a biological bandage. To date, we have obtained fast-growing and massively invasive tumours with one cSCC keratinocyte population. We are currently using this model to test whether PLK1 inhibitors identified specifically to induce cSCC toxicity *in vitro* are efficacious with topical application *in vivo*.

O2
Human basal cell carcinoma demonstrates telogen arrest

A. BenKetah, P. Bowden, S. Reed and G. Patel

Cardiff University, Cardiff, U.K.

Most cancers demonstrate partial or complete block in differentiation to aid proliferation. For example, retinoic acid induces differentiation and is used in the treatment of acute leukaemia, as well as in the prevention of squamous cell carcinoma. We have previously shown that human basal cell carcinoma (BCC) demonstrates hierarchical growth, driven by the presence of a small CD200+ cancer stem-cell population, resulting in progeny that differentiate along hair follicle (HF) lineages (Colmont CS Benketah A, Reed SH *et al*. CD200 expressing human basal cell carcinoma cells initiate tumor growth. *Proc Natl Acad Sci U S A* 2013; **110**: 1434–9). We hypothesize that factors that induce HF differentiation can be effective therapy for BCC. The human HF demonstrates complex inward and upward differentiation, resulting in concentric layers that express hair-specific keratins, resulting in the

© 2014 British Association of Dermatologists

formation of an emergent hair shaft. We first sought to determine the pattern of BCC differentiation by reverse-transcription polymerase chain reaction (RT-PCR) and immunofluorescence, using human hair-bearing normal skin (n = 6) and BCC tissue samples (n = 20), showing that BCC demonstrates the following: outer root sheath (K5, K14, K16, K17and K19), companion layer (K75), inner root sheath (K26, K27, K28, K71, K72 and K74), cuticle (K32, K35, K82 and K85); but not hair shaft differentiation (K31, K32 and K81). When we next colabelled tissue samples with specific keratins and the proliferation marker KI67, we observed concordance with proliferation rates observed in the HF with the exception of K17, which is specifically dysregulated in BCC. Likewise, transcriptional control of keratins, and thus differentiated cell phenotypes, was observed to be similarly regulated in the HF and BCC. The pattern of differentiation in BCC mirrored HF telogen arrest, a key step before anagen. Consistent with this hypothesis, BCC tissues exhibited high levels of bone morphogenetic protein (BMP)2 and BMP4 and low levels of transforming growth factor (TGF)-β. When noggin, a BMP antagonist and TGF-β were added to BCC cells in culture for 48 h, we were able to observe increased keratin expression by RT-PCR of the inner root sheath (K25), cuticle (K32) and medulla (K35). In conclusion, our findings demonstrate the presence of a complex pattern of HF differentiation in BCC, characterized by refractory telogen, which *in vitro* is amenable to potential therapeutic manipulation.

O3
Diverse roles for laminin-332 subunits in squamous cell carcinoma

M. Caley,[1] V. Martins,[1] K. Moore,[2] J. Marshall[2] and E. O'Toole[1]

[1]*Centre for Cutaneous Research and* [2]*Institute of Cancer, Barts and the London SMD, Queen Mary University of London, London, U.K.*

The basement membrane zone (BMZ), present in all epithelia, plays an important role in cancer spread, not only acting as a barrier to invasion but also signalling through cell-surface receptors. Components of the skin BMZ include the collagens Col IV, Col VII and Col XVII, as well as laminin-332 (Lam332), which is secreted in copious amounts by cancer cells. Patients with severe epidermolysis bullosa caused by mutations in BMZ components suffer from an increased incidence of aggressive squamous cell carcinoma (SCC). The role of Lam332 and collagen XVII in tumour development is poorly understood. This study dissects the effect of loss of individual subunits of Lam332 or collagen XVII in SCC. Using lentiviral shRNA we have generated four stable cutaneous SCC cell lines each lacking a different BMZ component, including collagen XVII and the three constituent parts of Lam322: LamA3, LamB3 and LamC2. Using our cell lines we studied their role in cell attachment, proliferation, motility, *in vitro* and *in vivo* invasion, integrin expression and cellular signalling. Loss of Col XVII increased cell attachment, whereas loss of any of the Lam332 chains reduced cell attachment. Loss of any of the BMZ components reduced cell proliferation. The loss of LamA3 and LamC2 increased cell motility, whereas loss of LamB3 and Col XVII had no significant effect on motility. We demonstrated increased invasion in both *in vitro* collagen gels and *in vivo* murine xenografts with loss of LamA3, LamC2 and Col XVII, with distinct patterns of invasion seen in each cell line. Each cell line demonstrated a unique pattern of integrin expression as determined by fluorescence-activated cell sorting analysis. Finally we have used quantitative polymerase chain reaction and Western blotting to identify changes in signalling pathways known to be involved in cancer progression, such as epithelial to mesenchymal transition, FAK signalling and tight junction formation. In conclusion, this study further refines the role of Lam332 and Col XVII in SCC invasion and proliferation *in vitro* and *in vivo*.

O4
The development of cutaneous squamous lesions in patients treated with vemurafenib: investigation of a possible role for human papillomavirus in addition to activated RAS

K. Purdie,[1] A. South,[2] M. Sommerlad,[3] H. Rizvi,[3] I. Leigh,[2] C. Proby[2] and C. Harwood[1]

[1]*Queen Mary University of London, London, U.K.,* [2]*Ninewells Hospital and Medical School, Dundee, U.K. and* [3]*Barts Health NHS Trust, London, U.K.*

Approximately half of metastatic melanomas have an activating mutation in the *BRAF* oncogene. The development of vemurafenib, a specific inhibitor of mutant BRAF, has been associated with tumour regression and improved overall survival in BRAF-positive patients with melanoma. However, one side-effect is the *de novo* development of benign and malignant squamoproliferative lesions in up to 25% of individuals. The putative mechanism involves paradoxical increased mitogen-activated protein kinase (MAPK) signalling by BRAF inhibitors in the context of mutated or activated RAS. However, cutaneous squamous cell carcinoma (cSCC) has been reported to develop in association with wart-like lesions, and upregulation of the MAPK pathway is known to facilitate human papillomavirus (HPV) replication, suggesting a possible role for HPV in the pathogenesis of these lesions. We have examined the *RAS* mutational status and the presence of HPV DNA in 45 skin biopsies from seven patients receiving vemurafenib (26 benign squamoproliferative lesions, 13 SCCs and six normal skin samples). A majority (80%, 36/45) of the samples were positive for beta (epidermodysplasia verruciformis-associated) HPV with a lower proportion (9%, 4/45) positive for cutaneous alpha HPV. However, only a minority of lesions [one of 11 SCCs (9%), six of 18 benign squamoproliferative lesions (33%), seven of 29 lesions in total (24%)] with apparent viral features at the clinicopathological level contained high levels of HPV DNA indicative of active infection. Activating *RAS* mutations were detected in 15/45 (33%) samples (eight of 26 benign squamoproliferative lesions and seven of 13 cSCCs). The majority of mutations (93%, 14/15) occurred in *HRAS*, with one mutation identified in *KRAS* and none in the *NRAS* gene. Nine of 15 (60%) activating *RAS* mutations occurred in codon 61, while three of 15 mutations (20%) were identified

 © 2014 British Association of Dermatologists

in each of codons 12 and 13; all three codons have been previously reported as mutational hotspots characteristic of vemurafenib-associated cSCC. The high frequency of *RAS* mutations in our sample series, together with the rapid time frame of their development, supports the hypothesis that these mutations are pre-existing and confer a selective advantage in the context of vemurafenib therapy. Our data suggest that HPV is unlikely to play a major aetiological role in these lesions.

O5
Characterization of peritumoral regulatory T cells in cutaneous squamous cell carcinomas

C. Lai,[1] R. Behar,[1] M. Polak,[1] M. Ardern-Jones,[1] J. Theaker,[2] A. Al-Shamkhani[3] and E. Healy[1]

[1]*Dermatopharmacology, University of Southampton, Southampton, U.K.,* [2]*Histopathology, University Hospital Southampton NHS Foundation Trust, Southampton, U.K. and* [3]*Cancer Sciences, University of Southampton, Southampton, U.K.*

Nonmelanoma skin cancer (NMSC) is the most common cancer in the U.K. and is increasing in incidence. Defective host immunity promotes the development of NMSCs, especially cutaneous squamous cell carcinomas (SCCs), and SCCs in immunocompetent individuals seem able to develop by avoiding immune-mediated destruction. Our previous work demonstrated that regulatory T cells (Tregs) accumulate around SCCs and that these Tregs could suppress peritumoral effector T-cell proliferation *in vitro*. In this study, further characterization was performed on Tregs isolated from human primary cutaneous SCCs. Flow cytometry showed expression of the costimulatory receptors OX40 and 4-1BB in SCCs predominantly by peritumoral forkhead box protein (FOXP)3+ Tregs (OX40 expression: 28·5% of CD4+ FOXP3+ vs. 4·4% of CD4 + FOXP3− and 0·2% of CD8+ T cells, n = 8 tumours, P = 0·001; 4-1BB expression: 10·3% of CD4+ FOXP3+ vs. 0·6% of CD4 + FOXP3− and 0·4% of CD8+ T cells, n = 6 tumours, P = 0·005). Peritumoral FOXP3+ Tregs also expressed higher levels of both OX40 and 4-1BB than Tregs from peripheral blood (percentage of CD4+ FOXP3+ Tregs expressing OX40: 28·5% in the tumour vs. 2·1% in peripheral blood, P < 0·0001, n = 8 tumours; percentage of FOXP3+ Tregs expressing 4-1BB: 10·3% in the tumour vs. 2·7% in peripheral blood, P = 0·0075, n = 6 tumours). Enzyme-linked immunosorbent spot assays showed that CD3+ CD4+ CD25highCD127low peritumoral Tregs sorted by fluorescence-activated cell sorting were able to suppress interferon-gamma production by phytohaemagglutinin-stimulated peritumoral effector T cells (mean suppression 27%, P = 0·049, n = 10 tumours). Immunohistochemistry demonstrated significantly more FOXP3+ Tregs in primary SCCs that subsequently metastasized than in SCCs that had not metastasized after 5 years of follow-up (49·3% vs. 23·5% of immune infiltrate, P < 0·0001, n = 29 and 26 tumours, respectively). Furthermore, there were fewer CD8+ T cells and lower CD8+ T cell : FOXP3+ Treg ratios in metastatic SCCs compared with nonmetastatic tumours (CD8+ T cells comprised 28·5% vs. 44·6% of the immune infiltrate, P < 0·0001; CD8 : FOXP3 ratio 0·63 vs. 2·68, P < 0·0001, n = 29 and 26, respectively). These data provide evidence that peritumoral Tregs in cutaneous SCCs are immunosuppressive and may play a role in promoting SCC metastasis.

O6
Cannabinoids hijack the autophagy pathway to promote melanoma cell death

J. Armstrong,[1] C. McKee,[2] D. Hill,[2] G. Velasco[3] and P. Lovat[2]

[1]*Faculty of Applied Sciences, University of Sunderland, Sunderland, U.K.,* [2]*Dermatological Sciences, Institute of Cellular Medicine Newcastle University, Newcastle upon Tyne, U.K. and* [3]*Complutense University, Madrid, Spain*

Metastatic melanoma remains largely untreatable, likely due to apoptotic resistance partly reflected by the ability of tumours to promote prosurvival signalling mechanisms including autophagy, the principle lysosomal mechanism for the degradation and recycling of damaged organelles and excess proteins. Coupled with observations that many chemotherapeutic drugs activate autophagy as a compensatory mechanism to counteract apoptotic signals, current therapeutic strategies have focused on the combined use of the lysosomal inhibitor, hydroxychloroquine (HCQ), to inhibit prosurvival autophagy. However, the capacity for sensitization to HCQ varies, and includes sensitization of normal cells to the cytotoxic effects of chemotherapy. As blocking autophagy may also promote secondary tumour development, the alternative use of autophagy-inducing drugs able to promote cell death, such as tetrahydrocannabinol (THC), the main biologically active component of cannabis, may represent a better therapeutic strategy. With this aim, the present study determined the contribution of autophagy to the cytotoxic effect of THC in melanoma. THC induced autophagic flux as shown by immunofluorescence/Western blotting of LC3 I–II conversion and caspase-dependent apoptosis of metastatic A375, CHL-1 and SK-MEL-28 melanoma cells, as demonstrated by significant inhibition of cell viability, increased caspase 3 cleavage and prevention of cell death by ZVAD-FMK (P < 0·001). Conversely, THC demonstrated little cytotoxicity to normal melanocytes. THC-induced apoptosis was also inhibited by knockdown of the autophagy regulatory genes *Atg7* and *Becn1* or combined treatment with chloroquine. In addition, THC activated lysosomal membrane permeability (LMP) as shown by release of cathepsin B, while specific inhibition of cathepsin B activity prevented both THC-induced cytochrome C release and loss of cell viability (P < 0·001). Collectively these data show that THC-induced autophagy in melanoma is cytotoxic, with the additional destabilization of LMP resulting in promotion of cytochrome C release and the enhanced induction of apoptosis. THC-induced autophagy may thus represent a more effective therapeutic strategy through which to harness autophagy modulation for the therapeutic benefit of metastatic melanoma.

© 2014 British Association of Dermatologists

O7
Transforming growth factor-β-mediated Ambra-1 downregulation in melanoma ulceration and metastasis

R. Ellis,[1] M.-E. Anagnostou,[2] S. Verykiou,[2] M. Elias,[2] S. Horswell,[3] N. Kirkham[4] and P. Lovat[2]
[1]*The James Cook University Hospital, Middlesbrough, U.K.*, [2]*Newcastle University, Newcastle upon Tyne, U.K.*, [3]*Cancer Research U.K., London, U.K.* and [4]*The Royal Free Hospital, London, U.K.*

Cutaneous metastatic melanoma remains a major world health problem, with an ever increasing mortality reflected by multiple deregulated cell-signalling mechanisms rendering tumours notoriously unresponsive to conventional chemotherapy. This highlights the acute need for novel targeted treatment strategies and personalized therapies. Ulceration is a major prognostic subfactor; however, the underlying biological relevance of epidermal breakdown remains enigmatic. Transforming growth factor (TGF)-β exerts pleiotropic effects on cellular differentiation and proliferation, and its secretion by melanoma tumours is associated with an invasive phenotype and poor survival, suggesting a potential role for specific TGF-β isoforms in tumour invasion and metastasis. TGF-β is also implicated in autophagy regulation, the principle lysosome-mediated mechanism for the degradation and recycling of damaged organelles/proteins, essential for the maintenance of cellular differentiation. The aim of the present study was therefore to test the hypothesis that melanoma secretion of specific TGF-β isoforms results in autophagy deregulation leading to epidermal breakdown and tumour progression. Optimization of semiquantitative immunohistochemical assays, and subsequent analysis of epidermal expression of the proautophagy regulatory protein Ambra-1 and tumoural expression of TGF-β2, was performed in a statistically powered retrospective cohort of formalin-fixed and paraffin-embedded benign naevi and melanomas of varying American Joint Committee on Cancer stage. The results revealed that the expression of Ambra-1 in normal, peritumoral epidermis increased in line with keratinocyte differentiation, while decreased or even complete loss of expression was observed in the epidermis overlying a subcohort of melanomas, which significantly correlated with decreased disease-free survival over 7 years ($P < 0{\cdot}05$). Increased tumoural TGF-β2 expression correlated significantly with decreased or no epidermal Ambra-1 expression ($P < 0{\cdot}01$), with the highest levels of TGF-β2 expression and strongest correlation with Ambra-1 loss observed in primary NRAS/BRAF mutated melanomas ($P < 0{\cdot}05$). *In vitro* analysis of calcium-induced differentiation of primary keratinocytes also resulted in an increase in Ambra-1 protein expression, paralleled by an increase in loricrin and decreased CK14 expression, the effects of which were reversed by cotreatment with recombinant TGF-β2. Collectively, these results suggest that TGF-β2 mediates the deregulation of autophagy in the peritumoral epidermis leading to downregulation of keratinocyte differentiation, epidermal ulceration and melanoma progression. Loss of epidermal Ambra-1 expression may thus represent a novel prognostic biomarker, identifying patients with melanoma at the highest risk of disease progression; targeting of TGF-β2 in the adjuvant setting of such patients may also provide a novel and more effective personalized therapeutic strategy.

O8
Prognostic significance of CXCR4 expression in cutaneous melanoma

A. McConnell,[1] R. Ellis,[1] G. O'Boyle,[2] R. Plummer,[3] S. Verykiou[1] and P. Lovat[1]
[1]*Dermatological Sciences, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, U.K.*, [2]*Faculty of Applied Sciences, University of Sunderland, Sunderland, U.K.* and [3]*The Northern Institute of Cancer Research, Newcastle University, Newcastle upon Tyne, U.K.*

Chemokines and chemokine receptors, a family of small chemoattractant cytokine-like proteins, facilitate directional movement of cancer cells to metastatic sites. In uveal melanoma, increased expression of cytoplasmic CXCR4 (cCXCR4) correlates with the increased likelihood of metastasis to the liver, while in cutaneous melanoma, increased cCXCR4 correlates with increased Breslow thickness and tumour ulceration. However, studies in melanoma have not distinguished between nuclear CXCR4 (nCXCR4) and cCXCR4 expression. Therefore the aim of the present study was to determine the differential expression cCXCR4/nCXCR4 in a retrospective cohort of 64 primary naevi and cutaneous melanomas of differing American Joint Committee on Cancer (AJCC) disease stage, using a semiquantitative immunochemistry assay, and to correlate expression with BRAF/NRAS mutational status and patient outcome over 7 years. The results revealed that total CXCR4 was consistently expressed in naevi and all AJCC disease stage melanomas to differing degrees, but was significantly increased in AJCC stage III/IV metastatic melanomas compared with stage I/II tumours (Mann–Whitney U-test, $P < 0{\cdot}05$). Univariate analysis of mean percentage CXCR4 expression vs. time to metastasis in melanomas of all AJCC stages revealed a stepwise decrease in disease-free survival (DFS) at 7 years. Stratification of DFS by AJCC disease stage demonstrated a significant decrease in DFS from 74% to 31% in AJCC stage II melanomas, suggesting that high CXCR4 expression may represent a novel biomarker of disease progression (hazard ratio 3·24, 95% confidence interval 1·08–9·73, $P = 0{\cdot}03$). Differential analysis of CXCR4 expression also revealed that nCXCR4 expression was significantly increased in NRAS/BRAF mutant tumours of all AJCC stages compared with wild-type melanomas ($P < 0{\cdot}001$, unpaired Student t-test), and was also significantly increased in metastatic vs. localized tumours ($P < 0{\cdot}05$, unpaired Student t-test). Collectively these data suggest a direct association between increased nCXCR4 expression and activation of NRAS/BRAF signalling, and that CXCR4 expression may represent a credible biomarker for the identification of a subset of high-risk melanomas, thereby enabling early therapeutic intervention. Furthermore, as studies in prostate cancer show that nCXCR4 functions as a ligand-responsive receptor, clinical antagonization of nCXCR4 may additionally represent a novel therapeutic strategy to prevent melanoma metastasis.

© 2014 British Association of Dermatologists

O9

Human circulating B cells express skin-homing receptors and accumulate within the skin at sites of infectious and neoplastic inflammation

I.U. Egbuniwe,[1] W. Alwan,[2] M.H. Rustin,[3] A.N. Akbar,[4] S.N. Karagiannis,[1] F.O. Nestle[1] and K.E. Lacy[1]

[1]*King's College London, London, U.K.*, [2]*Guy's and St Thomas' NHS Foundation Trust, London, U.K.*, [3]*Royal Free Hospital, London, U.K. and* [4]*University College London, London, U.K.*

It is well recognized that specific T cells are able to home from the peripheral circulation to the skin via expression of skin-homing receptors such as cutaneous lymphocyte antigen (CLA) and chemokine receptor (CCR)4. However, the trafficking of B cells to the skin has not previously been explored in detail. We sought to investigate the potential for B cells to home from the peripheral blood to the skin at sites of inflammation. Using flow cytometry, we first analysed peripheral blood from healthy donors (n = 45), where we identified a population of CD20+ B cells that expressed the homing markers CLA and CCR4. B cells formed two distinct populations expressing either CLA or CCR4 alone. Interestingly, the CLA+ but not the CCR4+ population was found to be predominantly IgG/IgE/IgA+CD22+ CD27+, indicative of a population of class-switched mature memory B cells primed to home into the skin. We next investigated whether B cells home into skin in response to an antigen challenge using a model of infective skin inflammation. For this we analysed healthy skin biopsies taken following intradermal challenge with varicella zoster virus (n = 5) and *Candida* antigens (n = 5) by immunohistochemistry. Populations of proliferating (CD20+ Ki67+) B cells were identified, and their numbers increased for up to day 7 postchallenge in parallel with the CD3+ T-cell infiltrate. Finally, we investigated B-cell recruitment in cutaneous melanoma as a model of neoplastic inflammation. Analysis of peripheral blood from patients with melanoma revealed a statistically higher proportion of CLA+ B cells (n = 63, mean of 6·31%) than in blood from healthy donors (n = 45, mean of 3·88%; P = 0·02). Comparison of CLA expression on CD20+ B-cell infiltrates in cutaneous metastatic melanoma biopsies and matched blood samples from the same patients (n = 10) revealed an enriched CLA+CD20+ B-cell population (P = 0·03, mean of 14·3) within cutaneous melanomas. This recruitment into tumours may be a response to the presence of melanoma antigens. *In vitro* functional assays revealed that CLA+ peripheral blood B cells expressed interferon gamma and tumour necrosis factor-β when cocultured with metastatic melanoma cells (A375), indicating their proinflammatory capabilities in the context of cancer. Our findings highlight previously unappreciated roles for B cells in cutaneous immune responses.

O10

A novel phosphatidylinositol-3-kinase inhibitor decreases activation of ribosomal protein S6 in psoriatic T cells

N. Yager,[1] C. Haddadeen,[1] A. Payne,[2] R. Allen[2] and E. Healy[1]

[1]*University of Southampton, Southampton, U.K. and* [2]*UCB, Slough, U.K.*

The phosphatidylinositol-3-kinase (PI3K) pathway represents an attractive therapeutic intervention for the treatment of inflammatory and immune-related disorders, as it is essential for cellular functions including protein synthesis, proliferation and cell survival. Psoriasis is characterized by a hyperproliferation of keratinocytes and an associated infiltrate of proinflammatory T cells. The PI3K pathway plays a key role in the differentiation of T cells, therefore inhibition of the PI3K pathway may be a useful approach to help treat psoriasis. We took 6-mm punch biopsies from the lesional skin of psoriatic subjects who had not received topical or systemic antipsoriatic treatment for at least 2 weeks, and 5-μm cryosections were stained. Confocal immunofluorescence imaging confirmed that the PI3K downstream biomarker ribosomal protein S6 (rpS6) was strongly phosphorylated at Ser235/236 throughout the epidermis of lesional skin, indicating activation of the PI3K pathway in psoriasis. In addition, we identified a proportion of CD3+ cells in the dermis colocalizing with phosphorylated rpS6. To further investigate PI3K signalling activity in CD3+ cells and the effect of PI3K pathway blockade, a phospho-specific flow cytometry assay was utilized. T cells that had emigrated from lesional skin biopsies from psoriatic subjects (n = 5) were preincubated with either vehicle or the selective PI3Kδ inhibitor UCB5857 prior to T cell receptor (TCR) stimulation. In dermal T cells, which were predominantly CD45RO+ memory T cells, 10 $\mu mol\ L^{-1}$ UCB5857 significantly decreased the median percentage of CD3+ phosphorylated rpS6+ cells by 70·3% ($P < 0{\cdot}005$), indicating effective blockade of the PI3K cascade. In peripheral blood mononuclear cells (PBMCs) from the same subjects, 10 and 1 $\mu mol\ L^{-1}$ UCB5857 significantly decreased the median percentage of CD3+ phosphorylated rpS6+ cells by 53·5% and 33·2%, respectively ($P < 0{\cdot}01$). Additionally, in a carboxyfluorescein succinimidyl ester assay, 10 $\mu mol\ L^{-1}$ UCB5857 strongly suppressed proliferation of TCR-stimulated PBMCs to levels that were comparable with unstimulated controls. These results suggest that targeting the PI3K pathway in skin and blood T cells of psoriatic subjects may offer a useful therapeutic option in the future treatment of psoriasis.

© 2014 British Association of Dermatologists

O11

The molecular mechanisms of modulation of human Langerhans cell function by epidermal cytokines

M.E. Polak,[1] C.Y. Ung,[1] J. Masapust,[1] E. Healy,[1] T. Freeman[2] and M. Ardern-Jones[1]

[1]*Clinical and Experimental Sciences, University of Southampton, Southampton, U.K. and* [2]*The Roslin Institute and Royal (Dick), School of Veterinary Sciences, University of Edinburgh, Edinburgh, U.K.*

Epidermal cytokines regulate function of Langerhans cells (LCs). Thymic stromal lymphopoietin (TSLP) is highly expressed in lesional atopic dermatitis (AD) and enhances the ability of LCs to stimulate Th2 T lymphocytes. In contrast, tumour necrosis factor (TNF)α is associated with Th1/Th17 polarization and effective induction of cytotoxic T cells. To understand the molecular mechanisms underpinning the regulation of LC function by epidermal cytokines we compared the behaviour of human LC molecular networks during the 24-h time course of stimulation with TSLP or TNFα. Migratory LCs were isolated from human skin and matured with TNFα or TSLP during or after migration (n = 3). Both cytokines induced LC migration from biopsies and mature phenotype (expression of CD40, CD70, CD80 and CD86). While both TNFα- and TSLP-matured LCs were able to activate T-lymphocyte proliferation and interferon-γ release, TSLP significantly augmented LC potential to prime and stimulate Th2-type responses (n = 3, $P < 0{\cdot}01$). Whole-transcriptome data were obtained with Affymetrix HGU219 microarrays at 0, 2, 8 and 24 h following cytokine stimulation (n = 3). LCs matured with TSLP showed the characteristic LC gene signature, with upregulation of genes involved in cell membrane reorganization and metabolism. Applying the MaSigPro algorithm, $P < 0{\cdot}05$, Benjamini–Hochberg corrected, we identified 527 genes differentially regulated by the two cytokines. The differences in TNFα-and TSLP-induced gene expression were time dependent (90 genes with signal difference > 1·25 at 2 h vs. 351 genes at 24 h). Delineation of the molecular networks involved in TNFα and TSLP signalling with BioLayout *Express*^{3D} (Markov Cluster Algorithm = 1·7, r = 0·85) revealed striking differences in regulation of genes involved in antigen capture (*CAV1*), intracellular trafficking (*SNX10* and *SNX11*) and formation of the immunoproteasome (*PSME2*, *PSME3*, *PSMB10*). In contrast to TNF , TSLP did not induce upregulation of genes involved in antigen processing and maintained the profile of freshly isolated LCs, lacking the antigen-processing-associated signature. Consistently, TSLP maturation diminished the potential of LCs to stimulate antigen-specific CD8 T cells. The decreased induction of the antigen-processing genes in TSLP LCs may be important for Th2 polarization, which has previously been shown to depend partially on the quality and quantity of antigen presented to T cells by the dendritic cells. The differential gene regulation as described here may explain the associated altered immune responses in TSLP-associated allergic skin inflammation.

O12

Cis-urocanic acid reduces the expression of monocyte-derived dendritic cell costimulatory molecules in a dose-dependent manner

C. Leitch,[1] C. Yu,[1] J. Schwarze,[1] S. Howie[1] and R. Weller[2]

[1]*MRC Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, U.K. and* [2]*Department of Dermatology, NHS Lothian, Edinburgh, U.K.*

Dendritic cells in the skin – comprising epidermal Langerhans cells (LCs) and dermal dendritic cells, both potent stimulators of T cells – play a key role in the adaptive immune response. In the absence of danger signals they may promote peripheral T-cell tolerance through induction of regulatory T cells. Urocanic acid (UCA), a breakdown product of the skin barrier protein filaggrin, is converted from *trans*- to *cis*-UCA at the skin surface by ultraviolet radiation. *Cis*-UCA has long been known to have immunosuppressive effects, but its precise mode of action has not yet been elucidated. The discovery of the association between common loss-of-function mutations in the filaggrin gene and atopic eczema has given a new significance to the potential role of filaggrin breakdown products in regulating the skin immune response. It has recently been demonstrated that levels of urocanic acid – measured by tape stripping – correlate with both filaggrin genotype and atopic dermatitis severity. We hypothesized that *cis*-UCA could have an immunosuppressive effect by modulating the expression of dendritic cell costimulatory molecules. We generated monocyte-derived dendritic cells from peripheral blood mononuclear cells from healthy volunteers. During culture, cells were exposed to *cis*-UCA (10–1000 $\mu g\ mL^{-1}$) or *trans*-UCA (10–1000 $\mu g\ mL^{-1}$). Lipopolysaccharide (LPS) was added to a portion of the cells 12 h prior to harvest. Live cells were analysed for the expression of human leucocyte antigen (HLA)-DR, CD11c, CD1a, CD40, CD80, CD86 and programmed death ligand (PDL)-1 by flow cytometry. While the expression of HLA-DR, CD11c, CD1a and CD80 as measured by mean fluorescence intensity was unaffected by the presence of *cis*- or *trans*-UCA, the expression of CD86, CD40 and PDL-1 in LPS-activated cells was reduced by up to 50% in a dose-dependent manner by the presence of *cis*-UCA. These results suggest that by reducing the expression of dendritic cell costimulatory molecules, *cis*-UCA may regulate adaptive immune responses in human skin. A lack of *cis*-UCA – as found in filaggrin-deficient individuals – could perturb normal mechanisms of tolerance to harmless antigens and thus predispose to inflammatory skin disease.

© 2014 British Association of Dermatologists

O13
Enhanced expression of interleukin (IL)-17, IL-10 and IL-1β in hidradenitis suppurativa

G. Kelly,[1] C.M. Sweeney,[1] R. Fitzgerald,[2] A. Lally,[1] A.M. Tobin[2] and B. Kirby[1]

[1]*Dermatology Research Group, St Vincent's University Hospital, Dublin, Ireland and* [2]*Dermatology Department, Adelaide and Meath Hospital, Tallaght, Dublin, Ireland*

Hidradenitis suppurativa (HS) is a relapsing, inflammatory disease characterized by recurrent abscesses, sinus tract formation and cribriform scarring in the axillary, inguinal, submammary and perineal areas. The exact aetiology of HS is unknown, but a role for immune dysregulation has been proposed. HS is associated with Crohn disease and pyoderma gangrenosum, which are recognized as conditions of immune dysfunction. Several reports have demonstrated therapeutic efficacy with antitumour necrosis factor-α agents. While the nature of the immune dysregulation in HS is unclear, recent studies have indicated a role for interleukin (IL)-17 and IL-17-associated cytokines in this disease. This study examined the expression of cytokines and inflammatory markers in the peripheral blood and skin of 20 patients with HS (five male, 15 female, mean age 38 years, Hurley stage I/II/III). We demonstrated significant systemic dysregulation in HS. The expression of highly sensitive C-reactive protein (hsCRP) and IL-10 was enhanced in the serum of patients with HS compared with healthy controls ($P < 0{\cdot}05$). Peripheral blood mononuclear cells (PBMCs) from patients produced more IL-10 and IL-17 ($P < 0{\cdot}05$) in response to CD3 activation than PBMCs from healthy controls. The mRNA expression of IL-17, IL-1β and IL-10 ($P < 0{\cdot}01$) was significantly enhanced in HS skin compared with control skin. These data suggest local and peripheral expression of T helper (Th)-17 and regulatory-cell-associated cytokines in HS. Complementary flow cytometry studies demonstrated that $CD45^+$ immune cells infiltrated the skin of patients with HS and produced IL-17, IL-10 and IL-1β. Whereas $CD4^+$ T cells produced IL-17 and IL-10, $CD1a^+$ dendritic cells produced IL-1β in HS skin. Our studies suggest a role for the inflammasome in HS, as the expression of NLRP3 was enhanced in HS skin ($P < 0{\cdot}05$). These data suggest that Th17 cells and their associated cytokines may be involved in HS and suggest that enhanced expression of IL-10 may be an attempt to compensate for inflammatory Th17 responses, but may also lead to local immune suppression and the development of secondary skin infections.

O14
Guttate psoriasis is associated with impaired epidermal Langerhans cell migration

L.H. Eaton,[1] L. Chularojanamontri,[2] F.R. Ali,[3] E. Theodorakopoulou,[3] R.J. Dearman,[1] I. Kimber[1] and C.E.M. Griffiths[3]

[1]*The Faculty of Life Sciences, The University of Manchester, Manchester, U.K.,* [2]*The Department of Dermatology, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand and* [3]*The Dermatology Centre, Manchester Academic Health Science Centre, The University of Manchester, Manchester, U.K.*

We have shown previously, using an epidermal explant model, that early-onset chronic plaque psoriasis (CPP) has virtually no Langerhans cell (LC) migration from the epidermis (compared with 20–30% for healthy individuals). Guttate psoriasis is an acute form of psoriasis occurring usually after a streptococcal pharyngitis or tonsillitis. Approximately 50% of such cases resolve, whereas 50% progress to CPP. The aim of this study was to determine whether guttate psoriasis, guttate psoriasis that had progressed to CPP, and resolved guttate psoriasis are associated with abnormal LC mobilization. Patient volunteers were divided into three groups; current guttate episode (five female; mean age 26 years), guttate psoriasis that had progressed to CPP (two female, four male, mean age 42 years) and resolved guttate psoriasis (one male, one female, mean age 21 years). Two 6-mm punch biopsies were taken, under local anaesthetic, from uninvolved skin on the buttocks. Epidermal sheets were isolated; one was fixed immediately in acetone (T0) and the other after being floated on medium for 24 h (T24). The frequency of epidermal LCs was assessed using fluorescence microscopy (staining for CD1a) and migration was calculated as the percentage loss of LC in the T24 compared with the T0 sample. Although LC migration was recorded in the active guttate psoriasis group, levels were lower than those seen previously with healthy controls ($8{\cdot}1 \pm 3{\cdot}8\%$ vs. $19{\cdot}6 \pm 8{\cdot}2\%$, respectively, $P < 0{\cdot}01$). The CPP group displayed even lower levels of LC migration compared with healthy individuals ($0{\cdot}5 \pm 1{\cdot}1\%$, $P < 0{\cdot}001$). In contrast, migration in the cleared group was restored to normal levels ($20{\cdot}9 \pm 2{\cdot}4\%$). These data confirm that patients with CPP have impaired LC migration, and moreover that guttate psoriasis is associated with an intermediate phenotype with LC mobilization occurring at a lower level than in patients who had resolved psoriasis. This suggests that LC migration in guttate psoriasis differs from that in CPP and returns to normal with clinical resolution. Taken together, these data may provide insights into factors that determine resolution of psoriasis.

O15
Histamine enhances keratinocyte-mediated resolution of inflammation by promoting wound healing and response to infection

D. Gutowska-Owsiak,[1] T.A. Selvakumar,[1] M. Salimi,[1] S. Taylor[2] and G.S. Ogg[1]

[1]*MRC Human Immunology Unit, NIHR Biomedical Research Centre, University of Oxford, Oxford, U.K. and* [2]*Computational Biology Research Group, University of Oxford, Oxford, U.K.*

Multiple cytokines are implicated in keratinocyte-mediated infection clearance and wound healing. However, little is known about histamine-mediated effects on keratinocytes in promoting resolution of inflammation. We performed a combined microarray/gene ontology analysis of histamine-stimulated keratinocytes. Functional changes were tested by apoptosis assessment and artificial injury assays. Histamine receptor (HR) involvement was assessed by blocking wound closure with specific HR antagonists. Histamine treatment had extensive effects on keratinocytes, including modulation of proinflammatory responses and cellular functions promoting

© 2014 British Association of Dermatologists

wound healing. At the functional level, there was reduced apoptosis ($P < 0.0001$) and enhancement of wound healing *in vitro* ($P < 0.05$ and $P < 0.0001$ for low and high histamine concentration, respectively). At the receptor level, we identified involvement of all keratinocyte-expressed HRs, with HRH1 blockage resulting in the most prominent effect (percentage of control gap closure, $P < 0.0001$). We conclude that histamine activates wound healing and infection clearance-related functions in keratinocytes. While enhancement of histamine-mediated wound healing is mediated predominantly via the HRH1 receptor, other keratinocyte-expressed receptors may also be involved. These effects could promote resolution of skin inflammation caused by infection or superficial injury.

O16
The role of type 2 innate lymphoid cells in the pathogenesis of atopic dermatitis

M. Salimi,[1] J. Barlow,[2] S. Saunders,[3] L. Xue,[1] D. Gutowska-Owsiak,[1] X. Wang,[4] L.-C. Huang,[1] D. Johnson,[5] S. Scanlon,[2] A. McKenzie,[2] P. Fallon[3] and G. Ogg[1]

[1]*MRC Human Immunology Unit, Oxford University, Oxford, U.K.,* [2]*MRC Laboratory of Molecular Biology, Cambridge, U.K.,* [3]*Trinity Biomedical Sciences Institute, Dublin, Ireland,* [4]*The Fourth Military Medical University, Shaanxi, China and* [5]*Oxford University Hospitals NHS Trust, Oxford, U.K.*

Type 2 innate lymphoid cells (ILC2, nuocytes, NHC) are a distinct population of ILCs that depend on the transcription factors retinoic acid receptor-related orphan receptor (ROR)α and GATA3 for their development. Here we show that human ILC2 cells express skin-homing receptors and infiltrate the skin after allergen challenge, where they contribute to the local production of the type 2 cytokines interleukin (IL)-5 and IL-13. These RORα^+ and GATA3$^+$ ILC2 cells express the IL-33 and IL-25 receptors, which are upregulated during activation and are enriched in lesional skin biopsies from atopic patients compared with healthy individuals (0·044–2·942% vs. 0–0·183%), and after allergen challenge of human skin (1·29- and 6·58-fold infiltration in nonallergic and allergic donors, respectively). Signalling via IL-33 and thymic stromal lymphopoietin (TSLP) induces expression of the effector type 2 cytokines and amphiregulin, and increases ILC2 migration. Using calcipotriol application to wild-type, IL-25R, IL-33R and TSLP receptor knockout models, we showed the dependence of ILC2 on the accompanying cytokines *in vivo*. E-cadherin is downregulated in atopic dermatitis lesions, and we showed that filaggrin knockdown decreases E-cadherin expression in HaCat cells. In addition, we demonstrated that E-cadherin ligation on human ILC2 dramatically inhibits their expression of IL-5 and IL-13. Therefore ILC2 may contribute to atopic inflammation of the skin, and ILC2 inhibition by E-cadherin may be a novel mechanism of sensing of an impaired barrier.Some of these data have been accepted for publication in *Journal of Experimental Medicine*.

O17
Clock genes PER1 and ARNTL modulate human hair follicle melanogenesis

J. Hardman,[1] I. Haslam,[1] Y. Al-Nuaimi,[1] B. Grimaldi,[2] D. Tobin[3] and R. Paus[4]

[1]*The Dermatology Centre, Salford Royal NHS Foundation Trust and the Institute of Inflammation and Repair, Manchester Academic Health Science Centre, University of Manchester, Manchester, U.K.,* [2]*Italian Institute of Technology, Genoa, Italy,* [3]*School of Life Sciences, University of Bradford, Bradford, U.K. and* [4]*Department of Dermatology, University of Münster, Münster, Germany*

The human hair follicle (HF) is a cyclic miniorgan that oscillates through three cycle stages throughout life. Coupled to this is melanogenesis, which leads to the formation of a pigmented hair shaft. However, while the hair cycle will continue to oscillate throughout life, melanogenesis declines with age leading to hair greying, which can have negative psychosocial implications for an individual. Hair pigmentation is a highly choreographed process occurring in those melanocytes found adjacent to the dermal papilla. While many candidates are now appreciated to be involved in hair pigmentation, the intrinsic control of this complex mechanism is yet to be fully uncovered. The recent demonstration that the peripheral molecular clock influences the hair cycle (silencing of which prolongs anagen in cultured HFs) and that melanogenesis is tightly coupled to the hair cycle suggests the possibility that the peripheral molecular clock may also mediate melanogenesis in human HFs. By utilizing a human HF culture model and *in situ* small interfering RNA-mediated gene knock-down, the role of the molecular clock in human melanogenesis was investigated. Immunohistochemical (Masson–Fontana) analysis demonstrated that silencing of core clock members (*ARNTL* or *PER1*) led to an increase in melanin content in anagen HFs. This was followed up by protein and transcript level analysis of the melanocyte marker GP100, showing that immunoreactivity was increased in both silenced groups. Using GP100 it was further possible to assess melanocyte number and dendricity, both of which increased in the gene-silenced groups. Finally, to elucidate how the molecular clock influences melanogenesis, tyrosinase activity was assessed in *ARNTL*- and *PER1*-silenced HFs and compared with a parallel scrambled oligo control. Both the mRNA levels of tyrosinase and enzymatic activity were significantly increased 24 h postsilencing, suggesting that the molecular clock mediates melanogenesis by influencing tyrosinase expression. Subsequent experiments will aim to elucidate the role of the molecular clock in melanogenesis using high-resolution light microscopy and both cell and skin culture models to decipher whether this is restricted to HF melanocytes or occurs in all melanocyte populations.

© 2014 British Association of Dermatologists

O18

Melanin reduces acute mitochondrial superoxide generation: a real-time amperometric study

S.J. Boulton and M. Birch-Machin

Newcastle University, Newcastle upon Tyne, U.K.

Despite its well-known role as a sunscreen molecule, melanin's role as a direct antioxidant is often overlooked. The production of melanin by melanocytes and subsequent transfer of melanosomes to keratinocytes is a key component in the protection of skin from the negative effects of ultraviolet radiation exposure. However, abrogation of damage mediated by reactive oxygen species (ROS) is more often attributed to cellular antioxidants such as glutathione and superoxide dismutase, while the potential contribution by melanin is largely ignored. This work aimed to elucidate the impact of melanin's presence on the mitochondria's capacity to generate ROS. It is known that melanin can act as an intracellular electron transducer and as such the polymer can act in a unique manner to 'mop up' electrons leaked in excess from inhibited or damaged electron transport complexes of dysfunctional mitochondria. These organelles are responsible for 90% of all intracellular-generated ROS. By functioning as an 'electron sponge' it is possible that melanin abrogates the acute generation of proximal ROS superoxide (O_2^-) by preventing the reduction of diatomic oxygen by free high-energy electrons, therefore diminishing intracellular damage and ROS-mediated signalling events. Using an amperometric technique recently described in our group, melanin's capacity to abrogate mitochondrial O_2^- generation was demonstrated for the first time. When incubated with synthetic melanin and subsequently treated with antimycin A, isolated mitochondria from the melanoma cell line CHL1 displayed a significant reduction ($P < 0{\cdot}05$) in complex III-mediated O_2^- formation (maximum current observed $341{\cdot}3 \pm 17{\cdot}93$ pA, $n = 3$) compared with nonmelanin-doped samples (maximum current observed $458{\cdot}0 \pm 50{\cdot}44$ pA, $n = 2$). Furthermore, pretreatment of the isolated mitochondria with melanosomes derived from the moderately pigmented FM55 melanoma cell line prevented O_2^- generation to an even greater extent, further demonstrating melanin's antioxidant effect (maximum current observed $149{\cdot}2 \pm 69{\cdot}79$ pA, $n = 2$). Modulation of O_2^- flux by melanin was also observed using the redox-sensitive fluorescent probes dihydrorhodamine 123 and dichlorofluorescein diacetate in response to inhibition of complexes I, II and III with rotenone, 3-nitroproprionic acid and antimycin A, respectively. This real-time electrical method of selective and highly specific superoxide monitoring has provided a tractable methodology for further studies including the possible mechanism by which melanin may act as an intracellular antioxidant. Application of the technique to mitochondrial scenarios of dysfunction that result in the uncontrolled production of free radicals could provide unparalleled insight towards the mode of cellular pathology in an array of disease states.

O19

Altered BRAF signalling is not necessarily associated with BRAF V600E mutations in either adult or childhood Langerhans cell histiocytosis

J. Selway, P. Harikumar, A. Chu and K. Langlands

Buckingham Institute of Translational Medicine, University of Buckingham, Buckingham, U.K.

The BRAF V600E mutation leads to constitutive signalling of the mitogen-activated protein kinase (MAPK)–extracellular signal-related kinase pathways and is associated with numerous cancers, including melanoma and colon cancer. Recent studies have identified BRAF V600E mutations in approximately 60% of cases of Langerhans cell histiocytosis (LCH), albeit in small cohorts. Moreover, these cohorts primarily consist of childhood disease with a focus on single sites, even when disease is disseminated. We have investigated the prevalence of BRAF V600E mutations in a primarily adult population. Furthermore, we were able to assess biopsies from multiple sites in a subgroup of five individuals, allowing us to assess the clonality of LCH in these cases, and thus establish a causal relationship between mutation and disease. Using genomic DNA and reverse transcription polymerase chain reaction from archival specimens, we found BRAF V600E mutations in only 10% of 21 biopsies. Critically, biopsies from all sites were found to carry the mutation when multiple samples from BRAF-positive individuals were available, consistent with a clonal origin of the disease. We went on to investigate altered BRAF in the context of a low background of specific mutations in adult LCH by examining the transcriptional profiles of a subgroup of patients, using the MetaCore analysis package. MAPK-regulated genes and processes indeed showed significant levels of disruption; for example, mitogenic signalling was significantly impacted when gene expression profiles in lesional cells were compared with normal epidermal Langerhans cell counterparts ($P = 6{\cdot}62 \times 10^{-9}$). In conclusion, we demonstrate that the prevalence of the BRAF V600E mutation in adult LCH appears to be significantly lower than in the childhood population, even though the MAPK pathway is consistently impacted in this disorder. This suggests that BRAF signalling remains a potential therapeutic target in LCH irrespective of an individual patient's mutation status.

© 2014 British Association of Dermatologists

O20

Dense genotyping and human leucocyte antigen imputation identifies two novel loci in late-onset psoriasis

H.L. Hebert,[1] J. Bowes,[1] R.L. Smith,[1] R. Parslew,[2] A. Alsharqi,[2] E. Flynn,[1] N.J. McHugh,[3] J.N.W.N. Barker,[4] C.E.M. Griffiths,[5] A. Barton[1] and R.B. Warren[5]

[1]*ARUK Epidemiology Unit, University of Manchester, Manchester Academic Health Science Centre, Manchester, U.K.,* [2]*Department of Dermatology, Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, U.K.,* [3]*Royal National Hospital for Rheumatic Diseases, University of Bath, Bath, U.K.,* [4]*Division of Genetics and Molecular Medicine, King's College, London, U.K. and* [5]*The Dermatology Centre, Salford Royal NHS Foundation Trust, University of Manchester, Manchester Academic Health Science Centre, Manchester, U.K.*

Psoriasis is a complex inflammatory skin disorder with a genetic component contributing to its aetiology. The most common form of psoriasis, chronic plaque, can be divided into two subtypes according to age at onset: early-onset psoriasis (EOP, < 40 years, approximately 75%) and late-onset psoriasis (LOP, ≥ 40 years, approximately 25%). Genetic studies have so far focused on EOP, identifying 36 loci in genome-wide association studies of white populations, but studies in LOP are lacking. Those that have compared the two types of psoriasis have reported no association of LOP with human leucocyte antigen (HLA)-Cw6 (the main genetic determinant for EOP), while *IL1B* gene variants appear to be exclusively associated with LOP. The aim of this study was to uncover further loci specific to LOP. In total, 543 white patients with LOP and 4373 white unaffected controls were genotyped on the Immunochip, a custom array containing 196 524 markers linked with autoimmune diseases including psoriasis. Samples and markers were subject to stringent quality-control measures based on previously published Immunochip studies (PLINK v1.07). Imputation and stepwise conditional logistic regression were carried out for markers spanning the HLA gene region using the SNP2HLA tool. Associations were considered significant at a threshold of $P < 2{\cdot}3 \times 10^{-5}$, calculated using Genetic type I Error Calculator. Two loci showed significant association that have not been identified in EOP. The first was at *SNRPC* on chromosome 6 [rs9469857, $P = 2{\cdot}82 \times 10^{-12}$, odds ratio (OR) 1·59, 95% confidence interval (CI) 1·40–1·81] and the second was at *IL1R1* on chromosome 2 (rs887998, $P = 8{\cdot}81 \times 10^{-6}$, OR 1·40, 95% CI 1·21–1·62). IL1R1 (interleukin 1 receptor, type 1) is a receptor for IL1β, which we have previously shown to be associated with LOP. SNRPC (small nuclear ribonucleoprotein polypeptide C) is a ribonucleoprotein involved in pre-mRNA splicing. Two loci were identified from the major histocompatibility complex region, the first from HLA-C (rs13191099; $P = 3{\cdot}73 \times 10^{-10}$, OR 1·72, 95% CI 1·46–2·02), which had a much smaller effect size than that seen in EOP (4·32), and the second from HLA-A/*HCG9* (rs2256919; $P = 2{\cdot}54 \times 10^{-6}$, OR 1·38, 95% CI 1·21–1·58). This is the largest study of genetic markers in LOP to date and demonstrates that this form of the disease has a distinct genetic signature from EOP. Future studies will involve validating these associations and finding the causative mutations.

O21

The effects of narrowband ultraviolet B (311 nm) phototherapy on epidermal barrier and differentiation markers in polymorphic light eruption

E.J. Pond,[1] C.A. O'Neill,[1] L.E. Rhodes[2] and N.K. Gibbs[1]

[1]*Centre for Dermatology, Institute of Inflammation & Repair, University of Manchester, Manchester, U.K. and* [2]*Photobiology Unit, Salford Royal NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, U.K.*

The exact aetiology of polymorphic light eruption (PLE) is unknown, but is thought to involve a delayed-type IV immunological reaction to unknown endogenous or exogenous 'photoallergens' formed after skin exposure to ultraviolet radiation (UVR). Paradoxically, it has been found that narrowband (NB) UVB phototherapy (PT) reduces the severity of PLE symptoms. The mechanisms of successful NB-UVB PT in PLE are unknown, and the histological effects of PT on PLE skin have not been reported. We therefore investigated epidermal structure, proliferation and differentiation in the skin of patients with PLE (n = 6, skin types I–III) before and after NB-UVB PT. Patients received whole-body NB-UVB PT (Philips TL-01, peak 311 nm) for 15 sessions over 5 weeks. Exposure doses started at 70% of the individual patient's minimal erythemal dose and increased in approximately 20% increments over the treatment course. Biopsies were taken from PLE skin before and after the PT course. Haematoxylin and eosin was used to stain epidermal structure, and immunofluorescence of keratin-14 (K14), K16 and Ki67 was used to investigate proliferation. The markers K10 and filaggrin were used to investigate differentiation. Results showed that the mean overall thickness of the epidermis was significantly reduced after PT ($P < 0{\cdot}001$). In contrast, the stratum corneum (SC) was significantly thicker ($P < 0{\cdot}05$) after PT. The increased SC thickness, but reduction of overall epidermal thickness, was explained by the finding that individual keratinocyte cell thicknesses were significantly thinner after PT ($P < 0{\cdot}05$). Expression of the proliferation marker K16 was significantly increased ($P < 0{\cdot}001$) after PT. K14, a marker of the proliferating basal layer, was also increased, but not significantly. However, the K14 expression was localized over more of the epidermis after PT ($P < 0{\cdot}05$). Furthermore, Ki67 was also increased, but not significantly. A marker of a mature, stratified epidermis, K10, was reduced (not significantly) after PT, whereas the terminal differentiation marker filaggrin was significantly reduced ($P < 0{\cdot}001$) after PT. These results suggest that NB-UVB PT has profound effects on epidermal morphology, differentiation and proliferation in PLE. The increase in SC thickness may compensate for the decrease in overall epidermal thickness and result in both increased photoprotection and a strengthened barrier against exogenous 'photoallergen' ingress.

© 2014 British Association of Dermatologists

O22
Molecular surgery for epidermolysis bullosa simplex

M. Aushev,[1] C. Mussolino,[2] T. Cathomen,[2] H. Torma[3] and J. Reichelt[1]
[1]Newcastle University, Newcastle, U.K., [2]University Medical Centre Freiburg University, Freiburg, Germany and [3]Uppsala University, Uppsala, Sweden

Inherited bullous skin disorders are characterized by blistering after minor trauma. Epidermolysis bullosa simplex (EBS) is the most common subtype of this type of disease. EBS is caused by heterozygous dominant–negative mutations in either the keratin 5 (KRT5) or keratin 14 (KRT14) genes. Mutant keratins integrate into the intermediate filament cytoskeleton, impairing filament resilience and resulting in skin fragility. Currently there is no cure for this spectrum of diseases. Gene therapy using molecular scissors (designer nucleases) to eliminate the disease-causing mutations is among the most promising approaches. We have previously shown that zinc-finger nucleases (ZFNs) can be used to inactivate an enhanced green fluorescent protein transgene in murine keratinocyte stem cells with 18% efficiency and without impairment of stem-cell properties. Transcription activator-like effector nucleases (TALENs) are a new generation of designer nucleases showing considerably higher site specificity than ZFNs. We aim to develop an *ex vivo* gene therapy for EBS using TALEN technology to inactivate the mutant *KRT5* allele. *KRT5*-specific TALEN plasmids are transiently transfected into keratinocytes where TALENs are expressed, and bind specifically to the target site creating a double-strand break (DSB). These DSBs are recognized by the cellular error-prone nonhomologous end-joining repair system, resulting in a high number of frameshift mutations causing nonsense-mediated mRNA decay and thus functional inactivation of the targeted *KRT5* allele. Our gene therapy approach using *KRT5*-specific TALENs is being validated in two immortalized patient-derived EBS keratinocyte lines carrying two distinct *KRT5* mutations causing either a mild or severe phenotype (EB21 and EB11, respectively). Immunofluorescent staining of K5 showed that both EBS keratinocyte lines display the disease phenotype characterized by fragile intermediate filaments in cell culture. *KRT5*-specific TALENs were designed and engineered using the Golden Gate assembly method. Keratinocytes were transfected using Xfect and grown clonally. Target-site modifications of individual clones are identified by pyrosequencing of allele-specific polymerase chain reaction products. Correctly modified clones are analysed by immunofluorescence and Western blotting for successful inactivation of the mutant *KRT5* allele.

O23
Characterization of SPINK5-transduced keratinocytes under good manufacturing practice conditions for Netherton syndrome

A. Petrova,[1] W.-L. Di,[1] W. Qasim,[1] L. Chan,[2] F. Farzaneh,[2] A. Thrasher[1] and J. Harper[1]
[1]Institute of Child Health, UCL, London, U.K. and [2]King's College London, London, U.K.

Netherton syndrome (NS) is a rare but debilitating autosomal recessive skin disorder, caused by mutations in the *SPINK5* gene. Clinically it is characterized by defective keratinization, hair-shaft defects, recurrent infections, atopy and a predisposition to skin malignancies. The prognosis is often poor, with high risk of life-threatening complications such as bronchopneumonia, sepsis and hypernatraemic dehydration secondary to severe water loss through the defective skin barrier. Currently, there is no curative treatment for this condition. We have previously reported the development of procedures for the production of lentiviral vector stocks and epithelial skin sheets under good manufacturing practice conditions. Concentrated lentiviral vector-encoding *SPINK5* was produced using a third-generation packaging system, which segregates gag/pol, rev and envelope genes from the therapeutic viral genome. Characterization of vector stocks included sterility, endotoxin testing, exclusion of adventitious pathogens and screening for replication-competent lentivirus. Here we report characterization of NS keratinocytes genetically modified with clinical grade vector. Keratinocytes from a mutation-proven patient with NS were isolated and expanded on irradiated feeder cells before being transduced with the *SPINK5* vector by a single round of vector exposure. The modified cells were seeded onto fibrin gels populated with autologous fibroblasts. The fibrin gels were then grafted onto artificially made wounds on nude mice for human–murine chimeric studies. In parallel, vector-mediated gene expression was confirmed at a molecular level using Western blotting and *in situ* staining of *SPINK5*-transduced NS keratinocytes. Vector copy number was measured by quantitative polymerase chain reaction using a standardized assay. These results indicate that autologous keratinocytes from patients with NS can be isolated, genetically modified and cultured as epithelial sheets for subsequent grafting. The procedures developed have been approved by the U.K. Medicines and Healthcare products Regulatory Agency, and the first-in-man clinical trial has been authorized to start in January 2014.

O24
Induction of dermal papilla properties in highly potent CD271+ CD90+ mesenchymal stem cells generated from human-induced pluripotent stem cells

O. Veraitch,[1] Y. Mabuchi,[2] Y. Matsuzaki,[3] M. Amagai,[1] H. Okano[4] and M. Ohyama[1]
[1]Department of Dermatology, Keio University, Tokyo, Japan, [2]Department of Biochemistry and Biophysics, Tokyo Medical and Dental University, Tokyo, Japan, [3]Division of Molecular and Cellular Biology, Tokyo Medical University, Tokyo, Japan and [4]Department of Physiology, Keio University, Tokyo, Japan

In our attempts to regenerate human hair follicles (HFs), we have been devising approaches to utilize human-induced pluripotent stem cells (hiPSCs). Recently, we successfully regenerated HF epithelial component using hiPSC-derived epithelial precursors. However, preparation of dermal papilla (DP) cells, an indispensable mesenchymal element for HF induction, from hiPSCs remains a challenge. Considering their multipotency, hiPSC-derived mesenchymal stem cells (hiPSC-MSCs) may be

© 2014 British Association of Dermatologists

programmed into DP cells. In this study, we established an original protocol to induce hiPSC-MSCs enriched for the highly proliferative and multipotent CD271+ CD90+ subset. Subsequently, CD271+ CD90+ hiPSC-MSCs were assessed for their capacity to differentiate into DP cells. MSC induction was performed by culturing hiPSCs in MSC medium supplemented with recombinant platelet-derived growth factor, transforming growth factor-β and fibroblast growth factor (FGF). The resultant cells uniformly expressed the human MSC markers CD90, CD166, CD44 and CD29 at levels similar to bone-marrow-derived MSC controls, could be serially passaged, and differentiate into osteocytes, adipocytes and chondrocytes, indicating that they were hiPSC-MSCs. Importantly, approximately 10–25% of hiPSC-MSCs were found to be CD271+ CD90+, at a rate more than 100-fold higher than that observed in bone marrow-derived MSCs. CD271+ CD90+ hiPSC-MSCs demonstrated greater clonogenicity and ability to differentiate into three mesenchymal lineages than CD271–CD90+ hiPSC-MSCs. When CD271+ CD90+ hiPSC-MSCs were exposed to retinoic acid and activators for the Wnt, bone morphogenetic protein and FGF pathways, which are crucial for DP property maintenance, they demonstrated DP-cell-like morphology and expressed classic (*ALPL*, *LEF1*, *WIF1*, *HEY1*, *NOG*) and recently added (*RGS2*, *SPRY4*, *LRP4*, *EDN3*, *BAMBI*, *GUCY1A3*) human DP signature genes, suggesting successful induction of DP substitute cells (DPSCs) from hiPSCs. To assess further whether hiPSC-DPSCs could mimic epithelial–mesenchymal interactions in the HF bulb, hiPSC-DPSCs were cocultured with human keratinocytes. Impressively, hiPSC-DPSCs exhibited DP properties in increasing expression of the HF-related genes *LEF1*, *TRPS1*, *MSX2* and *KRT75* in keratinocytes, and autonomously upregulating the DP markers *ALPL*, *LEF1*, *BMP4* and *IGF1*. Furthermore, these bidirectional interactions were potentiated by addition of minoxidil. In conclusion, the current study provides an efficient protocol to generate well-defined and potent MSC populations from hiPSCs and suggests the possibility of using these for generation of dermal cells with hair-inductive activity. This may prove a useful tool for new drug discovery for hair diseases and, eventually, facilitate regeneration of human HFs from hiPSCs.

O25
Lysophosphatidic acid-mediated signalling during human epidermal keratinocyte migration

D. Johnson,[1] C. Jahoda[2] and N. Reynolds[1]
[1]*Newcastle University, Newcastle upon Tyne, U.K. and* [2]*Durham University, Durham, U.K.*

Defects in cutaneous wound healing constitute a major health burden, highlighting the requirement for the identification of potential therapeutic targets for the treatment of acute and chronic wound healing. The lysophospholipid signalling mediator, lysophosphatidic acid (LPA) is released by platelets into acute wounds and is detectable in blister fluid. LPA has previously been shown to illicit promigratory responses in human epidermal keratinocytes (HEKs) via the activation of Ca^{2+} dependent activation of nuclear factor of activated T cells (Jans R, Mottram L, Johnson DL *et al.* Lysophosphatidic acid promotes cell migration through STIM1- and Orai1-mediated Ca^{2+} mobilization and NFAT2 activation. *J Invest Dermatol* 2013; **133**: 793–802), and has been shown to promote re-epithelialization *in vivo* in rodent skin (Demoyer JS, Skalak TC, Durieux ME. Lysophosphatidic acid enhances healing of acute cutaneous wounds in the mouse. *Wound Repair Regen* 2000; **8**: 530–7). As LPA activates β-catenin signalling in epithelial cells, and β-catenin signalling has been linked to the regulation of wound healing responses (Badri L, Lama VN. Lysophosphatidic acid induces migration of human lung-resident mesenchymal stem cells through the β-catenin pathway. *Stem Cells* 2012; **30**: 2010–19), we investigated the interaction between LPA wound healing and β-catenin signalling in HEKs. Scratch wounding of HEK monolayers activated β-catenin phosphorylation, which was detected by Western blotting (maximal at 30 min, n = 3). Consistent with this finding, wounding also increased activity of the β-catenin-dependent luciferase reporter plasmid TOPflash at similar time points (1·5-fold increase, one-way ANOVA with Dunnett's post hoc test, $P < 0{\cdot}05$, n = 6). In contrast, LPA treatment of HEKs also induced TOPflash activity but with a delayed time course, with phosphorylation still detectable at 24 h (twofold increase, $P < 0{\cdot}05$, one-way ANOVA with Dunnett's post hoc test, n = 3). To study the functional interaction between LPA and β-catenin-mediated re-epithelialization, we targeted the upstream regulator of β-catenin, glycogen synthase kinase (GSK)-3. We determined the effect of the GSK-3 inhibitor CT99021 on HEK migration by scratch wounding cellular monolayers, and measured the rate of wound closure using real-time imaging over 24 h. As expected, LPA induced rapid wound closure, and treatment with CT99021 blocked LPA-induced HEK migration completely (two-way ANOVA, Bonferroni post test, $P < 0{\cdot}001$, n = 6). In conclusion, these data further support a key role for LPA in regulating epithelial wound healing and show a complex time-dependent interaction between wounding and GSK-3/β-catenin signalling.

O26
Hidradenitis suppurativa: haploinsufficiency of γ-secretase components does not affect γ-secretase enzyme activity *in vitro*

A. Pink,[1] D. Dafou,[1] N. Desai,[2] O. Holmes,[3] C. Smith,[3] P. Mortimer,[4] M. Simpson,[1] R. Trembath[1] and J. Barker[1]
[1]*King's College London, London, U.K.,* [2]*St. John's Institute of Dermatology, Guy's & St Thomas' NHS Trust, London, U.K.,* [3]*Harvard Medical School, Boston, MA, U.S.A. and* [4]*St Georges University of London, London, U.K.*

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease that presents with painful cysts and abscesses in flexural areas. It can be inherited in an autosomal dominant manner, and heterozygous mutations have been reported in the -secretase genes *NCSTN*, *PSENEN* and *PSEN1*. The aim of this study was to identify the molecular mechanisms by which two previously identified -secretase gene mutations (*NCSTN* c.1125+ 1 G>A and *PSENEN* c.66_67insG) result in HS. Immunohistochemical analysis of axillary skin harvested from

 © 2014 British Association of Dermatologists

unaffected volunteers and mutation-positive patients revealed colocalization of nicastrin (NCSTN) and presenilin enhancer (PSENEN) in the hair follicle, sebaceous gland, apocrine gland and epidermis, with additional staining in fibroblasts and inflammatory infiltrates. *NCSTN* c.1125 + 1 G>A was found to result in aberrant splicing and skipping of *NCSTN* exon 9 (predicted protein p.Glu333_Gln367del). *PSENEN* c.66_67insG was predicted to result in a frameshift and an altered protein product (p.Phe23ValfsX98). *NCSTN* and *PSENEN* mRNA expression in dermal fibroblasts was reduced in respective mutation-positive patients vs. healthy volunteers. Treatment of mutant dermal fibroblasts with cycloheximide (for 6 and 24 h) incrementally increased mRNA expression, suggesting that the mutant alleles are subject to nonsense-mediated decay. Immunoblotting analysis revealed reduced NCSTN and PSENEN expression in respective mutant dermal fibroblasts compared with wild-type control fibroblasts. Enzyme activity assays were performed using solubilized cell-membrane protein derived from primary human fibroblasts *in vitro* (solubilized γ-secretase preparation). There was no significant difference in complex number, maturity or endopeptidase or carboxypeptidase-like enzyme activity in samples harvested from mutation-positive patients vs. unaffected volunteers. Overall, these studies revealed that both NCSTN and PSENEN are expressed and colocalize in the skin, and that haploinsufficiency of the γ-secretase components underlies some cases of HS. This conferred no demonstrable affect on γ-secretase enzyme activity *in vitro* under the conditions studied. However, haploinsufficiency may potentially be sufficient to affect enzyme activity under conditions of stress, which, in flexural sites, may include alterations in temperature, pH and salinity, and this hypothesis is worthy of further exploration.

O27
The sebaceous gland as a cycling skin appendage

E. Hinde,[1] I. Haslam,[1] M. Schneider,[2] T. Reis-Matos,[1] A. Imanishi,[1] K. Kawai,[3] J. Kloepper[4] and R. Paus[1]

[1]*University of Manchester, Manchester, U.K.,* [2]*Ludwig-Maximilians University, Munich, Germany,* [3]*Kagoshima University, Kagoshima, Japan and* [4]*Luebeck University, Luebeck, Germany*

Whether cross-talk occurs between the hair follicle (HF) and the sebaceous gland (SG), and whether one of these compartments of the pilosebaceous unit 'controls' the other, are long-standing questions, yet to be answered. It is well established that the HF and the SG act codependently, and that changes in HF cycle stage coincide with changes in skin architecture, namely thickness of the epidermis, dermis and the subcutis. However, the effect of HF cycling on the SG has been relatively neglected, and conflicting reports on this cloud the picture. The aim of this study was to assess quantitatively whether morphological and activity changes occur within the SG in conjunction with HF cycling. C57BL/6 mice were anagen induced by depilation during telogen and analysed post-depilation (PD) at days 1, 8, 17, 19, 21 and 32 in order to study SG morphology and enzyme activity/protein expression during one full HF cycle. Quantitative (immuno)histomorphometry showed that SG morphology changed significantly throughout the HF cycle, with smaller SGs, fewer sebocytes and smaller sebocytes found in telogen skin (days 1 and 32 PD, $P < 0{\cdot}001$), with the largest SGs, sebocytes and numbers of sebocytes found in mid-anagen skin (day 8 PD). Sebocyte proliferation *in situ* (Ki67 immunohistochemistry) was significantly increased from telogen to mid-anagen (day 8 PD, $P < 0{\cdot}05$) with similar levels maintained thereafter. Fatty acid synthase was maximally expressed during mid-anagen, and perilipin-2 was found to be highly expressed during mid-anagen and late catagen. Inversely, and surprisingly, lipid production assessed by Oil Red O histochemistry was greatest during the telogen and late anagen stages (day 17 PD, $P < 0{\cdot}001$). Thus, SG morphology and activities are strikingly dependent upon HF cycling, suggesting the possibility of an HF-controlled 'SG cycle'. That most SG morphological and activity changes reach a maximum during mid-anagen suggests that the SG reaches its maximal size and activity before the HF, indicating that the SG cycle and the HF cycle are closely coordinated, staggered events. We are currently examining whether this also occurs during spontaneous HF cycling. Moreover, future studies of SG biology need to take the HF cycle stage into account to avoid misleading interpretations of changes in SG morphology or function observed in test vs. control groups.

O28
Global transcriptomic changes in primary keratinocytes with loss of basement membrane genes

S. Marsh,[1] V. Martins,[1] M. Caley,[1] M. Barnes,[2] M. Donaldson[3] and E. O'Toole[1]

[1]*Centre for Cutaneous Research and* [2]*Genome Centre, Barts and the London SMD, Queen Mary University of London, London, U.K. and* [3]*Stiefel, a GSK company, Uxbridge, U.K.*

The basement membrane in skin has an essential structural role in aiding the tight attachment of the epidermis to the underlying dermis. Mutations in the genes encoding basement membrane proteins, such as *COL7A1*, *COL17A1* and *LAMA3*, result in the severe blistering disease epidermolysis bullosa. In this study we performed knock-down of the basement membrane proteins collagen IV, collagen VII, collagen XVII and the laminin 3 chain of laminin-332 in keratinocytes. Transient knock-down (lasting up to 10 days) was generated in primary foreskin keratinocytes using small interfering RNA and lentiviral short hairpin RNA. Stable knock-down was also performed in a telomerase-immortalized keratinocyte line. In order to analyse the role of the basement membrane in skin, three-dimensional organotypic cultures on a fibroblast-populated collagen gel were produced with keratinocytes with both stable and transient basement membrane protein knock-down. Basement membrane protein loss caused altered differentiation of the three-dimensional organotypic cultures, as determined by immunofluorescence staining of the epidermal differentiation markers cytokeratin 10 and involucrin. In addition, three-dimensional organotypic cultures of keratinocytes with loss of

© 2014 British Association of Dermatologists

collagen VII displayed blistering at the dermoepidermal junction. In order to analyse further the role of the basement membrane in cellular processes in the skin, we performed RNA sequencing analysis of the transcriptome of keratinocytes with loss of the above basement membrane proteins. Interestingly, knockdown of collagen IV produced relatively few gene expression changes, consistent with no known associated skin disease phenotype. A transcriptomic signature containing inflammatory cytokines and extracellular matrix genes was seen with knockdown of collagen VII, collagen XVII and the laminin 3 chain. Changes were validated in the proteases coded by *ADAM19*, *MMP15*, *SPARC* (a known regulator of wound healing and cancer) and *THY1* (involved in Wnt signalling). These data suggest that loss of basement membrane upregulates genes that accelerate matrix turnover facilitating epithelial cell invasion.Funding sources: BBSRC CASE studentship with Stiefel, a GSK company.

O29

The role of store-operated calcium entry in epidermal wound healing responses

L. Mottram,[1] M. Begg[2] and N. Reynolds[1]

[1]Newcastle University, Newcastle upon Tyne, U.K. and [2]GlaxoSmithKline, Stevenage, U.K.

Dysregulation of processes involved in cutaneous injury repair and abnormalities in epidermal proliferation and migration may impair wound closure and result in chronic wounds. An epidermal calcium gradient exists in skin, and the role of calcium in normal human epidermal keratinocyte (NHEK) growth and differentiation is established. Calcium release from internal stores, store-operated calcium entry (SOCE) and calcium oscillations have been in implicated in regulation of cell migration and proliferation. However, mechanisms regulating calcium signalling following wounding of NHEK have not been fully characterized. Live cell confocal imaging of Fluo-4-loaded NHEKs showed an immediate increase in the concentration of intracellular Ca^{2+} – $[Ca^{2+}]_i$ – at the wound edge, which then spread rapidly away from the wound edge as a calcium wave; the $[Ca^{2+}]_i$ gradually returned to baseline within 2 min. Cells at the wound edge showed the greatest rate of rise in $[Ca^{2+}]_i$; this rate of rise gradually decreased in cells progressively further back from the wound edge. Wounding in extracellular Ca^{2+} concentration – $[Ca^{2+}]_o$ – at 1·2 mmol L^{-1}, but not 0·06 mmol L^{-1}, resulted in later $[Ca^{2+}]_i$ signals after the initial wave, which were in the form of $[Ca^{2+}]_i$ oscillations. An average of 25·4 ± 1·5% of cells oscillated postwounding in 1·2 mmol L^{-1} Ca^{2+} compared with 0·87 ± 1·5% in 0·06 mmol L^{-1} ($P < 0·05$) over a 20-min imaging period. To confirm that wound-induced oscillations were dependent upon $[Ca^{2+}]_o$, NHEKs were scratched in 0 mmol L^{-1} or 0·06 mmol L^{-1} $[Ca^{2+}]_o$ and 4 min postwounding $[Ca^{2+}]_o$ was 'added-back' to a final concentration of 1·2 mmol L^{-1} $[Ca^{2+}]_o$. Upon 'add-back' of 1·2 mmol L^{-1} $[Ca^{2+}]_o$ to 0 mmol L^{-1}, oscillations were detected in 44·3 ± 2·1% of cells vs. 28·4 ± 1·3% following 'add-back' to 0·06 mmol L^{-1} $[Ca^{2+}]_o$. These results indicate that wound-induced calcium oscillations are dependent on calcium entry from external media, supporting the theory that there is an endoplasmic reticulum depletion mechanism feeding back to plasma membrane calcium pumps. To examine the potential role of SOCE, NHEKs were treated with the SOCE inhibitor, GSK-7975A. SOCE inhibition itself did not induce calcium oscillations. Moreover, pretreatment with GSK-7975A effectively blocked wound-induced calcium oscillations upon 1·2 mmol L^{-1} $[Ca^{2+}]_o$ 'add-back'. Together these data show that calcium oscillations postwounding occur through SOCE-mediated mechanisms. Future work will determine the role of SOCE and wound-induced calcium oscillations in regulating NHEK migration and wound closure.

O30

Sclerostin domain containing 1 downregulation is required for altered transforming growth factor-β responsiveness and increased cell migration in linear morphoea fibroblasts

S. Brown,[1,2] L. Weibel,[3] B. Way,[1,2] J. Harper[12] and R. O'Shaughnessy[1,2]

[1]UCL Institute of Child Health, London, U.K., [2]Livingstone Centre for Skin Research, London, U.K. and [3]Dermatology, University of Zurich, Zurich, Switzerland

Scleroderma is an autoimmune disease characterized by excessive extracellular matrix deposition in the skin, which if unresolved can result in destruction of underlying tissue and bone. Although the autoimmunity can be treated with immunosuppressants, the underlying disease commonly recurs, suggesting a persistent site-specific structural disorder. A better understanding of the molecular mechanisms underlying this disorder would lead to more directed treatments for scleroderma. To this end, we investigated linear morphoea (LM), a rare scleroderma subtype that appears in a well-demarcated banded pattern, commonly on the torso, limbs or head, with an appearance and inheritance suggesting that developmental mosaicism contributes to disease. To identify fibroblast-intrinsic disease mechanisms related to disease, we isolated fibroblasts from affected skin sites, site matched unaffected control sites, and compared their biology and gene expression. LM fibroblasts showed stable differences in migration in scratch assays, greater tolerance of contact inhibition, and insensitivity to transforming growth factor (TGF)-β-mediated growth arrest. Switching conditioned media between cell populations suggested that signalling by secreted factors may be controlling these phenotypes. Analysis of the LM fibroblast transcriptome found common downregulation of sclerostin domain containing (SOSTDC)1, a multivalent secreted signalling protein that alters Wnt, bone morphogenetic protein and TGF-β signalling. Downregulation of SOSTDC1 in fibroblasts recapitulated both the TGF-β insensitivity and the increased migration of LM fibroblasts. Taken together, this suggests two novel therapeutic avenues for the treatment of scleroderma: reintroduction of unaffected fibroblasts to an affected site and re-expression of SOSTDC1.

 © 2014 British Association of Dermatologists

O31
Parakeratosis causes profound changes in epidermal terminal differentiation

A. Naeem,[1] B. Verney,[1] S. Marmiroli[2] and R. O'Shaughnessy[1]

[1]*UCL Institute of Child Health, London, U.K. and* [2]*School of Medicine, University of Modena and Reggio Emilia, Modena, Italy*

Nuclear degradation is a key stage in keratinocyte terminal differentiation and the formation of the cornified envelope that comprises the majority of epidermal barrier function. Parakeratosis, the retention of nuclear material in the cornified layer of the epidermis, is a common histological observation in many skin diseases, but most notably in the epidermal barrier-defective diseases eczema and psoriasis. Nuclear degradation has not yet been well characterized and it is not known whether the retained nuclei contribute to the altered epidermal differentiation seen in eczema and psoriasis. Loss of *AKT1* function strongly correlated with parakeratosis both in pathological samples and in organotypic culture models, and also with reduced expression of both loricrin and filaggrin, key components of the keratinocyte cornified envelope. Although levels of DNAse 1L2, known to degrade the nucleus in differentiating keratinocytes, were unchanged, proteomic analysis revealed an increase in lamin A/C (LMNA) in differentiated keratinocytes with reduced *AKT1* activity. AKT is known to phosphorylate LMNA, targeting it for degradation. Consistent with this, LMNA was not being degraded during epidermal terminal differentiation, and LMNA was observed in the cornified layer of *AKT1* knockdown organotypic cultures, surrounding nuclear material. Using AKT-phosphorylation-dead LMNA constructs in our culture model we show that the retention of nuclear material by the prevention of LMNA degradation is sufficient to cause profound changes in epidermal terminal differentiation, specifically a reduction in loricrin, keratin 1 and keratin 10, and reduction and altered proteolytic processing of filaggrin. We therefore present a model where, in the absence of LMNA phosphorylation, passive DNA degradation processes, such as those mediated by DNAse 1L2, are prevented, leading to parakeratosis and changes in epidermal differentiation, which we propose are caused by continued aberrant transcription in the retained nuclei.

O32
Modelling melanoma metastasis using organotypic skin equivalent and zebrafish models

D. Hill,[1] S. Verykiou,[1] N. Robinson,[2] S. Przyborski,[3] J. Armstrong,[4] B. Chaudhry[1] and P. Lovat[1]

[1]*Newcastle University, Newcastle upon Tyne, U.K.,* [2]*Durham University, Durham, U.K.,* [3]*Reinnervate Ltd, Sedgefield, U.K. and* [4]*University of Sunderland, Sunderland, U.K.*

Accumulating evidence confirms the influence of microenvironmental factors on cancer cell behaviour. Currently, mouse models of melanoma are the only way to recreate features such as cellular proximity, nutrient availability and three-dimensional orientation. However, these are limited by their expensive and time-consuming nature, as well as by their ability to model only later stages of melanoma progression and only in humans. The aim of the present study was thus to develop improved models to monitor all stages of human melanoma growth, invasion and metastasis. To this aim we have created an *in vitro* full-thickness human melanoma skin equivalent (MSE) by seeding neonatal dermal fibroblasts onto a polymer scaffold (Alvetex) to create a dermal equivalent layer prior to the inclusion of keratinocytes and melanoma cells. Immunofluorescence studies confirmed the presence of collagens I and III throughout the dermal layer, and collagen IV at the dermoepidermal junction, indicating that fibroblasts produce their own extracellular matrix, which increases the longevity of this model. In addition, immunofluorescence studies confirmed the expression of cytokeratin XIV by basal layer keratinocytes, while cytokeratin I and involucrin are expressed by keratinocytes in the stratum spinosum/granulosum and stratum corneum, respectively, indicating the formation of a viable basal layer and differentiation of keratinocytes into an intact epidermis. Melanoma cells expressing melan-A formed nests at the dermoepidermal junction, which expand over time and invade through the basement membrane, demonstrating the ability of this MSE to recreate the natural skin microenvironment, and its applicability to investigate early stages of melanoma growth and invasion. To investigate migration/invasion in an *in vivo* microenvironment, as well as secondary tumour formation, we have also developed a zebrafish xenograft of human melanoma (ZMX) by microinjecting DiI-labelled melanoma cells into the yolk sac of 2-day-old anaesthetized fetal liver kinase-1–green fluorescent protein-tagged '*casper*' zebrafish embryos. Confocal fluorescence microscopy studies of melanoma cell migration/invasion *in situ* for up to 3 days revealed invasion of melanoma cells throughout the yolk sac and blood vessels, as well as movement of cells to the head and tail via the bloodstream, confirming the applicability of this ZMX for the investigation of melanoma metastasis *in vivo*. Collectively, the combined use of these *in vitro*/*in vivo* models allows for the investigation of melanomagenesis, early invasion and metastasis in a representative microenvironment. This is an ideal approach through which to study deregulated cell signalling in melanoma and the validation of novel therapeutic targets for generation of improved personalized treatment strategies.

O33
Dissecting the regulation of the inactive rhomboid protein 2/ADAM17 pathway in keratinocyte differentiation and barrier function

A. Chikh, M. Brooke, S. Etheridge and D. Kelsell

Blizard Institute, Queen Mary University of London, London, U.K.

We have recently described that mutations in *RHBDF2*, the gene encoding inactive rhomboid protein (iRHOM)2, are associated with the syndrome tylosis, with palmoplantar keratodermas and with a squamous oesophageal cancer, oral squamous cell carcinoma (Blaydon DC, Etheridge SL, Risk JM *et al.* *RHBDF2* mutations are associated with tylosis, a familial esophageal cancer syndrome. *Am J Hum Genet* 2012; **90**: 340–6).

© 2014 British Association of Dermatologists

iRHOM2 is a key regulator of ADAM17 and epidermal growth factor functions, with dominant iRHOM2 mutations leading to increased ADAM17 activity and associated substrate 'shedding', including the growth factor amphiregulin, cytokines such as tumour necrosis factor- and the transglutaminases (Brooke *et al.*, submitted for publication). Towards identifying the molecular mechanisms controlling the iRHOM2–ADAM17 pathway, we investigated putative regulators using the bioinformatics software from Genomatix to analyse the ADAM17 sequence from the University of California, Santa Cruz genome browser (http://genome.ucsc.edu), and found that ADAM17 contains a putative p63-like responsive consensus binding site in its promoter, but not in iRHOM2. Thus, we hypothesized that ADAM17 is a direct transcriptional target of p63. Immunohistochemical analysis showed an increased expression of p63 in the nuclei of the basal and suprabasal layers of tylotic epidermis compared with normal epidermis. Furthermore, p63 expression was increased in the immortalized tylotic keratinocytes compared with matched normal keratinocytes, as shown by immunoblot analysis. Depletion of p63 by small interfering (si)RNA significantly reduced levels of the endogenous ADAM17 protein. As it has been shown that the E3 ubiquitin ligase Itch controls the protein stability of p63, we investigated whether Itch affects ADAM17 expression, and discovered that Itch siRNA reduces both ADAM17 and iRHOM2 expression. Furthermore, to explore a link between Itch and iRHOM2/ADAM17, their respective interactions were shown by coimmunoprecipitation. These data demonstrates links between p63/Itch and the iRHOM2–ADAM17 pathway. The elucidation of novel regulators might provide further insights into the regulation of differentiation, wound healing and barrier function, in addition to future design of therapeutic approaches in oesophageal squamous cell cancer.

O34
Mutations in the grainyhead-like 2 transcription factor result in a novel autosomal recessive ectodermal dysplasia syndrome

G. Petrof,[1] A. Nanda,[2] M.A. Simpson,[3] T. Takeichi,[4] J. McMillan,[5] L. Ozoemena,[5] R. Begum,[6] H. Al-Ajmi,[2] M. Parsons[6] and J.A. McGrath[1]

[1]St John's Institute of Dermatology, King's College London, Guy's Hospital, London, U.K., [2]As'ad Al-Hamad Dermatology Center, Al-Sabah Hospital, Kuwait City, Kuwait, [3]Department of Genetics and Molecular Medicine, King's College London, Guy's Hospital, London, U.K., [4]Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan, [5]The National Diagnostic EB Laboratory, GSTS Pathology, St Thomas' Hospital, London, U.K. and [6]Randall Division of Cell and Molecular Biophysics, King's College London, Guy's Hospital, London, U.K.

Grainyhead-like 2, encoded by *GRHL2*, is one of three members of the highly conserved family of transcription factors that play essential roles in epidermal morphogenesis during embryonic development. A heterozygous nonsense mutation in *GRHL2* has previously been identified in a pedigree with autosomal dominant deafness, but no link to skin defects has been described. We investigated two unrelated but consanguineous Kuwaiti families in whom a total of three affected individuals had lifelong ectodermal pathology. The clinical features comprised nail thickening or nail loss, acral skin wrinkling/atrophy, focal palmoplantar keratoderma, hypoplastic dental enamel and a smooth geographical tongue with hyperpigmentation. In addition, one individual had sensorineural deafness. There was no blistering or impaired wound healing and none of the parents had any skin abnormalities or deafness. To identify the genetic basis of this ectodermal dysplasia syndrome, genomic DNA from the affected individuals was analysed by whole-exome sequencing. Sixteen novel homozygous mutations were identified (10 in one pedigree and six in the other), although the only gene containing novel variants in both was *GRHL2*. In both pedigrees there was a block of approximately 25 cM of homozygosity surrounding different novel homozygous missense mutations in *GRHL2*: c.1445T>A in exon 11, p.Ile482Lys; and c.1192T>C in exon 9, p.Tyr398His. Quantitative polymerase chain reaction amplification of cDNA from patient skin (p.Tyr398His) revealed a significant reduction in expression of *GRHL2*, as well as known targets of this transcription factor (*CDH1*, *CLDN4* and *TERT*). Skin histology showed mild acanthosis and hyperkeratosis, and electron microscopy revealed subtle widening of the intercellular spaces between keratinocytes with some desmosomes appearing small in size. Immunofluorescence for basement membrane and tight junction proteins showed similar expression compared with control skin. Keratinocytes isolated from one subject (p.Ile482Lys) showed altered cell morphology adhesion defects. Using whole-skin transcriptomics from two individuals we identified significant upregulation of 31 genes and downregulation of 47 genes implicated in cell adhesion and cell matrix adhesion process networks. Collectively, we report a novel ectodermal dysplasia syndrome that reveals a new role for *GRHL2* in skin development and homeostasis.

 © 2014 British Association of Dermatologists