DOI: 10.1111/pcmr.12622

PS.01.01 | Modeling of neural crest diseases in mouse – Human chimeras

Rudolf Jaenisch

Whitehead Institute for Biomedical Research and Department of Biology, MIT, Cambridge, MA, USA

The greatest promise of the iPS (Induced Pluripotent Stem) technology is its potential to study human diseases in the Petri dish. In this approach patient-derived cells are differentiated into cells affected in the patient with the goal to uncover a disease relevant phenotype in the dish. However, because numerous human diseases originate already in embryogenesis, i.e. are caused by disturbances of developmental processes, such an approach cannot recapitulate the developmental aspects of a disease because the test cells are not incorporated into the developing embryo and do not participate in normal developmental processes. Thus, a major challenge of the "disease in the dish" approach is establishing model systems that, using patient-derived iPS cells, allow for the investigation of human disease under long-term *in vivo* conditions. The talk will summarize our efforts to introduce human neural crest cells (NCCs) into the developing mouse embryo with the goal to set up a system that can model neural crest cell diseases under in the animal.

To study the potential of committed stem to functionally integrate into the developing mouse embryos we have differentiated mouse, rat and human ESCs or iPSCs into NCCs that were injected *in utero* into E8.5 albino wild-type and *c-Kit* mutated W^{sh}/W^{sh} embryos. Both the mouse and human NCCs migrated laterally under the epidermis and ventrally into deeper regions of the embryo. Importantly, analysis of postnatal animals derived from mouse, rat or human NCC-injected embryos displayed coat color pigmentation from the donor cells. Our results demonstrate that NCCs from different species can integrate into the developing mouse embryo, migrate through the dermis and differentiate into functional pigment cells in postnatal mice.

In recent experiments we have used human neural crest donor cells that carry neuroblastoma relevant mutations into gastrulating mouse embryos. We found that some of the chimeras develop tumors that resemble primary human neuroblastomas.

PS.01.02 | Chemiexcitation of electrons in melanin: A new route to disease

Sanjay Premi; Leticia C.P. Goncalves; Douglas E. Brash

Yale School of Medicine, New Haven, CT, USA

Mutations in sunlight-induced melanoma arise from cyclobutane pyrimidine dimers (CPDs), DNA photoproducts at adjacent thymines or cytosines usually created picoseconds after an ultraviolet photon is absorbed. In melanocytes, however, CPDs were generated for hours after UV exposure ended. These "dark CPDs" constituted the majority of CPDs in human melanocytes and mouse skin, and were most prominent in skin containing pheomelanin, the melanin responsible for blonde and red hair.

The mechanism was discovered to be chemiexcitation (chemical excitation of an electron), a process familiar in fireflies and jellyfish but unknown in mammals. Dark CPDs arose when UV-induced superoxide and nitric oxide combined to form peroxynitrite, one of the few biological molecules capable of exciting an electron. An electron in a carbonyl group of a melanin fragment was excited to a quantum triplet state that had the energy of a UV photon but induced CPDs by radiationless energy transfer to DNA. UVA and peroxynitrite also solubilized the melanin and allowed it to enter the nucleus. Melanin is evidently carcinogenic as well as protective. These findings may underlie the dependence of UV-induced and spontaneous skin cancers on melanin type, and they validate the long-standing suggestion that chemical generation of excited electronic states is important in mammalian biology.

Chemiexcitation may underlie diseases in tissue never exposed to UV: Inflammation also leads to superoxide and nitric oxide; these factors co-localize with melanin or neuromelanin in tissues susceptible to macular degeneration, noise-induced deafness, and Parkinson's Disease. A recent conference on Chemiexcitation in Human Disease at Cold Spring Harbor Laboratory therefore reviewed the prospects and challenges of the new field of human excited-state biology.

PS.01.03 | Translational approach in pigmentary disorders

Kyoungchan Park

Bundang Seoul National University Hospital, Seongnam, Korea

There are diverse pigmentary disorders. Among them, melasma will be a most important hyperpigmentary disorder and vitiligo will be a typical example of hypopigmentary disorder. In this presentation, I am going to introduce our experiences in translation research in melasma and vitiligo. Melasma is difficult to treat and is often refractory to multiple treatment modalities. Histologically, melasma is characterized by epidermal hyperpigmentation associated with underlying dermal pathology. It means that melasma is not only a disease of melanocytes but also a disease of surrounding environment. In addition, melanogenesis occurs through several mechanisms in melanosomes. Thus, strategies for regulating both intraceullar and extracellular interactions will be necessary in the treatment of melasma. Based on these mechanisms, translational approaches for the treatment of melasma

will be discussed. In addition, vitiligo also has complex pathogenesis and various approaches have been introduced for the treatment of vitiligo. But, imbalance of free radical status has been emerging as an important inducing factor and I am going to present the effects of nutritional supplementation in the management of vitiligo

PS.02.03 | Macrophage relay for long distance communication during Zebrafish adult pigment pattern development

Dae Seok Eom[1]; Larissa Patterson[1,2]; Emily Bain[1]; David M. Parichy1

[1]*University of Virginia, Charlottesville, VA, USA;* [2]*Rhode Island College, Providence, RI, USA*

Cellular communication over long distances is essential for normal development and homeostasis. In analyses of zebrafish adult pigment pattern formation, we have identified novel cellular projections, "airinemes," that extend from xanthophore precursors to melanophores. We show that airinemes promote Delta–Notch signaling and are required for organizing melanophores into stripes. We further demonstrate that airinemes are relayed from originating cells to target cells via interactions with wandering macrophages that patrol the tissue environment. This macrophage-dependent mode of long-distance communication may function in other tissues and could be relevant to cancer and other disease states.

CS.01.01 | TFAP2 paralogs regulate melanocyte differentiation in parallel with MITF

Hannah Seberg[1]; Eric Van Otterloo[2]; Stacie Loftus[3]; Derek E. Gildea[3]; Huan Liu[1]; Juan Sananta[1]; Robert J. Manak[1]; William Pavan[3]; Trevor Williams[2]; Robert Cornell[1]

[1]*University of Iowa, Iowa City, IA, USA;* [2]*University of Colorado, Denver, Aurora, CO, USA;* [3]*NIH, Bethesda, MD, USA*

The gene regulatory network (GRN) governing differentiation of melanocytes may also regulate phenotype switching in melanoma cells. Members of the Transcription Factor Activating-enhancer binding Protein 2 (TFAP2A-TFAP2E) family govern induction of neural crest in the neural plate. Indicating that they may also contribute to later steps of melanocyte development, patients with mutations in the TFAP2A gene exhibit premature hair graying. Moreover, zebrafish loss-of-function mutants of tfap2a, and mice with Tfap2a deleted in neural crest, exhibit deficiencies in melanocyte migration and, in the case of zebrafish, differentiation. Finally, data at the Cancer Genome Atlas reveal that expression of the TFAP2A gene is downregulated in stage 4 melanoma in comparison to in benign nevi. The contribution of TFAP2A to melanocyte development has been unclear because a closely related paralog, Tfap2b, is expressed with Tfap2a in neural crest (in mouse). Here we show that mouse embryos with *Wnt1-Cre*-mediated deletion of *Tfap2a* and *Tfap2b* in the neural crest almost completely lack melanocytes but retain neural crest-derived sensory ganglia. To determine the position of TFAP2A in the melanocyte GRN we carried out TFAP2A ChIP-SEQ in mouse and human melanocytes. TFAP2A is bound at a majority of enhancers and promoters that are active in melanocytes. Interestingly there is extensive co-occupation of such elements by TFAP2A and MITF. We also observe a genetic interaction between *tfap2a* and *mitfa* in zebrafish. We find that artificially elevating expression of *tfap2a* does not increase levels of melanin in *mitfa* hypomorphic or loss-of-function mutants, suggesting TFAP2A is a weak transcriptional activator and that it has some other function at enhancers; we are examining the possibly it regulates chromatin accessibility. In conclusion, differentiation of melanocytes is controlled by MITF together with other transcription factors, expanding the number of potential targets for therapeutically manipulating differentiation status in melanocytes or in melanoma cells.

CS.01.02 | Single cell expression profiling identifies pigment cell differentiation trajectories from partially-restricted intermediate pigment progenitor cell

Tatiana Subkhankulova[1]; Masataka Nikaido[1]; Artem Kasianov[2]; Leonid Uroshlev[2]; Gerardo Aquino[3]; Hartmut Schwetlick[1]; Tatjana Sauka-Spengler[4]; Andrea Rocco[3]; Vsevolod Makeev[2]; Robert N. Kelsh[1]

[1]*University of Bath, Bath, UK;* [2]*Vavilov Institute of General Genetics, Moscow, Russia;* [3]*University of Surrey, Guildford, UK;* [4]*University of Oxford, Oxford, UK*

The neural crest (NC) is a major model for understanding stem cell differentiation, in particular how multipotent progenitors generate a balance of different derived cell-types. Controversy over how individual fates are specified from the NC concerns whether fate choice proceeds via a Direct Fate or Progressive Fate Restriction model. The latter model posits sets of partially-restricted intermediates, but these remain poorly characterised *in vivo*. The zebrafish neural crest generates three distinct pigment cell types – melanocytes (black), iridophores (silver) and xanthophores (yellow) - which have been proposed to share a common cellular origin, either a partially-restricted chromatoblast, or a bipotent melanoiridoblast. We have proposed that the former is characterised by *ltk* expression (Lopes et al., 2008, PLoS Genetics 4, e1000026). We are using NanoString technology to profile expression of 45 neural crest/pigment cell genes in FACS-isolated freshly *ex vivo* single NC-derived cells. Clustering analysis of the expression profiles identifies cells showing expression patterns consistent with multipotent NC cells, differentiated cell-types (melanocytes, iridophores), and common precursors for all pigment cells, as well as genes marking these cell-types. Dimension reduction with 3D tSNE displays well-separated melanocyte and less markedly separated, but still distinguishable, iridophore cell-types. Analysis of shortest paths in the shared nearest neighbour graph identifies a likely course of differentiation of pigment cells via these intermediates. Consistent with our unpublished fate-mapping data of *ltk*-expressing cells, these intermediates appear also to have

neural potential. Subsequent studies using qRT-PCR profiling of isolated single cells using 13 molecular markers for early pigment cell, neuronal, glial and skeletogenic NC markers confirm the overlap of *ltk* and neuronal markers, identifying a highly multipotent partially-restricted intermediate in vivo. Together these data give us a unique view of the mechanisms of pigment cell development from NC cells *in vivo*.

CS.01.04 | Autologous human melanocytes derived from induced pluripotent stem (iPS) cells and in vivo generation of melanin pigmentation

Tamihiro Kawakami[1]; Munenari Itoh[2]; Tomohisa Hirobe[3]; Kowichi Jimbow[4]

[1]*Department of Dermatology, St. Marianna University School of Medicine, Tokyo, Japan;* [2]*Department of Dermatology, Jikei University School of Medicine, Tokyo, Japan;* [3]*National Institute of Radiological Sciences, Department of Molecular Imaging and Theranostics, Chiba, Japan;* [4]*Institute of Dermatology & Cutaneous Sciences, Sapporo, Japan*

Induced pluripotent stem (iPS) cells have the ability to differentiate into multiple cell types in the body and have an unlimited growth potential. However, iPS cell-derived melanocytes produced by existing protocols have significant limitations in developing novel strategies for regenerative medicine and cell therapies of pigmentation disorders in humans, because they involve culture in media containing fetal bovine serum (FBS) and non-physiologic agents. In this study, we established a new *in vitro* approach to generate iPS cell-derived human melanocytes which have higher proliferation rates and increased melanin production compared to melanocytes prepared by previously reported approaches. Importantly, our iPS cell-derived human melanocytes are prepared in FBS-free culture conditions that do not contain any non-physiologic agents. We designed two original methods, transferring black colonies by pipet and recovering black cell pellets from centrifuged medium, and numerous human iPS cell-derived melanocytes proliferated in gelatinous dishes coated with Matrigel after 12 days. We also succeeded in inducing melanin pigmentation in nude mouse skin *in vivo* using those human iPS cell-derived melanocytes. We propose that this new method using iPS cells established from T cells in the blood of normal human volunteers could be applied clinically to develop regenerative medicine and cell therapies for various forms of human pigmentation disorders.

CS.01.05 | Multilineage-differentiating stress enduring (Muse) cells-derived melanocytes have functional roles in 3D reconstituted muse-skin

Takeshi Yamauchi; Kenshi Yamasaki; Saaya Koike; Setsuya Aiba

Tohoku University Graduate School of Medicine, Miyagi, Japan

Multilineage-differentiating stress enduring (Muse) cells naturally exist as a endogenous stem cells and are non-tumorigenic pluripotent stem cell. Muse cells can be isolated as SSEA3-positive cells from bone marrow, dermis and fat tissue. We demonstrated that Muse cells obtained from adipose tissues could differentiate into melanocytes by culturing with Wnt3a, stem cell factor, endothelin 3 for 6 weeks (Muse-MC) (J Dermatol Sci 2017). In this study, we asked if Muse-MC could have functional roles in 3D reconstituted skin with Muse-derived skin component cells (Muse-skin). We differentiated Muse cells into melanocytes as well as into keratinocytes by culturing with 0.6 nM BMP4 and 1 mM ATRA (Muse-KC) and into fibroblasts by culturing with 10 ng/mL TGF-β2 and 0.3 mM ascorbic acid (Muse-Fb). Muse-MC and Muse-KC at a ratio 1:5 were seeded onto the collagen layer containing Muse-Fb. The sheets were cultured in Humedia KG2 with a gradually increased Ca2+ concentration for 7 days. The Ca2+ concentration was 0.15 mM on Day 1, 0.5 mM on Day 2 through 3, 1.0 mM on Day 4 through 5 and 1.5 mM on Day 6 through 7. Then, the medium was changed to DMEM and the reconstituted sheets were cultivated at air expose for 7 days. Immunofluorescence analysis confirmed generated 3D skin expressed keratin 14 in epidermis and collagen 3 in dermis in. We observed gp100-positive cells and tyrosinase activity on basal layer, indicating Muse-MC existed in basal layer. Moreover, we observed that 3D reconstituted skin contained melanin in epidermis of Muse-KC. These data showed that Muse-MCs reside in basal layer of reconstituted skin similarly to human epidermal melanocytes and transfer melanin to neighboring keratinocytes in 3D reconstituted skin. This Muse-MC containing 3D skin is useful model to understand melanocyte activity and to treat diseases with skin defect or depigmentation like vitiligo.

CS.01.06 | Control of the pigment pattern formation in Japanese quail

Masafumi Inaba; Cheng-Ming Chuong

University of Southern California, Los Angeles, CA, USA

Pigment patterns on the animal skin are a good model to study the mechanism of biological pattern formation. Japanese quail (JQ), one of the model animals in birds, has longitudinal dark and yellow stripes on the embryonic and juvenile dorsal skin. The dark stripes are formed by the melanocytes with eumelanin; yellow stripes are formed by melanocytes with pheomelanin. Although melanocyte progenitor cells (mitf+) are homogeneously distributed in both dark and yellow stripe regions, the progenitor cells in the prospective dark stripes are already specified as melanocytes with eumelanin (MelEM+) before melanin synthesis begins. This implies that the mechanism of the stripe patterning is independent of that of the pigment synthesis. However, both mechanisms remain unclear. To investigate the patterning mechanism, we identified the cell type responsible for the stripe patterning by chimera experiments. When we orthotopically transplanted JQ neural crest into white leghorn embryos, which have no pigment pattern, the host showed JQ like pigment pattern. This result suggests that JQ stripe pattern is formed by melanocytes autonomously. Next,

we examined the expression of Agouti, which switches the pigment synthesis pathway from eumelanin to pheomelanin. Agouti were expressed in the feather mesenchyme corresponding the prospective yellow stripes. We propose two-step pigment pattern formation: the stripe pattern is formed by the melanocyte-autonomous mechanism; this spatial information is converted into the expression pattern of Agouti that produces yellow pigment.

CS.02.01 | Photoreactivity and phototoxicity of melanin granules of the aging human retinal pigment epithelium

Magdalena Olchawa; Justyna Furso; Anna Pilat; Grzegorz Szewczyk; Tadeusz J. Sarna

Jagiellonian University, Krakow, Poland

Human retinal pigment epithelium (RPE) is a unique pigmentary system, in which melanin is synthesized during fetal development and afterwards shows little or no metabolic turnover. However, with aging, RPE melanin can undergo considerable physicochemical changes, aggravated by chronic exposure to short-wavelength visible light and oxidative conditions that may compromise biological functions of the pigment. In this study, photoreactivity and phototoxicity of pigment granules isolated from RPE of younger and older donors were analyzed in model systems and in ARPE-19 cells using an array of advanced biophysical methods and standard cell biology techniques. The results have shown that RPE melanin and melanolipofuscin granules from older donors exhibit higher photochemical reactivity than those from younger donors, as demonstrated by the pigment granule ability to photogenerate superoxide anion and singlet oxygen, and to induce photooxidation of extracellular and cellular proteins. Phototoxic effects were examined in ARPE-19 cells, loaded with melanin and melanolipofuscin granules by phagocytosis, upon excitation with blue light. Consistent with the photochemical results, phototoxicity mediated by pigment granules from older donors was higher than that from younger donors. Distinct phototoxic effects mediated by melanin and melanolipofuscin granules, particularly from older donors, were also observed at sub-lethal doses of the photic stress: the specific phagocytic activity of ARPE-19 cells was reversibly inhibited by blue light in a dose-dependent manner. Importantly, functional specific phagocytosis of RPE is critical for survival of photoreceptor and other retina cells. The phototoxic effects mediated by melanin granules could be reduced by supplementation with zeaxanthin and a-tocopherol. Our study has demonstrated for the first time that one of the most important functions of the RPE, its specific phagocytosis, can be impaired by sub-lethal photic stress mediated by the aging pigment granules and that the effect can be reversed by natural antioxidants.

CS.02.02 | Spectro-electrochemical reverse engineering applied to probe redox and radical scavenging property of melanin

Eunkyoung Kim[1]; Zheng Wang[2]; Lucia Panzella[3]; William E. Bentley[1]; Alessandra Napolitano[3]; Gregory F. Payne[1]

[1]University of Maryland, College Park, MD, USA; [2]Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC, USA; [3]Department of Chemical Sciences, University of Naples, Naples, Italy

Melanin is proposed to perform various functions in biology. The conventional approach to understand a material's functions is to start by characterizing its structure, but this approach has been difficult to apply to melanin because of its complexity. We are developing an alternative, reverse engineering approach that focuses on properties. Importantly, our reverse engineering uses electrochemistry to probe melanin's redox and radical-scavenging properties that are believed to be integral to many of melanin's proposed protective functions. In this reverse engineering method we: entrap melanin in a hydrogel film adjacent to an electrode surface; add diffusible mediator(s) that can shuttle electrons between the electrode and the melanin; impose a complex sequence of voltages to the electrode to oxidize/reduce the mediators; and measure/analyze the resulting currents to understand if, and under what voltages the melanin donates or accepts electrons. This electrochemical approach can also be coupled to spectral analysis to allow observation of the generation/quenching of free radicals.

Through a series of (spectro)electrochemical reverse engineering studies, we have shown:

- All melanins tested are redox-active and can be repeatedly oxidized and reduced.
- Pheomelanin appears to have a more oxidative redox potential (vs eumelanin) which may explain its pro-oxidant activities.
- The agricultural chemical paraquat can transfer electrons to melanin through redox-cycling reactions. While the medical significance is uncertain, it is important to note that exposure to paraquat is linked to Parkinson's disease through a proposed redox-cycling, oxidative-stress-inducing mechanism, while the neurons selectively vulnerable in Parkinson's disease are rich in melanin.
- All melanins tested are able to scavenge free radicals either by donating or accepting electrons, and the radical-scavenging activity is linked to melanin's redox state.

In summary, (spectro)electrochemical reverse engineering allows melanin's redox and radical-scavenging activities to be readily characterized and this could assist in discerning melanin's complex and diverse biological functions.

CS.02.03 | A new insight into the origin of eumelanin chromophore

Lucia Panzella[1]; Raffaella Micillo[1]; Mariagrazia Iacomino[1]; Orlando Crescenzi[1]; Kenzo Koike[2]; Alessandra Napolitano[1]; Marco d'Ischia[1]

[1]University of Naples "Federico II", Naples, Italy; [2]Kao Corporation, Tokyo, Japan

Eumelanins, the chief photoprotective pigments in men and mammals, owe their black color to an unusual broadband absorption spectrum whose origin is still a conundrum. The contributions of structural and redox disorder in particular have still remained uncharted.

We report herein a new entry to eumelanin chromophore dynamics based on a comparative spectrophotometric investigation of the oxidation mixtures of a collection of eumelanin precursors featuring different substituents, molecular size and shape. The obtained results uncovered the impact of the structural scaffold on eumelanin optical properties, disproving the widespread assumption of a universal monotonic chromophore. Moreover, intermolecular interactions leading to redox equilibration and stacking were found to play an important role in chromophore broadening in an oxidation independent manner.

Based on these data, eumelanin chromophore buildup can be described as a three-step process involving the rapid generation of oxidized oligomers (melanochromes) (*step I*), followed by a slow oxidant-independent band broadening (*step II*) leading eventually to scattering (*step III*).[1]

Although the relevance of these results to eumelanin optical properties *in vivo* within the melanosomes remains to be assessed, the new background of structure-property relationships emerging from this study may guide the rational design of eumelanin-inspired functional materials tailored to specific biomedical, dermocosmetic or technological applications.

CS.02.04 | Mechanistic study of melanogenesis: Binding of cysteine to dopaquinone

Ryo Kishida[1]; Hideaki Kasai[2]

[1]Department of Applied Physics, Osaka University, Suita, Japan; [2]National Institute of Technology, Akashi College, Japan, Akashi, Japan

Melanogenesis involves a step which consumes cellular thiols, including cysteine (Cys-SH). This is due to binding of thiols to dopaquinone (or similar *o*-quinones) generated by tyrosinase-catalyzed oxidation of tyrosine/dopa (or similar phenols/catechols). The binding of Cys-SH proceeds via binding of sulfhydryl sulfur to an aromatic carbon. 5- and 2-carbon (C5, C2) have been respectively identified as the major and sub-major site for the binding whereas C6 has been identified as the very minor site [1]. The binding of thioglycolic acid (an ammonium-free Cys-SH analogue) has been reported to form an unstable intermediate as compared to the case of Cys-SH [2]. C5 and C2 are adjacent to the carbonyl group. Thus, interaction between the ammonium proton in Cys-SH and the carbonyl oxygen in dopaquinone would stabilize the corresponding Cys-SH-attacked intermediates.

In the present study, we investigated the initial processes of Cys-SH binding to dopaquinone using density functional theory based calculations. We calculated the binding energies of $Cys\text{-}S^-$ on aromatic carbons (C1, C2, C5, C6, and C3-C4 bridge). We found that C2 and C6 show comparable binding energies. Thus, the less yield of C6-adduct cannot be explained by energetic consideration. We also found that C3-C4 bridge shows the highest binding energy to $Cys\text{-}S^-$. Based on the results, we propose a part of mechanism to form the C5- or C2-adduct; $Cys\text{-}S^-$ is first recruited by C3-C4 bridge and then migrated to the adjacent C5 or C2. Furthermore, we found that the ammonium proton in $Cys\text{-}S^-$ bound on C5 (C2) can be transferred to O3 (O4) with a fairly small activation energy. This proton transfer results in strong stabilization of the $Cys\text{-}S^-$-attacked intermediates.

[1] S. Ito, G. Prota, Experientia 33 (1977) 1118.

[2] G.N.L. Jameson et al., Org. Biomol. Chem. 2 (2004) 777.

CS.02.05 | Tyrosinase is ubiquitinated by RNF152, a membrane associated ubiquitin ligase

Hideaki Fujita[1]; Rina Hashimoto[1]; Shiyoh Takahashi[1]; Mizuki Hama[1]; Yuki Fujii[1]; José C. Menezes[1]; Hirotaka Takahashi[2]; Hiroyuki Takeda[2]; Tatsuya Sawasaki[2]; Tomonori Motokawa[3]; Kenzo Tokunaga[4]

[1]Faculty of Pharmaceutical Sciences, Nagasaki International University, Sasebo, Nagasaki, Japan; [2]Proteo-Science Center, Ehime University, Matsuyama, Ehime, Japan; [3]Cutaneous Drug Research Department, POLA Chemical Industries, Inc., Yokohama, Kanagawa, Japan; [4]Department of Pathology, National Institute of Infectious Diseases, Shinjyuku-ku, Tokyo, Japan

Tyrosinase, a key enzyme for melanin production, is synthesized as a membrane protein, transported to melanosomes and finally degraded in lysosomes. The molecular mechanism that redirects tyrosinase from melanosomes to lysosomes has not yet been fully elucidated. In the presence of lysosomal protease inhibitors, the degradation of tyrosinase is retarded and its ubiquitination is detected in B16 melanoma cells. These results suggested that the ubiquitination of tyrosinase contributes to its sorting to lysosomes. Cell-free based protein array analyses suggested that RNF152, a membrane associated ubiquitin ligase, potentially interacts with tyrosinase. Co-immunoprecipitation analyses revealed that RNF152-myc specifically interacted with tyrosinase-HA but Tyrp1-HA. Overexpression of RNF152-myc enhanced ubiquitination of tyrosinase-HA. Immunofluorescence analyses revealed that tyrosinase-HA and RNF152-myc are colocalized to the vesicular structures possibly corresponding to the late endosomes. These results suggest that RNF152 is a specific ubiquitin ligase for tyrosinase and may be responsible for targeting it to lysosomes.

CS.03.01 | Microbial dysbiosis of the gut accelerates depigmentation in a mouse model of vitiligo

Emilia Dellacecca[1]; Suhail Akhtar[1]; Steven W. Henning[1]; Victor H. Engelhard[2]; I. C. Le Poole[1]

[1]*Loyola University Chicago, Maywood, IL, USA;* [2]*University of Virginia School of Medicine, Charlottesville, VA, USA*

Vitiligo is mediated by skin infiltrating, cytotoxic T cells reactive with melanocytes. Disease development may be influenced by environmental factors that trigger autoimmunity. We studied the impact of the microbiome on vitiligo development in FH mice, where depigmentation occurs in presence of recombinant HLA-A2 and tyrosinase-reactive cytotoxic T cells. FH mice (>8 per group with or without the AAD transgene) were split into 3 groups and exposed to no antibiotics, ampicillin or neomycin in their drinking water at the first signs of pregnancy and beyond. Pelage depigmentation was followed among offspring by image analysis. DNA was isolated from fecal pellets to evaluate total bacterial DNA content and measure enterobacteriaceae and segmented filamentous bacteria by qRT-PCR. Gut permeability was measured by estimating aspartate aminotransferase activity in the serum. Microbial content of the total gut, ileum and colon and the skin was analyzed in representative animals. IL-17 among gut proteins was estimated by ELISA. In untreated control mice with juvenile depigmentation, pigment loss did not expand to the pelage, whereas mice in the ampicillin group were about 1/3 depigmented at 30 weeks. Depigmentation was associated with significantly reduced bacterial content in fecal pellets from ampicillin or neomycin treated mice. Gut permeability increased to approximately 2-fold in neomycin, and 3-fold in ampicillin treated animals. A pilot experiment revealed that segmented filamentous bacteria were diminished at the expense of enterobacteriaceae, with no changes to the skin microbiome. Less IL-17 was observed by ELISA of protein isolated from gut tissue. Thus, FH mice depigmentation was accelerated by ampicillin exposure, accompanied by dysbiosis of the gut. These observations differ from those reported for other models of autoimmunity that are less dependent on IFN-γ for disease development. The results suggest that maintaining a healthy diet to avoid gut dysbiosis might delay vitiligo onset.

CS.03.02 | Non immunological aspect in vitiligo pathogenesis

Maria Lucia Dell'Anna; Daniela Kovacs; Emanuela Bastonini; Monica Ottaviani; Barbara Bellei; Giorgia Cardinali; Mauro M. Picardo

Istituto San Gallicano, Rome, Italy

The mechanisms causing the progressive loss of melanocytes in vitiligo are still being discussed. Several factors including genetic, autoimmune, oxidative and metabolic alterations have been considered but their distinctive contribution is still under investigation. Intrinsic metabolic abnormalities leading to a higher vulnerability to oxidative stressors are believed to activate immune and autoimmune responses, which progressively result in melanocyte degeneration and loss. Oxidative insults, when persistent, also promote the acquisition of a stress-induced premature senescent-like phenotype, which is not only restricted to melanocytes but is extended to the entire skin. It is believed that the triggering intrinsic cellular defect is in charge of mitochondria, which display an increase in their mass associated with energetic defects. Moreover, recent evidence is proving the presence of modifications even in normal appearing skin, suggesting the existence of alterations before the onset of clinically established lesions. In this context, non lesional fibroblasts also show a senescence-associated phenotype characterized by an increased production of aging-associated secreted proteins including HGF, IL-1b and IL-6, able to affect melanocyte functionality. Collectively, vitiligo appears as a global disease affecting all skin cell populations, not only in depigmented areas but also in normal appearing skin, and mainly related to degenerative and senescence-associated processes.

CS.03.03 | International initiative for outcomes (Info) for vitiligo-results of workshop with patients and progress so far

Viktoria Eleftheriadou[1]; Khaled Ezzedine[2]

[1]*Centre of Evidence Based Dermatology, University of Nottingham, Nottingham, UK;* [2]*UPEC-Université Paris-Est Créteil, Paris, France*

Currently, no cure or firm clinical recommendations exist for the treatment of vitiligo. One of the main issues identified by the Cochrane systematic review is the heterogeneity of outcomes and measures used in RCTs for vitiligo.

A systematic review on outcomes in vitiligo trials showed that 25 different outcomes had been measured in 54 randomised controlled trials.

Following this, an international e-Delphi consensus on core outcomes set for vitiligo was completed. A 101 from 25 countries worldwide took part including patients, clinicians, representatives of regulatory authorities and journal editors. Three outcomes were identified as essential: repigmentation, cessation of spread and side effects.

A second e-Delphi, with an aim to gain consensus on how to measure repigmentation was conducted. Although this e-Delphi reached a response rate of 94%, no agreement was found on the best outcome measure to assess repigmentation in target lesions.

The VGICS (Vitiligo Global Issues Consensus Group) group, which works on outcomes for vitiligo since 2011, decided that a workshop with patients need be conducted in June 2017 in Detroit, USA in order to address the above.

Aim: This workshop (lead by Dr V Eleftheriadou and Prof. K. Ezzedine) is needed in order to overcome the difficulties and disagreements between various stakeholders groups raised during e-Delphi on

repigmentation outcome measure. Issues to be addressed: definition of repigmentation (for target lesion) and successful repigmentation from patients' point of view. Patients' feedback on how and when should we evaluate and measure repigmentation and its characteristics.

Results: This workshop is currently in preparation. Date: 25th of June 2017 (Detroit, USA). The results of this crucial workshop will be feedback to the VGICS group and broader audience. A discussion will follow and initiation of a second e-Delphi in order to identify the best outcome measure for repigmentation for target lesions.

CS.03.04 | Unraveling the genetic architecture of vitiligo in an isolated, Romanian village

Genevieve H. Andersen[1]; Stanca Birlea[1,2]; Stephanie Santorico[1,4]; Richard Spritz[1,3]

[1]Human Medical Genetics and Genomics, University of Colorado, Anschutz Medical Campus, Aurora, CO, USA; [2]Department of Dermatology, University of Colorado School of Medicine, Aurora, CO, USA; [3]Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA; [4]Mathematical and Statistical Sciences, University of Colorado, Denver, CO, USA

Vitiligo is a genetically complex disease in which patches of depigmented skin result from autoimmune destruction of melanocytes. By genome-wide association studies (GWAS) of vitiligo in European-derived whites (EUR), we identified 48 vitiligo susceptibility loci. While proteins encoded at these loci highlight a framework for melanocyte-directed autoimmunity, the overall picture of vitiligo pathobiology remains incomplete. Here, we report analyses of an isolated, inbred Romanian village with vitiligo prevalence ~8 times higher than in the general EUR population, suggesting an enrichment of genetic and/or environmental risk factors. Due to the small number of ancestral founder genomes (~23), villagers are expected to have reduced causal allele heterogeneity and perhaps fewer causal alleles overall, thus constituting a less genetically complex subset of the general EUR population. We have genotyped 53 cases and 121 unaffected villagers for 591,361 SNPs genomewide, and we have used these genotypes to compare genetic architectures under three alternative models of vitiligo pathogenesis: (1) a polygenic model with unusually high prevalence of common EUR risk variants detected by GWAS; (2) a mono- or oligogenic model in which a highly-penetrant variant rare in the general EUR population explains most vitiligo risk; or (3) both. Specialized association analyses of the 48 loci known susceptibility loci are being used to assess common, genetic risk variants under hypothesis 1, and genome-wide linkage analysis is being used to identify variants consistent with hypothesis 2. Our combined approach of association and linkage within this remarkable isolated village provides a unique opportunity to ascertain the genetic architecture of vitiligo in a relatively simple, homogeneous population. Implications of this study will be to highlight key pathobiological pathways, either by identifying a combination of common risk variants that confer particularly high risk, or by identifying a novel vitiligo susceptibility locus that confers unusually high disease risk.

CS.03.05 | NKG2D is highly expressed on IFNy producing skin CD8+ effector memory T cells in vitiligo

Clément Jacquemin[1]; Christina Martins[1]; Alain Taieb[1,2]; Katia Boniface[1]; Julien Seneschal[1,2]

[1]INSERM U1035, Immuno-dermatology, Bordeaux, France; [2]Department of Dermatology and Pediatric Dermatology; Hôpital Saint-André, Bordeaux, France

Vitiligo is an autoimmune disease that results from the loss of epidermal melanocytes. During progression of the disease, Vitiligo skin is infiltrated by IFNy-producing CXCR3+ CD8+ Effector Memory T (TEM) cells that contribute to disease pathogenesis. Natural Killer (NK) Group 2D (NKG2D) is an activating receptor mainly found on immune cells, including NK and CD8+ T cells. NKG2D+ CD8+ T cells are important during immune surveillance and are involved in inhibition of tumor growth. However, the involvement of NKG2D in Vitiligo pathogenesis remains unknown. Here, we show that expression of NKG2D, that also defines a Tc1 profile, is up-regulated on skin CD8+ TEM in progressive Vitiligo compared to stable Vitiligo or psoriasis. A thorough analysis of extracted skin T cells found that NKG2D+ CD8+ TEM cells express markers of residency such as CD69, CD49a and/or CD103 and produce elevated levels of both IFNy and TNF-α. Moreover, we found a positive correlation between expression of CXCR3 and NKG2D on CD8+ TEM cells. In additional in vitro experiments, isolated Vitiligo skin NKG2D+ CD8+ TEM cells displayed higher capacity of activation, proliferation and survival compared to NKGD2- CD8+ TEM cells. All together, these data highlight NKG2D as a potential marker of pathogenic CD8+ TEM cells important for Vitiligo progression. Therefore, developing strategies that target NKG2D expressing CD8 TEM cells could be interesting for Vitiligo, a disease with high unmet needs.

CS.03.06 | Immunosuppressive activities in the target tissue of autoimmune vitiligo-susceptible but non-expressing brown line chickens

Daniel M. Falcon; Kristen A. Byrne; Gisela F. Erf

Department of Poultry Science, University of Arkansas, Fayetteville, AR, USA

Vitiligo is a depigmentation disorder driven by the autoimmune targeting and elimination of melanocytes in the skin. There is no cure for vitiligo and preventative measures are difficult to design due in part to limited data obtained before visual onset. The purpose of this study was to examine the immunological mechanisms underlying the initiation of autoimmune vitiligo in Smyth chickens (SL). Growing feather (target-tissue) samples were taken from SL and vitiligo-susceptible Brown line (BL) parental-control chickens starting at 1 day post-hatch and collected twice per week until 16 weeks of age. Selected chickens were also monitored for visible de-pigmentation of feathers (indicative of vitiligo onset) for the duration of the study. In SL chickens characteristic infiltration of CD45+ leukocytes, including CD4+ and

CD8+ T-cells, was observed prior to vitiligo onset and was sustained throughout disease progression. Gene expression analysis suggested active recruitment (CCL19, CCR7) of lymphocytes prior to onset and a sustained Th1-like gene signature (IFN-γ, FASLG, GZMA) throughout disease progression. Spectratype analysis of CDR3 regions of T-cell receptor cDNA suggested skewing of the T-cell repertoire prior to visual onset indicative of a clonal T-cell response. Unexpectedly, while no BL chickens showed any signs of depigmentation, in some individuals a transient recruitment and infiltration of CD4+ and CD8+ T-cells was observed, with CD4+ cells being the dominant population. In contrast to SL however, infiltration was accompanied by elevated expression of immunosuppressive genes (CTLA-4 and IL-10) without increases of IFN-γ, FASLG or GZMA. These results reveal, for the first time, what appear to be immunoregulatory activities in vitiligo-susceptible BL chickens. Taken together these data suggest triggering of melanocyte-specific immune responses in growing feathers of both SL and BL chickens with the latter responding in an immunosuppressive manner and the former progressing to a sustained cell-mediated immune response.

CS.03.07 | Dickkopf-1 expression in vitiligo patients and its in vitro effect on the cultured melanocytes

Seema Rani[1]; Davinder Parsad[2]; Vijay L. Sharma[1]; Ravinder Kumar[1]

[1]*Panjab University, Chandigarh, CH, India;* [2]*PGIMER, Chandigarh, CH, India*

Various factors secreted by the dermal fibroblasts during epidermis-dermis crosstalk play very important role in the maintenance and regulation of skin pigmentation. In the dermis, fibroblasts secrete many melanogenic factors which affect the melanocytes located at the basal layer of the epidermis. Fibroblasts also secretes factor, DKK-1 which exerts an inhibitory effect on melanocyte proliferation and pigment production. Therefore, this study was designed to check the role of fibroblasts derived factor dickkopf-1 (DKK-1) in vitiligo patients. Expression of DKK-1 was analysed in the vitiligo skin by qRT-PCR and immunohistochemistry. Effect of different concentrations (50 ng/ml & 100 ng/ml) of DKK-1 was checked on the cultured melanocytes by proliferation assay, melanin content assay, tyrosinase assay and melanocytes specific gene expression studies by qRT-PCR. Melanocytes culture treated with DKK-1 was observed morphologically and melanocyte senescence was checked by β-galactosidase staining kit and further confirmed by qRT-PCR of senescence markers (p16, p21, hp1). We observed increased expression of DKK-1 both at mRNA level and protein level in lesional dermis of vitiligo patients. DKK-1 treatment decreased the growth and pigmentation of melanocytes. Relative gene expression of melanocyte specific genes (MITF, TYR, c-KIT, DCT) was found to be decreased with DKK-1 treatment. Importantly we found higher number of senescence cells in the DKK-1 treated melanocytes. These altered expressions of fibroblasts derived factors might be playing important role in the vitiligo pathogenesis and treatment.

CS.04.02 | Melanoma: Immune therapy

Reinhard Dummer

Universitäts Spital, Zurich, Switzerland

For decade, melanoma was the paradigm for Immunotherapy. Unfortunately, vaccination approaches did not produce convincing results.

However, the introduction of checkpoint inhibitors indicated a revolution in melanoma therapy.

Checkpoint inhibitors such as Pembrolizumab, Nivolumab and Ipilimumab target surface proteins that control the activation of immune cells, especially T cells.

This implies that these treatments are associated with significant autoimmune toxicities including dermatitis, colitis, thyreoiditis and many others.

Response rate are around 50% for the combination Anti-PD1 antibody and Anti-CTLA-4 antibodies. The major promise is in response duration. There is the possibility that a number of patients are cured by these treatment approaches.

The major steps of these developments will be described and potential developments outlined for the future.

One central issue is the development of biomarkers that allow precision immunotherapy. It appears reasonable to assume that these biomarkers will be compose by a number of different molecular markers and clinical features.

CS.04.03 | Final results of a phase II multicenter trial of HF10, oncolytic virus immunotherapy, and ipilimumab combination treatment in patients with stage IIIB-IV melanoma

Robert H. I. Andtbacka[1]; Merrick Ross[2]; Sanjiv S. Agarwala[3,4]; Matthew Taylor[5]; John Vetto[5]; Rogerio I. Neves[6]; Adil Daud[7]; Hung T. Khong[1]; Richard S. Ungerleider[8]; Maki Tanaka[9]; Kenneth F. Grossmann[1]

[1]*Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA;* [2]*The University of Texas MD Anderson Cancer Center, Houston, TX, USA;* [3]*St. Luke's Medical Center, Easton, PA, USA;* [4]*Temple University, Philadelphia, PA, USA;* [5]*Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA;* [6]*Penn State Hershey Cancer Institute, Hershey, PA, USA;* [7]*UC San Francisco, San Francisco, CA, USA;* [8]*Theradex Oncology, Princeton, NJ, USA;* [9]*Takara Bio. Inc, Shiga, Japan*

Background: HF10 is a bioselected replication-competent oncolytic virus derived from HSV-1. Herein, we report the safety and efficacy data of HF10 + ipilimumab (ipi) combination treatment in a Phase II trial in melanoma.

Methods: Ipi naïve patients (pts) with Stage IIIB-IV unresectable melanoma were enrolled. HF10 injected into single or multiple tumors (1 x 10^7 $TCID_{50}$/mL/dose, up to 5 mL depending on tumor size and number); 4 injections qwk; then up to 15 injections q3 wk. Ipi was administered intravenously (3 mg/kg), q3 wk for 4 doses. Tumor responses assessed per irRC at 12, 18, 24, 36 and 48 wks.

Primary endpoint was Best Overall Response Rate (BORR) at 24 wks.

Results: Of 46 pts enrolled and treated: 59% men, median age 67 yrs (range 28 to 91); disease stage: 20% IIIB, 44% IIIC and 36% IV; 57% were treatment naïve and 43% had ≥1 prior cancer therapies. Most HF10-related AEs were ≤G2, similar to HF10 monotherapy. 37% had ≥G3 AEs, the majority due to ipi. HF10-related ≥G3 AEs (n = 3) were embolism, lymphedema, diarrhea, hypoglycemia, and groin pain. Of 44 efficacy evaluable pts per irRC, BORR at 24 wks was 41% (18% irCR, 23% irPR); disease stability rate was 68% (27% irSD). BORR at 48 wks was 45% (18% irCR, 27% irPR). As of Apr 19, 2017, median PFS was 19 months and 1-year overall survival rate was 85%. HF10+ipi treatment resulted in a decrease in lesion size by ≥50% in 57% of injected lesions (N = 148), 39% of never injected non-visceral lesions (N = 41), and 14% of never injected visceral lesions (N = 22). Complete resolution of lesions occurred in 30% of injected lesions and 20% of never injected non-visceral lesions.

Conclusion: The combination HF10 and ipi treatment demonstrated a favorable benefit/risk profile and encouraging antitumor activity in both injected and non-injected lesions in pts with unresectable or metastatic melanoma.

CS.04.04 | Surgical approaches to melanoma

Vernon K. Sondak

Moffitt Cancer Center, Tampa, FL, USA

The past decade has seen transformative changes in the management of metastatic melanoma, changes that legitimately introduce the possibility of cure to a substantial minority of patients for whom no curative options existed previously. So how do these changes impact on surgery for resectable melanoma? Overall, the importance of high-quality surgical management of localized and regionally metastatic melanoma within a multidisciplinary care context has never been greater. Recommendations for excision margins in patients with invasive primary cutaneous melanomas of the trunk and proximal extremities, based on the highest levels of evidence from prospective randomized clinical trials remain the gold standard and have been largely unchanged for decades. While minor changes and incremental improvements have been seen in selection criteria for and techniques of sentinel lymph node biopsy, the biggest change has been in the surgical management of the node-positive patient. Today, even if one is an acknowledged master of the procedure of regional lymph node dissection, the technical ability to perform the procedure must be tempered by the judgement to know when the procedure should and should not be performed. Patients with microscopic metastatic disease in the sentinel node(s) will increasingly be managed by expectant observation, while patients with bulky but resectable regional nodal metastases are ideal candidates for neoadjuvant multimodality therapy. Oligometastatic melanoma, even if technically resectable, should generally be treated with upfront systemic therapy, with surgery reserved for specific situations including persistent or progressive disease in a single site despite overall resolution of tumors elsewhere. The next decade will be exciting for surgeons and patients alike, and everyone interested in the research into and management of melanoma should be familiar with how the surgical approaches to this disease are changing and evolving.

CS.05.01 | Neural crest derived melanocytes regulate aortic valve elastogenesis

Joshua Hutcheson; Xiaoshuang Li; Lidia Kos

Florida International University, Miami, FL, USA

A precise extracellular matrix (ECM) microarchitecture of the aortic valve leaflets dictates appropriate valvular function. A specialized cell population known as valve interstitial cells (VICs) resides within valve leaflets and maintains the ECM. Pigmented Neural Crest (NC)-derived melanocytes appear in the aortic valve leaflets of C57BL/6 mice and pigmented regions of tissue exhibit increased mechanical stiffness compared to non-pigmented regions in C57BL/6 mouse valves, suggesting that melanocytes may play a role in valve biomechanics. We hypothesized that melanocyte VICs contribute to aortic valve structure and correspond to a similar NC-VIC population in human aortic valves. We used wild-type (C57BL/6), melanocyte-deficient (Kit-/-), and mice with an overabundance of cardiovascular melanocytes (K5-Edn3), to access changes in aortic valve ECM through two-photon microscopy, immunohistochemistry/immunofluorescence, and gene expression. Immunofluorescence assessed the relationship between melanocyte VICs and markers associated with neural cell phenotypes. Autopsy specimens were used to compare the murine phenotypic observations to human aortic valves. Tyrosinase-positive melanocytes in murine aortic valves exhibit phenotypic markers of both neuronal (Tuj1) and glial cells (GFAP). Movat's pentachrome staining along with immunohistochemistry showed significant ($p < 0.05$) neuronal VIC ($p < 0.05$) association with elastin and glial VIC association with glycosaminoglycans in C57BL/6 mice. Two-photon imaging of intact murine aortic valve leaflets revealed a complete loss of elastin fibers in Kit-/- mice and increased, disorganized elastin fibers in K5-Edn3 mice. Gene expression analyses corroborated these findings, revealing a 42% decrease in elastin expression in Kit-/- mice and a 114% increase in elastin expression in K5-Edn3 mice. Immunohistochemistry analyses of human aortic valves show Tuj1-positive VICs in elastin-rich regions and GFAP-positive VICs in glycosaminoglycan-rich regions, suggesting that similar NC-VIC populations exist in human aortic valves. Given the importance of elastin in aortic valve homeostasis and disease, the role of melanocytes and NC-VICs in elastin regulation warrants further investigation.

CS.05.02 | Establishment of primary melanocyte eQTL dataset for analysis of melanocyte genome regulation

Stacie K. Loftus[1]; Tongwu Zhang[2]; Jiyeon Choi[2]; Laura L. Baxter[1]; Kevin Brown[2]; William J. Pavan[1]; NISC Comparative Sequencing Program

[1]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; [2]National Cancer Institute, National Institutes of Health, Gaithersburg, MD, USA

Characterization of variation in gene expression levels is fundamental to understanding phenotypic diversity and disease states. To assess the contribution of regulatory variation on gene expression in melanocytes, we have established an expression Quantitative Trait Loci (eQTL) dataset derived from >100 primary melanocyte cultures. EQTLs identify genomic variants that are correlated with changes in a gene's expression and can mark a regulatory SNP directly, or indirectly via marking SNPs in linkage disequilibrium with the regulatory SNP. Thus identification of eQTLs in melanocytes can facilitate the analysis of the causal variants that regulate gene expression variation linked to altered cellular functions associated with pigmentation, disease states and human health. We performed cis-eQTL analysis, following the GTEX computational pipeline using RNA-seq (>80,000,000 mapped reads/sample) and SNP genotyping (Illumina 700K SNP array) analyses from primary melanocytes. This yielded 5009 cis-eQTL GENE:TOPSNP pair associations (p-value of < 0.05), known here as EGENEs. In addition, this analysis identified over 500,000 individual SNPS significantly correlated with gene expression variation in melanocytes. Contained within the 5009 EGENEs were 114 genes directly known to be associated with visible pigmentation-related phenotypes of the eye, hair and skin in humans and animal models. These 114 genes were identified utilizing a newly annotated set of 585 pigmentation phenotype genes, which we generated by integrating annotations from Mouse Genome Informatics (MGI), Online Mendelian Inheritance in Man (OMIM), International Mouse Phenotyping consortium (IMPC) and Gene Ontology (GO) databases. Taken together, this analysis provides a foundational dataset for establishing the regulatory variation underlying melanocyte genome regulation and assessing the impact of this on the diverse pigmentation-related phenotypes, disorders and human health.

CS.05.03 | The role of p53 in UV signaling

Tamara Terzian; Nema Sobhani; Rohan Mylavarapu; Rohan Mylavarapu; Colleen Little; Manale El-Kharbili; Ethan Krauspe

Department of Dermatology, University of Colorado Denver, Aurora, CO, USA

Exposure to ultraviolet radiation (UVR) in sunlight activates both defensive and repair pathways in the skin. As the major cell type affected by UVR, keratinocytes coordinate critical aspects of the skin response. Molecularly, the transcription factor p53 is induced by UVR in keratinocytes leading to their cell death by apoptosis (peeling). p53 also stimulates the expression of paracrine melanocyte growth factors involved in tanning. Here we investigate the role of p53 in tanning that is to date not well-defined. For this we are using the hyperpigmented *Sooty Foot Ataxia* (SFA) mouse model that presents with hyperpigmentation of the extremities due to increased melanocyte number and melanin production. Interestingly this mouse has high p53 levels in keratinocytes and loss of p53 completely abrogates the hyperpigmentation phenotype. Therefore, this mouse represents a good tool to study the paracrine effects of keratinocyte p53 on melanocyte behavior and function, tanning and melanomagenesis. To mimic the effect of sunburn in humans and elucidate the role of the p53 response in melanomagenesis, we exposed these mice to high doses of UVB and followed melanocyte proliferation and the expression of keratinocyte-induced melanocyte growth factors. The data generated from our experiments demonstrates strong evidence of the cross-talk between keratinocytes and melanocytes and supports the notion that keratinocyte p53 promotes melanocyte proliferation through the release of paracrine factors.

CS.05.04 | Transgenic Dct-promoter driven Mitf expression rescues eye structure and Uveal, but not cutaneous, melanocytes in Mitf-deficient mice

Chi-Ping Day[1]; Helen T. Michael[1]; Cari Graff-Cherry[2]; Kapil Bharti[3]; Heinz Arnheiter[4]; Glenn Merlino[1]

[1]National Cancer Institute, NIH, Bethesda, MD, USA; [2]Frederick National Laboratory for Cancer Research, Frederick, MD, USA; [3]National Eye Institute, NIH, Bethesda, MD, USA; [4]National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, USA

Microphthalmia-associated transcription factor (Mitf), the lineage-determining factor of pigment cells, drives the expression of melanin-producing genes, such as Dct, Tyrp1, and tyrosinase. It is required for the maturation and survival of melanocytes, and mice deficient of Mitf (e.g. vga9 mice) have striking phenotypes, including loss of overall pigmentation, shrunken and closed eyes, blindness, and deafness. Mitf is frequently downregulated in melanoma despite gene amplification being identified in a significant fraction of metastatic disease. To address the role of Mitf in the development of melanocytes and melanoma, we crossed Dct-rtTA and TRE-Mitf mice into the Mitf-null vga9 background, thus generating a Dct promoter-driven, doxycycline-inducible Mitf (DMV) mouse. Activation of Mitf by constant doxycycline treatment initiated from Embryonic day 1, did not rescue the coat color of DMV mice. However, the treatment restored the appearance of eyes in DMV mice, including open eyes, normal size, and partial pigmentation. In contrast, DMV mice in doxycycline-free control group had the same phenotypes as vga9 mice. Histological studies of Mitf-induced DMV mice showed that melanocytes were absent from the skin, including hair follicles, epidermal and dermal regions. Retinal pigment epithelium (RPE) was partially restored, but neither the photoreceptors nor the vision of the mice was rescued.

Interestingly, Sox10-positive pigmented cells were identified in choroid and iris, indicating they were uveal melanocytes that partially rescue the eye color. Our results suggest that Dct may be activated by an Mitf-independent pathway in the early stage of uveal melanocyte development, implying uveal and cutaneous melanocytes are derived from distinct sub-lineages of neural crest and explaining why uveal and cutaneous melanomas are dominated by different driver mutations (BRAF/NRAS and Gnaq/Gna11, respectively). Because of the specificity in rescuing pigment cells in the eyes, DMV mice provide a potential model to study uveal melanocytes and melanoma.

CS.05.05 | Delineating the role of MITF isoforms in melanogenesis

Jessica L. Flesher[1]; Elyse K. Paterson[2]; Priya Vasudeva[1]; Grant R. MacGregor[1]; Jonathan Neumann[1]; Anand K. Ganesan[1]

[1]University of California, Irvine, Irvine, CA, USA; [2]Oregon Health and Science University, Portland, OR, USA

9-*cis* retinoic acid stimulates melanogenesis through upregulation of melanocyte specific gene expression, protein levels and pigment accumulation. The biological effects of 9-*cis* retinoic acid are mediated through the activation of nuclear receptors, the retinoid X receptor (RXR) and the retinoic acid receptor (RAR). Here we show through chromatin immunoprecipitation that RXR and RAR bind to the promoter region of *MITF-A*, an MITF isoform expressed in melanocytes. Further validation revealed that RXR and RAR agonists induce activity of the *MITF-A* promoter indicating that RXR and RAR regulate the expression of *MITF-A*. Finally, we have generated novel *Mitf-A* and *Mitf-M* isoform specific knockout mice utilizing CRISPR/Cas9 techniques. The loss of Mitf-A results in a subtle pigment phenotype with no visible difference on a black coat. However, the loss of Mitf-M results in a dramatic loss of pigment in the coat, but pigment is retained in the eyes. The distinct pigment phenotypes of Mitf knockout mice illustrate the importance of studying specific Mitf isoforms in pigmentation. Taken together these studies showcase the potential for isoform specific deletion in mouse models and identify a novel role for MITF-A in the regulation of melanogenesis.

CS.05.06 | The mechanism and timing requirement for endothelin 3 regulation of murine coat color

Alexander Durango; Javier Pino

Florida International University, Hialeah, FL, USA

Melanocortin Receptor 1 (MC1R) signaling is essential for proper pigment production. In its absence, mice are unable to produce eumelanin and display a yellow coat color as seen in lethal yellow mice (Ay). We have previously shown that Endothelin Receptor b (EdnrB) signaling can compensate for the lack of MC1R in pigment production by crossing Ay mice with hyperpigmented inducible transgenic mice that over-express the EndrB ligand endothelin 3 in the skin (K5-Edn3). The goal of this study was to determine the mechanism of action and timing requirement for Edn3 in rescuing the coat color phenotype of Ay mice. To determine if the darker coat color of *K5*-Edn3 mice results from an increase in the number of follicular melanocytes, we performed immunofluorescence with Trp1 antibody. The numbers of follicular melanocytes in transgenic and control mice were not significantly different. We used qRT-PCR to evaluate if the darkened coat color produced by Edn3 is a result of melanogenic gene regulation in follicular melanocytes. There was at least a two fold increase in the expression of Mitf, Tyrosinase and Trp1 in the hair follicles of A^y:*K5*-Edn3 mice when compared to those of control animals. To establish the timing requirement for the maintenance and rescue of the darker coat seen in A^y;*K5*-Edn3 mice, we treated A^y;*K5*-Edn3 mice with doxycycline (DOX) for six weeks after birth to turn off transgene expression, The darkened coat color of the transgenic animals gradually receded until they were undistinguishable from their Ay littermates. A^y;*K5*-Edn3 mice that were treated with DOX during the entire gestational period (E0-P) were only capable of producing pheomelanin, and only showed a darker coat color after depilation and the start of a new hair cycle. Together these results show that Edn3 should be considered as an important player in pigment production regulation.

CS.06.03 | A genetic/epigenetic model for melanoma initiation and progression: Status in 2017

Dorothy C. Bennett

St George's, University of London, London, UK

A first genetic model for cutaneous melanoma development and progression was published in 2003. It proposed initiation of clonal proliferation by an oncogenic activation, producing a naevus; then came further changes needed to evade senescence and apoptosis, to generate a metastatic melanoma. Since then, the model has been tested in many ways and much information has been gathered about the genetic changes observed during progression and the order in which they occur, and about the genetic basis of familial melanoma which is also relevant. Some aspects of the initial model proved correct. For example benign nevi display the oncogenes also found in melanoma. Disruption of the p16/RB pathway and *TERT* reactivation are both important for advanced melanoma (being observed in the great majority of these), and so is repression of apoptosis. Others were incorrect; for example cellular immortality does not usually seem to be acquired at the onset of malignancy, but later on.

The current update of the model will be presented, with key evidence that has led to updates. This includes the potential roles of epigenetic regulation, and data from familial melanoma genetics, melanoma deep exomic sequencing, microdissection-based sequencing, gene expression studies and proteomics.

CS.06.04 | Regulation of melanosome pH determines skin and hair pigmentation

Dalee Zhou[1]; Koji Ota[1]; Kazumasa Wakamatsu[3]; Jonathan H. Zippin[1]

[1]*Weill Cornell Medicine, New York, NY, USA;* [2]*Yale School of Medicine, New Haven, CT, USA;* [3]*Fujita Health University, Nagoya, Japan*

Pigmentation is dependent on the pH of melanosomes, the melanocyte organelle that produces melanin. Melanosomes in lighter-skinned people are more acidic than those of darker-skinned people. It has been proposed that melanosome pH controls human pigmentation by regulating tyrosinase, the pH-sensitive, rate-limiting enzyme in melanin production. However, the signaling pathways that regulate melanosome pH are poorly understood, and the signaling mechanisms leading to the myriad mammalian eye, skin and hair colors remain unclear. Here we show that soluble adenylyl cyclase (sAC, *ADCY10*), a known regulator of lysosomal pH, defines a signaling cascade that controls pigmentation by regulating the pH set point of melanosomes. sAC was localized at melanosomes, and genetic and pharmacologic inhibition of sAC rapidly elevated melanosome pH, tyrosinase activity, and pigmentation in human and mouse melanocytes both in culture and *in vivo*. In human melanocytes, the frequency of sAC localization at melanosomes and the ability of sAC to alter pH and pigmentation depended upon the skin color of the melanocyte donor. sAC regulation of melanosomal pH was independent of Protein Kinase A (PKA), the most widely studied effector of cAMP; instead, sAC modulated melanosomal pH and pigmentation via the exchange protein activated by cAMP (EPAC). Thus, we now define a novel cAMP signaling cascade in melanocytes that regulates human pigmentation by modulating melanosome pH. Our elucidation of a signaling cascade that directly regulates melanosomal pH helps explain the breadth of human pigmentation, and has led to the discovery of a new class of drugs capable of elevating pigmentation *in vivo*.

CS.06.05 | Mahogunin ring finger 1 as a novel regulator of cell shape, motility and differentiation in melanocytes

Cecilia Herraiz[1]; María Castejón-Griñán[1]; Idoya Martínez-Vicente[1]; Marta Abrisqueta[1]; Julia Sirés-Campos[1]; Elena V. Sviderskaya[2]; Dorothy C. Bennett[2]; Conchi Olivares[1]; Celia Jiménez-Cervantes[1]; José C. García-Borrón[1]

[1]*Department of Biochemistry, Molecular Biology and Immunology, School of Medicine, University of Murcia and Instituto Murciano de Investigación Biosanitaria IMIB-LAIB, El Palmar, Murcia, Spain;* [2]*Molecular and Clinical Sciences Research Institute, St George's, University of London, Cranmer Terrace, London, UK*

Mahogunin Ring Finger 1 (Mgrn1) is an E3 ubiquitin ligase whose downregulation by the *mahoganoid* mouse mutation is associated with fur darkening, heart defects and neurodegeneration. In this study, we examined the molecular mechanisms underlying the physiological actions of Mgrn1 in melanocytic cells. We performed gene expression profiling of genetically-defined mouse melanocytic cells, a Mgrn1-null melanocytic cell line (melan-md1) and the littermate control line (melan-a6). Gene set enrichment analysis pointed to the regulation of cell migration and cytoskeleton remodelling, as an important process differentially regulated in these cell lines. Given that cell motility and cytoskeleton dynamics are critical in normal development and in melanoma progression, we compared the phenotype of Mgrn1 wild-type melan-a6 melanocytes and mutant melan-md1. We found that melan-md1 cells showed a dramatically different shape, with a highly differentiated, neuronal-like phenotype and were much more pigmented than control melanocytes. Moreover, medium derived from melan-md1 cells induced a more differentiated phenotype in control cells. Melanocytes from *mahoganoid* mice displayed a decreased rate of proliferation compared to control cells, as shown by 2D cell proliferation and by the behaviour of 3D spheroids embedded in collagen. Mgrn1-deficient melanocytes showed a lower migration and less invasion potential, and higher adhesion properties on collagen and Matrigel compared to control melanocytes. Further molecular analysis showed that loss of Mgrn1 had no significant effect on key Rho-GTPases activation. We have now focused on analysing Rho-GTPase effector molecule Rock2, which is downregulated in melan-md1 cells, as well as the role of integrins-α4 and -α5, which are upregulated in mutant cells. In addition, a phosphoarray analysis performed on control and melan-md1 cells revealed an important contribution of Src activation and other regulators of the cytoskeleton in Mgrn1-null melanocytes. Thus, we have preliminarily identified Mgrn1 as a new regulator of cell shape, motility and differentiation in melanocytic cells.

CS.06.06 | Effects of thioredoxin reductase 1 knockdowns in the human melanocyte-derived cell lines: Antioxidant response and growth in 2D and 3D cultures

Chelsey D. Kline; Madeleine Anderson; Matthew Honeggar; John Basset; Sancy Leachman; Pamela Cassidy

Oregon Health & Science University, Portland, OR, USA

UV-induced oxidative stress is known to contribute to DNA damage in melanocytes, the pigment producing cells in the skin. If not repaired properly, this damage can result in mutations and subsequent evolution of melanoma. Our laboratory is interested in understanding how detoxification of reactive oxygen species (ROS) produced by exposure of the skin to the sun's UV radiation is controlled by the antioxidant networks regulated by glutathione (GSH) and the selenoprotein, thioredoxin reductase 1 (TR1). Our group has evidence to show that the antioxidant selenoproteins and GSH are able to reduce UV-induced oxidative stress thereby mitigating the risk for melanoma posed by exposure to UV light. However, recently we found that TR1 expression levels vary in human melanoma tissues, with increased expression being associated with advanced stages of melanoma and increased metastasis. In order to directly evaluate the role of TR1 and GSH in melanoma and melanocyte cell lines, we have used shRNAs, CRISPR Cas9 and small molecule inhibitors to modulate TR1 activity and GSH levels. Antioxidant capacity

at baseline, growth rates in 2D culture and response to simulated solar radiation were then evaluated. We also employed hanging droplet cultures to generate 3D spheroid models for a better understanding of TR1's role in migration and invasion. In these studies, we hope to elucidate the mechanisms by which TR1 levels affect the response of the complex antioxidant networks of the melanocytes to UV-induced oxidative stress and oncogenic transformation. Results from these studies could lead to the identification of biomarkers of malignant transformation and the development of new melanoma chemoprevention agents.

CS.07.01 | A rational approach to macular pigmentation of uncertain aetiology

Prasad Kumarasinghe

Fiona Stanley Hospital and University of Western Australia, Nedlands, WA, Australia

Acquired hyper-pigmentation of the skin without an identifiable cause, without textural change(macular pigmentation of uncertain aetiology/MPUA/MPUE) is not an uncommon dilemma in clinical dermatology. Visible patches of hyperpigmentation can be distressing to the patient. It is more obvious in the dark skinned persons of Fitzpatrick skin Types III-V. Ashy dermatosis(AD), erythema dyschromicum perstans(EDP), lichen planus pigmentosus(LPP), idiopathic eruptive macular pigmentation(IEMP) and Riehl's melanosis are some terms that have been used to describe these cases. However the literature is confusing as there has been no real consensus on the use of the terms. One of the main problems is that the histopathology is rather non specific, with dermal melanophages(causing the ashy grey colour) and sparse dermal lymphocytic infiltrates. Interface dermatitis is seen in some cases, but not in all. The histopathology can be indistinguishable from post inflammatory hyperpigmentation, burnt out lichen planus, graft versus host reactions, fixed drug eruptions and several other conditions. It is very important to rule out various forms of drug induced hyperpigmentation, frictional melanoses, macular amyloidosis, endocrine causes etc. in diffuse and patchy acquired pigmentations. It is best to consider various causes that can lead to macular pigmentation of uncertain aetiology(MPUA) in a rational way before labelling as ashy dermatosis, LPP, EDP, Riehl's melanosis or IEMP. Various causes of acquired patchy pigmentation and a rational approach to diagnosing and managing these difficult cases will be discussed. The deliberations of the global forum on acquired macular pigmentation of uncertain aetiology will also be discussed.

CS.07.03 | MC1R highly selective small α-MSH analogs as topical sunless pigmentary agents with multiple clinical applications

Leonid Koikov[1]; Viki Swope[1]; Renny Starner[1]; Kendall Schick[1]; Abbas Raza[2]; Zalfa Abdel-Malek[1]

[1]University of Cincinnati, Cincinnati, OH, USA; [2]University of Minnesota, Minneapolis, MN, USA

α-Melanocyte stimulating hormone (α-MSH) is a major regulator of constitutive and facultative human pigmentation. There has been considerable interest in developing analogs of α-MSH as sunless tanning agents. The best known analog is the tridecapeptide [Nle^4, D-Phe^7]-α-MSH (NDP-MSH; melanotan 1; afamelanotide), which is more potent and stable than the native α-MSH. Clinical trials proved that NDP-MSH induces tanning without any sun exposure, reduces phototoxicity in patients with erythropoietic protoporphyria or polymorphic light eruption, prevents actinic keratosis, and repigments vitiligo skin. However, NDP-MSH has to be delivered systemically, and has side effects due to its binding to other melanocortin receptors, besides MC1R that is expressed on melanocytes. We developed tetra- and tripeptide α-MSH analogs, which include the 6-9 or 6-8 amino acid residues, with substitution of L-Phe^7 by D-Phe, and various N-capping modifications. Five analogs had unique selectivity for MC1R, including two tetrapeptides that were 100 fold more potent than α-MSH, and equally potent to NDP-MSH, and three tripeptides that were only 10 fold less potent than α-MSH in activating the MC1R, as measured by increasing cAMP levels and tyrosinase activity. These peptides, similar to α-MSH, also reduced DNA photoproducts independently of increasing pigmentation. Testing of the two tetrapeptides and one tripeptide on cultured skin substitutes resulted in increased pigmentation and enhanced repair of DNA photoproducts in melanocytes and the entire epidermis within 10–15 days of treatment, without affecting skin morphology or melanocyte number. The tripeptide could be applied topically on skin explants, resulting in increased pigmentation after daily treatment for 6 days. Therefore, our peptides can serve as effective topical sunless tanning agents, with the added benefits of activating DNA repair pathways to reduce sun-induced genotoxicity and photocarcinogenesis, as well as stress-induced apoptosis of melanocytes in vulnerable individuals. These peptides can be effective for melanoma prevention and vitiligo treatment.

CS.07.04 | Mosaicism for a KITLG mutation in linear and whorled nevoid hypermelanosis

Arthur Sorlin[1]; Annabel Maruani[2]; Marie-Hélène Aubriot-Lorton[1]; Yannis Duffourd[1]; Jean-Baptiste Rivière[1]; Laurence Faivre[1]; Pierre Vabres[1]

[1]CHU Dijon Bourgogne, Djon, France; [2]CHU Tours, Tours, France

Linear and whorled naevoid hypermelanosis (LWNH) - hyperpigmented macular swirls and streaks following Blaschko's lines - has often been considered as a nonspecific manifestation of mosaicism. It has sometimes been mistaken with the pigmentary stage of incontinentia pigmenti. In a few patients, various X chromosome rearrangements have been reported, but the molecular basis of LWNH has remained unknown.

We performed deep exome sequencing on skin DNA from a 6 year-old patient with congenital non-progressive linear naevoid hyperpigmentation following Blaschko's lines on his trunk and limbs, without other cutaneous or neurosensory symptoms. We identified

a postzygotic heterozygous *KITLG* c.329A>G (p.Asp110Gly) mutation, predicted to be deleterious in-silico, confirmed by targeted deep sequencing in skin fibroblasts (28% of reads) and blood (18%). Paraffin-embedded skin section immunostaining using anti-KITLG polyclonal antibody showed intense nuclear and cytoplasmic staining of basal and spinous layer keratinocytes. In addition, c-KIT staining showed increased epidermal expression in basal keratinocytes.

KITLG (c-KIT Ligand, or Stem Cell Factor) regulates skin pigmentation through control of melanocyte migration, proliferation and survival, and melanin synthesis. Germline *KITLG* mutations have been reported in patients with Familial Progressive Hyper-and Hypopigmentation (FPHH), and in patients with isolated hearing loss or Waardenburg syndrome type 2A. This is the first report of a genetic basis for LWNH, which can be considered a mosaic presentation of a Mendelian disorder, FPHH. Hyperpigmentation associated with the p.Asp110Gly *KITLG* mutation appears to result from primary functional alteration of keratinocytes, rather than melanocytes, with autocrine and paracrine upregulation of c-KIT resulting in increased melanogenesis in melanocytes.

CS.07.05 | The unfolded protein response, mediated by PERK and IRE1α signaling, contributes to vitiligo pathogenesis

Omotayo A. Arowojolu; Nada Elbuluk; Seth J. Orlow; Prashiela Manga

The Ronald O. Perelman Department of Dermatology, New York University School of Medicine, New York, NY, USA

Interfollicular epidermal melanocytes are continually subjected to environmental challenges and activate protective stress responses for survival. Dysregulation of these responses may increase susceptibility to autoimmune-mediated destruction resulting in progressive skin depigmentation typical of vitiligo. We have shown that challenging melanocytes from normally pigmented individuals (NMs) with chemicals known to trigger vitiligo, such as monobenzone, results in activation of the unfolded protein response (UPR). In this study, we investigated the impact of the PERK-eIF2α (activated PERK phosphorylates eIF2α) and IRE1α-XBP1 (activated IRE1α promotes splicing and expression of XBP1) axes of the UPR on melanocyte viability and sensitivity to monobenzone.

NMs exhibited high basal PERK-eIF2α signaling compared to keratinocytes and dermal fibroblasts, and PERK knockdown substantially reduced melanocyte viability ($p < 0.01$), even in the absence of challenge. PERK inhibition increased sensitivity to monobenzone, while inhibition of IRE1α kinase activity, did not affect melanocyte toxicity. NMs that survive PERK knockdown were used to establish long-term cultures (shPERK^{LT}), which exhibited a paradoxical increase in phospho-eIF2α with reduced sensitivity to monobenzone. Sustained eIF2α phosphorylation was reduced with downregulation of PKR and GCN2, alternative eIF2α kinases, suggesting a role for these kinases in melanocyte adaptation.

Melanocytes from individuals with idiopathic vitiligo (VMs) exhibited increased sensitivity to monobenzone compared to NMs. VMs markedly activated the IRE1α/XBP1 pathway, reflected by an increase in XBP1 splicing. VMs also did not phosphorylate eIF2α in response to monobenzone treatment. Dysfunction of this protective response in VMs, in combination with increased IRE1α/XBP1 activity which promotes expression of chemokines, such as interleukin 6, that recruit immune cells to the skin, may contribute to the onset of autoimmunity in vitiligo. The UPR may thus represent a novel therapeutic target for vitiligo.

CS.07.06 | Cole disease: Role of ENPP1 in regulation of pigmentation and epidermal differentiation

Véronique Guyonnet-Duperat[2]; Eli Sprecher[1]; Alain Taieb[2]; Muriel Cario-Andre[2]

[1]*Department of Dermatology, Sourasky Medical Center, Tel Aviv, Israel;* [2]*Inserm 1035, Bordeaux University, Bordeaux, France*

Cole disease is a rare autosomal dominant disorder characterized by hypopigmented macules and hyperkeratosis. However, patient may also have hyperpigmented macules. In hypopigmented macules, a normal number of pigmented melanocytes but decreased melanin content in keratinocytes, suggesting an impairment of melanosome transfer, have been reported. Five mutations in somatomedin-B-like domains of EctoNucleotide Pyrophosphatase /Phosphodiesterase 1 (ENPP1) have been identified in five families with Cole disease. To study the role of ENPP1 in pigmentation and skin differentiation, we, first, reconstructed skin with cells from one patient with Cole disease and surprisingly Cole melanocytes were able to induced thickening of epidermal reconstructs. Since this disease is rare, we transduced melanocytes and keratinocytes with lentivectors coding wild-type (WT) ENPP1 or coding the first three mutations (M) identified in ENPP1. In melanocytes, at the protein level, expression of TRP-1 and tyrosinase but not of MITF seemed inversely correlated to the level of expression of mutated ENPP1. However, increased ENPP1 mRNA was not associated with decrease in MITF, tyrosinase, TRP-1 and TRP-2 mRNA. We could even observed an increase in these enzymes. Our results suggest that melanin synthesis modulation by ENPP1 is complex and may induced hyper or hypopigmentation. Reconstructions with melanocytes transduced with mutated ENPP1 were not thicker than those reconstructed with melanocytes transduced with WT melanocytes. But reconstructions with cells co-transduced with WT and mutated ENPP1 forms seemed thicker than those with WT alone. We also used pharmacological inhibitor of ENPP1 on monolayer cell culture. Secretome from melanocytes treated with ENPP1 inhibitors modulated expression of keratin 5 in keratinocytes whereas direct inhibition of ENPP1 in keratinocytes seemed less effective. Thus mutations of ENPP1 seemed directly implicated in establishing and sustaining hypo or hyperpigmentation in Cole Disease. Furthermore

melanocytes seemed implicated in establishment of hyperkeratosis in Cole Disease.

CS.07.07 | Diminished autophagy activity in the epidermis causes hyperpigmentation accompanied by epidermal differentiation disorders

Akira Hachiya[1]; Daiki Murase[1]; Ayumi Kusaka-Kikushima[2]; Rachel Fullenkamp[3]; Tadashi Hase[4]; Atsushi Oouchi[1]; Tamotsu Yoshimori[5]

[1]Kao Corporation, Haga-Gun, Japan; [2]Kao Corporation, Odawara, Japan; [3]Kao Corporation, Cincinnati, OH, USA; [4]Kao Corporation, Sumida-Ku, Japan; [5]Osaka University, Suita, Japan

Melanin in the epidermis transferred from melanocytes to keratinocytes defines skin color even while its variation associated with ethnic skin diversity is accelerated. Recently we have clarified the essential role of autophagy in determining skin color by regulating melanosome degradation in epidermal cells, which results in racial skin color differences. Apart from the racial differences, skin color unevenness, such as hyperpigmentation, becomes obvious within individuals especially according to aging and that is psychologically distressing. Since mechanistic analyses of hyperpigmentation have been performed especially from the viewpoints of cytokine networks, the impaired autophagic activity was elaborately examined using punch biopsies of human skin from hyperpigmented areas observed at sun-exposed and/or joint areas in the current study. Immunohistological analysis demonstrated the decreased expression of LC3-II, a marker of autophagy, and the disorganized expression of epidermal differentiation markers, such as transglutaminase, loricrin, filaggrin, etc., at hyperpigmented areas. Those areas were characterized by remarkable melanin accumulation as well as by hypertrophic epidermis and stratum corneum although similar levels of Pmel17 expression were observed in the hyperpigmented and peripheral areas. Consistently, a flux assay quantitatively confirmed the remarkably diminished epidermal autophagic activity at hyperpigmented areas compared to peripheral areas. Additionally, when Torin 1, an inducer of autophagy, was administered to human skin cultured ex vivo even for 5 days, epidermal melanin deposition was significantly reduced and disordered epidermal differentiation with a thicker epidermis and stratum corneum was substantially restored accompanied by a significantly enhanced expression of LC3-II in the epidermis. Taken together, our data reveal that autophagy plays a pivotal role in epidermal homeostasis at least by regulating melanosome degradation and by controlling epidermal differentiation in epidermal cells. These results suggest that autophagy might be an appropriate target to restore skin problems characterized by melanosome deposition and/or disordered epidermal differentiation.

CS.08.01 | Vitamin D and melanoma: What is new under the sun

Andrzej T. Slominski[1]; Michal Zmijewski[2]; Anna Brozyna[3]; Cezary Skobowiat[1]; Tae-Kang Kim[1]; Allen Oak[1]; Wojciech Jozwicki[3]; Anton Jetten[4]; Rebecca Mason[5]; Wei Li[6]; Robert Tuckey[7]

[1]UAB, Birmingham, AL, USA; [2]AMG, Gdansk, Poland; [3]Department of Tumor Pathology and Pathomorphology, Oncology Centre - Prof. Franciszek Łukaszczyk Memorial Hospital, Bydgoszcz, Poland; [4]National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; [5]Sydney Medical School, The University of Sydney, Sydney, NSW, Australia; [6]University of Tennessee Health Science Center, Memphis, TN, USA, 7. University of Western Australia, Perth, WA, Australia

VB (290–320 nm) is a well-recognized etiological factor in malignant transformation of melanocytes and melanoma development, and is also necessary for the production of vitamin D3 (D3). In the canonical pathway D3 is sequentially hydroxylated in position C25 and C1 to produce 1,25$(OH)_2$D3. We have discovered non-canonical pathways activated by CYP11A1, which generate several D3-hydroxyderivatives with a main product represented by 20(OH)D3 that is further metabolized to several di- and tri-hydroxy metabolites. In addition, after the absorption of UVB energy, CYP11A1 derived Δ7-sterols/steroids, including 7-dehydropregnenolone and their hydroxylated products or Δ7-androsta-steroids, are transformed to the corresponding secosteroids with or without a full-length side chain and to compounds with a lumisterol-like configuration. Both canonical 1,25$(OH)_2$D3 and non-canonical hydroxysecosteroids or lumisterol/lumisterol-like derivatives show anti-melanoma activities *in vitro* and protective properties against UVB-induced damage in melanocytes. Importantly, 20(OH)D3 injected intraperitoneally inhibits growth of human melanoma cells transplanted subcutaneously into immunodeficient mice. It is accepted that low levels of 25(OH)D3 are associated with more advanced melanomas and shorter patient survival with single nucleotide polymorphisms of the vitamin D receptor (VDR) affecting development or progression of melanoma, or disease outcome. An inverse correlation of VDR and CYP27B1 expression with melanoma progression has been found, with low or undetectable levels of these proteins being associated with poor disease outcomes. Unexpectedly, increased expression of CYP24A1 correlated with better prognosis for melanoma patients. Furthermore, a decreased expression of retinoic acid orphan receptors (ROR) A and C correlated with melanoma progression and shorter disease-free and overall survival time. Therefore, we propose that educated targeting of VDR and RORs receptors by novel secosteroidal and lumisterol/lumisterol-like derivatives may provide new strategies in melanoma therapy. We also believe that optimal vitamin D management should be beneficial for melanoma patients or in melanoma prevention.

CS.08.02 | UV irradiation modulates maintenance and differentiation of hair follicle-derived neural crest stem cells via miR-200c/Bmi1 pathway

Min Jiang[1]; Chengfeng Zhang[1]; Ruifeng Yang[2]; Xiaowei Xu[2]; Leihong Flora Xiang[1]

[1]*Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China;* [2]*Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA*

The neural crest (NC) is a transient embryonic structure that invades the embryo, and ultimately generates a wide variety of cell types. Previously our group successfully isolated multipotent precursor cells with NC characteristics from bulges of hair follicles. These neural crest stem cells (NCSCs) are considered as a key resource for melanocyte regeneration. In the present work, we expanded and characterized primary NCSCs from human hair follicles. Then we showed that miR-200c expression was obviously up-regulated by UV irradiation in vitro. Further, overexpression of miR-200c dramatically suppressed NCSCs proliferation/self-renewal, and induced apoptosis and senescence, these phenomena were also induced by UV irradiation and were partially rescued by miR-200c inhibition. Meanwhile miR-200c increased the spontaneous differentiation of NCSCs, including melanogenesis and adipogenesis. Moreover, miR-200c target Bmi-1 messenger RNA and protein were downmodulated by UV irradiation and by miR-200c overexpression. We revealed that Bmi-1 downmodulation is required for miR-200c-mediated cell arrest.

Bulge-derived NCSCs are critical for skin repigmentation. Maintenance of NCSCs may be a promising approach for dermatological clinic. UV irradiation modulates NCSCs stemness via miR-200c/Bmi1 pathway. Altogether, this study demonstrates the possibility to use miR-200c/Bmi-1 as a potential target in pigmentary diseases.

CS.08.03 | Pro-oxidant activity of mouse hair pheomelanin is promoted by UVA irradiation

Hitomi Tanaka[1]; Yui Yamashita[2]; Shosuke Ito[2]; Kazumasa Wakamatsu[1,2]

[1]*School of Health Sciences School of Health Research, Graduate School of Health Sciences, Fujita Health University, Toyoake, Japan;* [2]*Department of Chemistry, Fujita Health University School of Health Sciences, Toyoake, Japan*

Background and Objectives: Melanocytes produces the black to dark brown eumelanin (EM) and reddish brown to yellow pheomelanin (PM). Melanin pigments, especially PM, are sensitive to UVA, giving rise to reactive oxygen species (ROS) that indirectly damage DNA. It was recently shown that PM exhibits pro-oxidant activity even in the dark. In this study, to examine the effect of UVA on the pro-oxidant activity of PM, we irradiated hairs from C57 BL recessive yellow (*e/e*), black (*a/a*), and albino (*c/c*) mouse hair with UVA in the presence of reduced glutathione (GSH). Those mice produce almost pure PM, EM, and no melanin, respectively.
Methods: HPLC method was used for measurement of GSH and GSSG and spectrophotometric method was used for H_2O_2. 1 mg/mL hair suspension (NaPB, pH 6.8) was irradiated with UVA (325 – 400 nm; 3.5 mW / cm^2;) for 3, 7, and 24 hr in the presence of 1000 μM GSH.
Results: The results show that PM in the yellow hair consumed all of GSH (1000 μM), the level significantly greater than those in the black (800 μM) and in the white (750 μM) hair. Most (ca. 80%) of GSH was oxidized to GSSG. Hairs not irradiated with UVA did not oxidize GSH (160, 290, 140 μM, respectively). H_2O_2 was produced at a high level (107 μM) in the yellow hair while it was found at trace levels in the black (23 μM) and in the white (25 μM) hair. The benzothiazine (BT) structure of PM is reduced to the dihydrobenzothiazine (DHBT) structure through UVA-promoted oxidation, while GSH is oxidized to GSSG with a concomitant production of H_2O_2.
Conclusion: These results suggest that UVA plays an important role in promoting the pro-oxidant activity of PM.

CS.08.04 | GNAQ/GNA11 pathway mutations are involved in malignant transformation in a mouse model of UV-induced melanocytic lesion progression

Helen T. Michael; Chi-Ping Day; Antonella Sassano; Aleksandra Michalowski; Howard Yang; Maxwell Lee; Glenn Merlino

National Cancer Institutes, Bethesda, MD, USA

Progression of melanocytic nevi to malignant melanoma is rare, but contributes substantially to melanoma development. Early diagnosis and accurate differentiation of atypical nevi and early melanomas is important since clinical stage and melanoma thickness at the time of diagnosis correlate with patient prognosis. Ultraviolet light (UV) exposure, especially childhood sunburn, is associated with nevus formation and increased risk of melanoma, although the exact mechanisms remain unclear. Most melanomas show MAPK pathway activation, and mutations in BRAF or NRAS are present in most cutaneous melanomas and nevi. A small number of wildtype BRAF/NRAS cutaneous and most uveal melanomas have mutations in GNAQ/11, which signal through MAPK and other pathways. The long latency before malignant progression and low progression rate make studying nevus progression in the human population difficult. Mice are resistant to UV-induced melanoma formation partly because melanocytes are restricted to the hair follicles. Our laboratory developed the hepatocyte growth factor (HGF) transgenic mouse model with humanized epidermal-dermal junctional distribution of melanocytes. Following a single, clinically-relevant dose of neonatal UV, HGF mice on either FVB or C57Bl/6 backgrounds develop melanocytic nevi, some of which progress to melanoma. Exome sequencing of nevi, radial growth phase and vertical growth phase melanomas in this model reveals significantly higher mutations in the vertical growth phase melanomas than radial growth phase melanomas or nevi. Over 60% of melanomas contained GNAQ/11 pathway mutations, suggesting a role in malignant transformation. While the remaining 30% do not have an obvious driver. The GNAQ/11 pathway may be driving the neonatal UV HGF melanoma model. We anticipate our UV HGF model

will be useful for evaluating the role of the GNAQ/11 pathway in malignant transformation of UV-induced melanocytic lesions and for the discovery and novel and potentially more efficacious targets for treating GNAQ/11 mutant and other BRAF/NRAS wildtype melanomas.

CS.08.05 | Assessing the photoprotective property of skin colour against SSR-induced CPD damage

Damilola Fajuyigbe; Robert Sarkany; Antony R. Young

St John's Institute of Dermatology, King's College London, London, UK

Ultraviolet radiation (UVR) causes epidermal DNA damage, which initiates skin carcinogenesis. Epidemiological studies show a lower incidence of skin cancer in people with pigmented skin compared to fair skin. This is attributed to photoprotection by epidermal melanin. Some authors have shown that the degree of UV-induced DNA damage is influenced by constitutive pigmentation with greater protection (maximum of 6 fold) afforded by dark skin. We seek to understand how skin cancer markers differ in Caucasian(SPT I/II) and West African(SPT VI) subjects. Upper buttocks of subjects (6 SPT I/II and 6 SPT VI) were exposed to solar simulated radiation (SSR) dose series. Exposures were based on standard erythema doses and biopsies taken immediately after exposure were stained with monoclonal antibodies. Using the same antibody, monocytes extracted from blood taken from 7 SPT I/II and 7 SPT VI were assessed for DNA repair 12 and 24 hr post SSR exposure. *In vivo*, we found comparable amount of damage when the SSR dose in SPT VI is 10 fold greater than in SPT I/II thus indicating an overall epidermal melanin DNA protection of 10, much higher than previously recorded. We then made comparisons at different epidermal regions especially in the basal layer because lesions in the basal layer (contains proliferative stem cells) may be relevant to greater susceptibility to skin cancer. Unlike SPT I/II, SPT VI showed a clear effect of epidermal region with less DNA damage with increasing epidermal depth. The protective effect of melanin in the basal layer was 40 fold; a figure much closer to the 32 fold difference in melanoma incidence in black and fair skinned individuals. These results as well as our lack of skin colour dependent difference in DNA repair *in vitro* raises the possibility that factors besides genetic differences are important in determining skin cancer susceptibility.

CS.08.06 | Photo-protection assessment through gene expression modulation induced by UV exposure on a Chinese reconstructed full-thickness skin model

Yang Liu[1]; Ranran Wang[1]; Xiaofeng He[1]; Hongbin Dai[1]; Richard Betts[1]; Claire Marionnet[2]; Francoise Bernerd[2]; Emilie Planel[2]; Xiuxia Wang[1]; Hicham Nocairi[2]; Zhenzi Cai[1]; Jie Qiu[1]; Chunmei Ding[1]

[1]L'Oréal Research and Innovation, China, Shanghai, China; [2]L'Oréal Research and Innovation, France, Aulnay-sous-Bois, France

Chronic exposure to sub-erythemal doses of ultraviolet (UV) rays is strongly associated with an increase in cutaneous malignancies and a change in some skin aesthetical aspects (spots, wrinkles, etc). As such, daily photo-protection is an important area of research for optimizing the prevention of skin damage. The development and evaluation of new photo-protection actives and UV filters requires accurate biological evaluation techniques prior to clinical proof-of-concept studies and determination of protection factors. Three dimensional reconstructed skin models are powerful tools for this purpose since classical cell cultures poorly reproduce the 3D architecture and tissue organization of human skin, and are not suitable for formulation evaluation.

Within the study, we developed a full-thickness, 3D reconstructed skin model of Asian origin, and then subsequently examined the transcriptomic response of the model following daily UV radiation using QuantigenePlex, a novel transcriptomic analysis platform, finally validated this process for photoprotection evaluation. Our data demonstrates that the features of this reconstructed skin model are similar to native human skin, showing a well-stratified fully differentiated epidermis with a stratum corneum and a living dermis underneath, suitable for topical application of active ingredients and formulations. Response of fibroblasts and keratinocytes in the model to daily UV exposure was studied, revealing transcriptomic alterations linked to different UV-induced pathways, including oxidative stress, inflammation and extracellular matrix remodeling, etc. Changes observed at the gene level were further confirmed via protein expression, demonstrating the robustness of the daily UV response of the model. The approach has been validated for the evaluation of photoprotective properties of various sunscreen products, showing a close positive correlation with protection factors as established clinically on Asian volunteers.

These findings demonstrate that assessing UV-induced gene modulation within the Asian reconstructed skin model is a biologically-relevant approach with sufficient sensitivity and predictivity to evaluate photoprotection actives and products.

CS.08.07 | TLR3 stimulation regulate phagocytosis activity of epidermal keratinocytes though PAR-2/ Rho signaling pathway

Saaya Koike; Kenshi Yamasaki; Takeshi Yamauchi; Setsuya Aiba

Tohoku University Graduate School of Medicine, Sendai, Japan

Innate immune stimuli restlessly influence epidermis where human keratinocytes and melanocytes reside. We previous reported that TLR3 agonist poly(I:C) and UVB irradiation increased melanin release from melanocytes through increasing Rab27A expression and cell peripheral accumulation, which facilitate melanosome transportation to cell membrane of melanocytes. To further explorer machineries involved in melanosome transfer by innate immunity, we examined the effect of innate immune stimuli on keratinocytes functions involving melanosome transfer. To examine if keratinocyte enhance

melanosome uptake by TLR agonists and UVB, we isolated melanosome from normal human melanocytes and added the isolated melanosome to the keratinocytes pre-treated with TLR agonists or UVB irradiation. Poly(I:C) and UVB stimuli increased melanosome positive keratinocytes and siTLR3 inhibited the uptake of melanosome. To explorer factors promote phagocytosis of keratinocytes, we focused on PAR-2/Rho signaling pathway since PAR-2 promotes the keratinocyte phagocytic capability by actin reorganization and morphological change of cell surface. We examined PAR-2 expression in keratinocytes and observed increase in PAR-2 by Poly(I:C) stimuli or UVB irradiation. SiTLR3 inhibits the increase of PAR-2 expression induced by Poly(I:C) and UVB. Poly(I:C) also increased Rho1 mRNA expression, the downstream factor of PAR-2. Because PAR-2 also activates Rac and Cdc42, which activate phagocytosis by developing pseudopodia and reconstruction of actin filaments, we examined and observed that Poly(I:C) increases Cdc42 but not Rac1 mRNA expression in keratinocytes. These results suggest that TLR3 stimuli increase melanosome uptake by promoting phagocytosis activity of keratinocytes through PAR-2 and its downstream effectors Rho1 and Cdc42.

CS.08.08 | Convergence of endothelin B receptor and melanocortin 1 receptor signaling on key components of the DNA damage response pathway of melanocytes

Viki Swope[1]; Renny Starner[1]; Ranjan Perera[2]; Zalfa Abdel-Malek[1]

[1]University of Cincinnati, Cincinnati, OH, USA; [2]Sanford-Burnham Prebys Medical Discovery Institute, Orlando, FL, USA

Endothelin-1 (End-1) and α-melanocyte stimulating hormone (α-MSH) are two keratinocyte- derived paracrine factors that are essential regulators of human melanocyte (HM) proliferation, survival, and pigmentation. We showed that both factors reduce oxidative stress and enhance repair of DNA photoproducts in UV-irradiated HM. End-1 binds to the Endothelin B receptor (EndBR) on HM, and activates intracellular Ca^{2+} mobilization and PKC. α-MSH binds the melanocortin 1 receptor (MC1R), and activates the synthesis of cAMP. We report that End-1 and α-MSH enhance the DNA damage response of HM to UV by posttranslational and transcriptional mechanisms. End-1 and α-MSH increase the rapid phosphorylation and activation of the DNA damage sensors ATR and ATM, the translocation of the DNA repair protein XPA to chromatin, and the levels of γ-H2AX, which facilitates the recruitment of DNA repair proteins to DNA damage sites. Additionally, End-1 and α-MSH increase the phosphorylation of JNK and p38, and the levels and transactivation of p53. These effects of α-MSH are mediated by activation of MC1R, as they are markedly diminished in HM expressing loss-of-function *MC1R* variants, or upon treatment with the MC1R antagonist ASIP or the PKA inhibitor H89. The effects of End-1 were independent of *MC1R* genotype. End-1 and α-MSH affect the expression of a common set of miRNAs, and reverse the effects of UV on some of these miRNAs. The Gene Ontology pathways mostly affected by these miRNA are cellular metabolism, cell cycle, and apoptosis. Our results strongly suggest that End-1 signaling compensate for the loss of MC1R function, which is associated with increased risk for melanoma. A common downstream effector of MC1R and EndBR, e.g. a miRNA that regulates expression of genes involved in maintenance of genomic stability, can potentially be targeted for melanoma prevention.

PS.03.01 | Pathways of melanoma development: Prevention and therapeutic implications

David E. Fisher[1,2,3]

[1]Massachusetts General Hospital, Boston, MA, USA; [2]Harvard Medical School, Boston, MA, USA; [3]MGH Cancer Center, Boston, MA, USA

Melanocytes produce pigments that may participate actively in UV protection. Their synthesis arises from constitutive and adaptive pigmentation pathways, both of which are regulated by the action of MITF, a signal-responsive transcriptional inducer of the melanogenesis machinery. The pathway through which UV induces MITF has been elucidated, and sheds light on how red pheomelanin and dark eumelanin synthesis are controlled. Animal models of the red hair phenotype have identified carcinogenic roles for pheomelanin, which in addition to its chemical toxicity is challenging to clinically visualize in the epidermis. The use of topically administered small molecules may permit rescue of eumelanin synthesis, thereby potentially providing a novel prevention strategy. A molecular byproduct of the UV-pigment response is beta-endorphin, which has been found to contribute behavioral effects to UV exposure. UV is also trigger formation of signature mutations, some of which produce neoantigens that may be recognized and targeted in melanoma cells. The roles of neoantigens in melanoma immunotherapy will be discussed, as well as novel approaches to overcome neoantigen deficiency in melanoma therapy.

PS.03.03 | Ubiquitin ligase RNF5 coordinates immune and microbiome control of melanoma

Ze'ev Ronai

SBP Discovery Medical Research Institute, La Jolla, CA, USA

To date, the success of immune checkpoint inhibitors in cancer therapy has been limited to a few targets and tumor types, underscoring the need for greater understanding of immune checkpoint control. Here, we establish a link between the ubiquitin ligase RNF5, composition of the gut microbiota and immune checkpoint control. Compared with $Rnf5^{wt}$ mice, growth of mouse melanoma cells *in vivo* is attenuated, while tumor infiltration of $CD4^+/CD8^+$ T cells and dendritic cells is increased in $Rnf5^{-/-}$, resembling anti-CTLA-4 treatment. This phenotype was linked to reduced villi length and increased ER stress in intestinal epithelial cells. Notably, co-housing of $Rnf5^{-/-}$ and WT mice

largely abolished these phenotypes. Computer simulations identified the requirement of key metabolites that can reproduce the observed abundances bacterial taxa in the *Rnf5*$^{-/-}$, compared with the WT mice. Significantly, pre-biotic-fed WT mice phenocopied *Rnf5*$^{-/-}$ mice, as they exhibited increased tumor infiltration of immune cells and reduced tumor growth. The ability of select prebiotics to phenocopy changes identified in the *Rnf5*$^{-/-}$ mouse offer a new paradigm in understanding mechanisms underlying gut microbiome-immune checkpoint control.

PS.04.01 | Human pigmentation genetics for the clinic

Richard A. Sturm

University of Queensland, Bribane, QLD, Australia

Host pigmentation characteristics play an important role in the effects of sun-exposure, skin cancer induction and disease outcomes. Several of the genes most important for the diversity seen in human populations are involved in the regulation and distribution of melanin pigmentation or enzymes involved in melanogenesis itself within the melanocyte cell present in the skin, hair and eyes. The single nucleotide polymorphisms (SNPs) and extended haplotypes within and surrounding pigmentation and naevogenic genes have been identified as risk factors for skin cancer, in particular melanoma. These same polymorphisms have been under evolutionary selective pressure leading towards lighter pigmentation in European and Asian populations in the last 5000–20,000 years that have driven the increase in frequency in modern populations. Although pigmentation is a polygenic trait, due to interactive and quantitative gene effects, strong individual phenotypic associations are readily apparent for several genes with predictive value. The major genes with significant effect for skin, hair and eye colour, freckling and naevogenesis have now been identified, with at least 15 genes having common SNP alleles or haplotypes known to influence these interrelated phenotypes. However, few of these genes have high penetrance for all of these traits combined, the exception being *TYR* the enzyme critical for melanogenesis and the transcription factor *IRF4* that can regulate *TYR* expression. In contrast, some genes appear to have large effects for only one or two tissues such as *OCA2* for eye, *MC1R* for hair and skin, and *SLC45A2*/*SLC24A5* for skin colour. With the move toward personal or precision medicine the knowledge of how these common pigmentation and naevogenic alleles in our populations directly influence skin types and presentation of skin lesions must be actively incorporated into clinical practice.

PS.04.02 | The genetic architecture of human hair pigmentation

Michael D. Morgan[1,2]; David Sims[1]; Jonathan Rees[3]; Albert Tenesa[3]; Ian J. Jackson[3]

[1]*University of Oxford, Oxford, UK;* [2]*Sanger Institute, Cambridge, UK;* [3]*University of Edinburgh, Edinburgh, UK*

UK Biobank is a cohort of over 500,000 individuals who have been extensively phenotyped, including self-assessed hair colour. The participants have also been genotyped at over 800,000 SNPs, from which a total of approximately 7,000,000 SNPs can be imputed. We measured the impact of *MC1R* variants on red hair and can quantify the variable penetrance of the different alleles, some of which have exceedingly low penetrance. We performed genome wide association studies for red vs non-red hair and for blonde versus black and brown hair. We identify additional red-hair associated variants at *MC1R*, including non-coding variants, in addition to SNPs near the *ASIP* (agouti) locus which are themselves associated with variation in expression of *ASIP*. We also identify novel red hair associated genes. Whilst the genetic architecture of red hair is relatively simple, the blonde hair GWAS identifies 77 regions of association. Some of these are at genes previously shown to be involved in pigmentation in humans or model organisms, whilst others are novel. 15 loci are linked with gene expression variation in neighbouring genes. We constructed a polygenic score for hair colour which shows that blonde to black hair colour lies on a continuum, where increased numbers of variants shifts hair colour along the spectrum, whilst red hair colour lies in a separate dimension. Finally we analysed the associated SNPs for enrichment of gene expression regulatory genome annotations and find enrichment of regulatory domains in melanocytes, fibroblasts and whole skin but not in other tissues. The genetics of hair colour represents an excellent model for the dissection of complex genetic phenomena. Furthermore, our study is discovering novel genes with a role in melanocyte biology.

PS.04.03 | The genetics of vitiligo

Richard Spritz[1]; Ying Jin[1]; Stephanie Santorico[2]

[1]*University of Colorado School of Medicine, Aurora, CO, USA;* [2]*University of Colorado - Denver, Denver, CO, USA*

Vitiligo is a common depigmenting disease caused by autoimmune loss of melanocytes. Vitiligo causation is complex, involving many genes and unknown environmental triggers. By genomewide studies in various world populations, we have discovered ~50 vitiligo susceptibility loci in European-derived Caucasians. For many, the corresponding causal gene, variants, and pathogenetic mechanism have been identified. Together, these genes highlight a network of immune and apoptotic regulation, melanocyte function, and autoimmune targeting. Several vitiligo loci are also implicated in other autoimmune diseases, accounting for their epidemiologic association with vitiligo. Some vitiligo loci are relatively population-specific while others are shared, highlighting both differences and similarities in vitiligo pathogenesis between different populations.

As first steps towards personalized medicine for vitiligo, we have investigated phenotypic and genetic "endotypes" that define different vitiligo subgroups. We previously showed that vitiligo age-of-onset is associated with the MHC class II region, with mean onset ~25 years in both males and females. However, more detailed analysis defines two overlapping endotypes; an earlier-onset subgroup (mean ~11 years) and a later-onset subgroup (mean ~34 years). In the MHC class II region,

both subgroups are strongly associated with a super-enhancer located between *HLA-DRB1* and *HLA-DQA1* (OR ~1.8). However, the earlier-onset subgroup has additional strong association with another enhancer in the MHC class II region that confers extreme disease risk (OR ~5.0), consistent with our previous association of earlier vitiligo onset with greater risk to relatives. Similarly, the later-onset subgroup is specifically associated with a novel locus on chromosome 3p. Genomewide analyses classify vitiligo susceptibility loci as those associated principally with earlier onset, later onset, or both, thus defining two distinct vitiligo endotypes with both distinct and shared genetic underpinnings. Future studies will investigate other phenotypic correlations with these two endotypes, and may identify additional endotypes that facilitate personalized approaches to vitiligo diagnosis and treatment.

PS.05.01 | Germline MC1R status and somatic mutation burden in melanoma

David Adams[1]; Daniela Robles-Espinoza[1]; Nicola Roberts[1]; Tim Bishop[2]

[1]*Wellcome Trust Sanger Institute, Hinxton, UK;* [2]*University of Leeds, Leeds, UK*

The role of germline variants in influencing the somatic mutation profile of normal tissues and cancers has been poorly explored. The major genetic determinants of cutaneous melanoma risk in the general population are disruptive variants (*R* alleles) of the melanocortin 1 receptor (*MC1R*) gene. These alleles are also linked to red hair, freckling, and sun sensitivity, all of which are known melanoma phenotypic risk factors. By the analysis of the genomes of melanomas sequenced by the TCGA and the Yale Melanoma Genome Project we find that for somatic C>T mutations, a signature linked to sun exposure, the expected single-nucleotide variant count associated with the presence of an *R* allele is estimated to be 42% (95% CI, 15–76%) higher than that among persons without an *R* allele. This figure is comparable to the expected mutational burden associated with an additional 21 years of age. We also find significant and similar enrichment of non-C>T mutation classes supporting a role for additional mutagenic processes in melanoma development in individuals carrying *R* alleles. In addition to our analysis of *R* allele carriers I will also present a detailed analysis of tri-nucleotide mutational signatures found in melanomas and discuss potential mechanisms of mutagenesis.

PS.05.02a | Genetics of pigmentation in a longitudinal study of nevus development

Colleen Little[1]; Jenny Aalborg[2]; Nancy Asdigian[2]; Anna Baron[3]; Jeffrey Dunn[1]; Victoria Gonzalez[1]; Tamara Terzian[1]; Darren Lynn[1]; Robert Dellavalle[1]; Stefan Mokrohisky[1]; Joseph Morelli[1]; Lori Crane2; Neil Box[1]

[1]*Dermatology, University of Colorado Denver, Aurora, CO, USA;* [2]*Preventive Medicine and Biometrics, University of Colorado Denver, Aurora, CO, USA;* [3]*Department of Biostatistics and Informatics, University of Colorado Denver, Aurora, CO, USA*

Ultraviolet radiation (UVR) in sunlight and genetic and phenotypic risk factors are strongly correlated with melanoma incidence. Hair, skin and eye color, nevus count, freckle score and propensity to sunburn are associated with melanoma risk. To study how these factors interact with sun exposure, we have established the Colorado Kids Suncare Program (CKSP). The CKSP is a longitudinal study with annual data collection that began with around 1100 participants, with some recruited at birth and others since age 6 with strong data available from age 6 to 16. Both pigmentation phenotype and sun exposure information were collected over this time period, including number of sunburns, waterside vacations and hours outdoors in the middle of the day in summer. We have also collected pigmentation, nevus, freckle and melanoma risk genotypes on these subjects. We note that in Caucasians, blonde and light brown hair colors that appear in early childhood frequently darken as the child ages. Here we report the genotype/phenotype relationships observed in the CKSP, and the association between hair color genes and childhood changes in hair color that occur over time.

PS.05.02b | Gene-UV interactions determining facial sun damage

Darren Lynn[1]; Elizabeth Meyer[1]; Jenny Aalborg[2]; Nancy Asdigian[2]; Colleen Little[1]; Anna Baron3; Tamara Terzian1; Stefan Mokrohisky[1]; Joseph Morelli[1]; Robert Dellavalle[1]; Lori Crane[2]; Neil Box[1]

[1]*Dermatology, University of Colorado Denver, Aurora, CO, USA;* [2]*Preventive Medicine and Biometrics, University of Colorado Denver, Aurora, CO, USA;* [3]*Department of Biostatistics and Informatics, University of Colorado Denver, Aurora, CO, USA.*

Skin cancers that form after ultraviolet (UV) light exposure occur more frequently than any other cancer type, and in the case of melanoma, they are often fatal. UV exposure induces these cancers through DNA mutation, and causes damage such as aging, solar elastosis and hyperpigmentation. Ultraviolet (UV) facial photographs obtained using the Canfield Visia UV camera may detect sub-visible skin damage caused by UV exposure, and could be used to help track and minimize the accumulation of sun damage. Nevertheless, it is not known how these skin damage scores relate to melanoma risk factors such as UV exposure history or to genetic factors that may predispose to their formation. We are currently following a group of 1,145 children with annual skin exams and telephone interviews and collecting a comprehensive longitudinal set of skin cancer risk factor information. We have used facial UV photography to generate a sun damage score on 550 children. Here we report that facial sun damage scores correlate with known skin cancer and melanoma risk factors such as UV exposure history and genetic risk factors such as MC1R, IRF4, and TYR (among others). Thus we show that UV photography may be used to accurately identify, based on genetic makeup, sun damage associated with an individual's UV exposure history. Moreover, we report those loci that interact with sun exposure history and those that act in an additive fashion to maximize

the effects of UV exposure on sun damage. We are working to determine if this technology may be used to identify unique high risk groups that may benefit from dermatologic surveillance and preventive action.

PS.05.04 | Loss of keratinocytic RXRα combined with activated CDK4 and oncogenic NRAS generates spontaneous and acute neonatal UVB induced malignant metastatic melanomas

Arup K. Indra[1,2,3]; Sharmeen Chagani[4,5]; Rong Wang[3,4]; Evan Carpenter[4]; Christiane Löhr[5]; Gitali Ganguli-Indra[2,4,6]

[1]Department of Pharmaceutical Sciences, OHSU-OSU, Department of Dermatology, OHSU, Corvallis, OR, USA; [2]Knight Cancer Institute, OHSU, Portland, OR, USA; [3]Linus Pauling Institute, OSU, Corvallis, OR, USA; [4]Department of Pharmaceutical Sciences, OHSU-OSU, Corvallis, OR, USA; [5]College of Veterinary Medicine, OSU, Corvallis, OR, USA; [6]Molecular and Cellular Biology Program, OSU, Corvallis, OR, USA

Elucidation of the molecular mechanisms involved in formation of cutaneous malignant melanoma is critical for improved diagnosis and treatment. Keratinocytic nuclear receptor Retinoid X Receptor α (RXRα) has a protective role and is known to regulate keratinocyte and melanocyte homeostasis following acute ultraviolet (UV) irradiation. Here we report that a trigenic mouse model system (*RXRα*$^{ep-/-}$| *Tyr-NRAS*^{Q61K} | *CDK4*$^{R24C/R24C}$) with an epidermis-specific knockout of the *Retinoid X Receptor alpha* (*RXRα*$^{ep-/-}$), combined with oncogenic *NRAS*^{Q61K} and activated *CDK4*$^{R24C/R24C}$ develops spontaneous melanoma, and exposure to a single neonatal UVB treatment further reduces the tumor latency in those mice compared to control mice with functional RXRα. Melanomas from the trigenic *RXRα*$^{ep-/-}$ mice are larger in size, have a higher proliferative capacity and increased antigenicity, exhibit increased expression of malignant melanoma markers and exhibit enhanced vascularization. Altered expression of several biomarkers including increased expression of activated AKT, p21 and cyclin D1 and reduced expression of pro-apoptotic markers such as BAX and Pro-Caspase 3 was observed in the tumor adjacent normal (TAN) skin from our acute UVB treated trigenic *RXRα*$^{ep-/-}$ mice. Interestingly, we observed an increase in p21 and Cyclin D1 and downregulation of Bax expression in the TAN skin of unirradiated trigenic *RXRα*$^{ep-/-}$ mice, suggesting that those changes might be direct consequences of loss of functional RXR in the melanoma microenvironment. Loss of epidermal RXRα in combination with oncogenic *NRAS*^{Q61K} and *CDK4*$^{R24C/R24C}$ mutations significantly enhances invasion of melanoma cells to draining lymph nodes in the trigenic mice compared to the controls with functional RXRα. Above studies demonstrate a crucial role of keratinocytic RXRα to (1) suppress the formation of spontaneous and acute UVB-induced melanomas, and (2) prevent their progression to malignant melanomas in combination with driver mutations such as activated *CDK4*$^{R24C/R24C}$ and oncogenic *NRAS*^{Q61K}. Targeting melanoma microenvironment would be critical for treatment of UV-induced melanomas.

CS.09.01 | Understanding and measuring repigmentation in vitiligo

Alain Taieb[1]; Emily Gan[2]; Nanja Van Geel[3]; Mauro Picardo[4]

[1]University of Bordeaux, Bordeaux, France; [2]National Skin Institute, Singapore, Singapore; [3]University of Ghent, Ghent, Belgium; [4]Istituto Dermatologico San Gallicano, Rome, Italy

The Vitiligo Global Issues Consensus Conference (VGICC), through an international e-Delphi consensus, concluded that 'repigmentation' and 'maintenance of gained repigmentation' are essential core outcome measures in future vitiligo trials. In a recent position paper (Gan et al, Pigment Cell Melanoma Res 2017; 30:28–40) the VGICC summarized three years of international work addressing repigmentation patterns and color match, mechanisms and characteristics of vitiligo repigmentation, as well as discussions on outcomes measures based on e-Delphi surveys. No agreement was found on the best outcome measure for assessing target or global repigmentation, highlighting the limitations of e-surveys in addressing clinical measurements. Some results of the VGICC December 2017 Rome San Gallicano workshop, where selected measurement instruments were tested, will be discussed.

CS.09.02 | Harnessing the balance between UV-induced DNA damage and proliferation of hair follicle bulge melanocyte precursors to enhance repigmentation in human vitiligo

Stanca A. Birlea[1,2]; Nathaniel B. Goldstein[1]; Michael J. Wright[1]; Maranke I. Koster[1,2]; Kenneth L. Jones[3,4]; Bifeng Gao[5]; Chauncey C. Caldwell[1]; Laura G. Hoaglin[1,2]; Steven Robinson[6]; William Robinson[6]; Dennis R. Roop[1,2]; David A. Norris[1,2,7]

[1]Department of Dermatology, University of Colorado, Aurora, CO, USA; [2]Gates Center for Regenerative Medicine, University of Colorado, Aurora, CO, USA; [3]Department of Hematology, University of Colorado, Aurora, CO, USA; [4]Department of Pediatrics, University of Colorado, Aurora, CO, USA; [5]Sequencing and Microarray Core, University of Colorado, Aurora, CO, USA; [6]Division of Oncology, University of Colorado, Aurora, CO, USA; [7]Denver Department of Veterans Affairs Medical Center, Denver, CO, USA

The impact of UV-induced DNA damage on melanocyte precursors activation in the hair follicle (HF)-bulge during vitiligo repigmentation has not been studied to date. To better understand this process, we developed a strategy that combines immunostaining with laser capture microdissection of melanocytes and separately keratinocytes from HF-bulge, using skin from untreated vitiligo patients and narrow band UVB (NBUVB)-treated vitiligo patients ($N = 6$), followed by RNA isolation, RNA sequencing, and gene expression analysis. Our results revealed:

1. The presence of DNA damage (as determined by abundant cyclobutane pyrimidine dimers expression by immunohistochemistry) in the HF-bulge of NBUVB-treated vitiligo skin compared with untreated skin.

2. The *TP53* cascade appears to coordinate repigmentation, *TP53* being the top upstream regulator of NBUVB effects in the bulge melanocytes and keratinocytes [Ingenuity pathway analysis-(IPA): (Z-score = 2.3; *P*-value = 6.8E-18). This was associated with increased transcription levels of key pro-proliferative keratinocyte paracrine factors downstream of *TP53* in the NBUVB-treated bulge keratinocytes, together with their corresponding receptors in the melanocytes (*TGFβR1*, *FZD7*, *EDNRA*, *EDNRB*, *KIT*, *FGFR2*, and *MET*).
3. A pro-proliferative state in the bulge melanocytes of vitiligo NBUVB-treated skin as compared with vitiligo untreated skin, revealed by: a. Activation of Integrin-linked kinase (ILK) signaling, a pro-mitotic HF stem cell regulator, the top activated pathway in the NBUVB-treated bulge [(IPA:Z-score = 3.3; *P*-value = 5.2E-06)]. b. Upregulation of *CTNNB1*(*β-catenin)*, a major effector of ILK (+2.0-fold; *P*-value = 4.0E-02) in the NBUVB-treated HF-bulge melanocytes, which we confirmed by qRT-PCR (+5.8-fold; *P*-value = 2.3E-02) in an independent group of NBUVB-treated and untreated vitiligo patients (*N* = 6). c. Upregulation of *KCTD10* (with attributed pro-proliferative roles by forming complex with *PCNA*), which was the top signal in the NBUVB-treated bulge melanocytes (+9.7-fold; *P*-value = 1.2E-03), result validated by qRT-PCR (+2.1-fold; *p*-value = 2.0E-03) and fluorescent-*in-situ*-hybridization. We believe that our model of studying the balance between DNA damage and transcription activation of pro-proliferative molecules in the HF-bulge will lead to a better understanding of vitiligo repigmentation.

CS.09.04 | Impact of graft cell density and viability on repigmentation upon non-cultured autologous cell suspension transplantation in vitiligo

Sanne E. Uitentuis[1,2]; Karina J. Willemsen[1]; Albert Wolkerstorfer[1,2]; Rosalie M. Luiten[1,2]

[1]Academic Medical Center, Amsterdam, Netherlands; [2]Netherlands Institute for Pigment disorders, Amsterdam, Netherlands

Non-cultured autologous cell suspension transplantation (CST) is an upcoming treatment method in stable vitiligo and piebaldism. The relation between the graft cellular composition and repigmentation is still uncertain and could be useful to define the donor recipient ratio and to evaluate different donor site harvesting techniques.

Our objective was to determine the relation between the quantity and viability of transplanted cells and repigmentation after CST.

Patients with piebaldism or segmental vitiligo were recruited for CST. The cell suspension grafts were produced from split-skin grafts using a cell-harvesting device (ReCell®, Avita Medical, London, UK). The number of cells, viable cells, melanocytes and viable melanocytes transplanted per mm^2 acceptor site were correlated to the percentage of repigmentation six months after transplantation, as analyzed by digital image analysis.

Eighteen patients were included in this study, they had a median repigmentation of 82.2% and a mean repigmentation of 69.4%, of which 12 patients had a repigmentation higher than 70%. We found significant positive correlations between both cell numbers and viability (Spearman's rank correlation coefficient of 0.52, $p = 0.03$ and 0.51, $p = 0.03$ respectively). No significant correlations were found for the number of (viable) melanocytes and repigmentation.

Our study shows that the number and viability of cells transplanted per mm^2 acceptor site significantly correlate with repigmentation rate upon CST. However, this correlation was not found for (viable) melanocytes, which may imply a beneficial effect of other cells like keratinocytes on successful repigmentation. These findings deserve further investigation for confirmation in a larger study with more patients and to determine the potential relation to (viable) melanocyte density.

CS.09.05 | Skin repigmentation in a swine model of vitiligo

Steven W. Henning[1]; Manuel F. Fernandez[1]; James Mahon[1]; Richard Duff[3]; Farshid Azarafrooz[3]; I. Caroline Le Poole[1,2]

[1]Oncology Research Institute, Loyola University Medical Center, Maywood, IL, USA; [2]Departments of Pathology and Medicine, Loyola University Medical Center, Maywood, IL, USA; [3]Comparative Medicine Facility, Loyola University Medical Center, Maywood, IL, USA

Though vitiligo development has been extensively studied in tissue samples from patients concerned about their depigmenting skin, the process of repigmentation remains poorly understood. We have been working with a subline of Sinclair swine that exhibit progressive depigmentation of the skin and spontaneously regressing metastatic melanoma to test the use of modified $HSP70i_{Q435A}$ for vitiligo treatment. We applied plasmid DNA by jet injection to perilesional skin of 6 vitiligo lesions weekly for one month and observed significant repigmentation ($^*p < 0.05$) when compared to 4 PBS-treated lesions during the 6-month follow-up period. These studies provide us with sequential biopsies from repigmenting, human-like skin and serum samples to study repigmentation. Serum was evaluated via ELISA for HSP70 and anti-HSP70 antibodies. Skin cryosections were evaluated for CD3, CD8, FoxP3, c-KIT and TRP-1 expression. Remarkably, skin repigmentation was not associated with melanoma expansion. Distant lesions in treated swine displayed a trend toward repigmentation. HSP70 and anti-HSP70 titers fluctuated independent of treatment. Antibodies Ta99 and 2B8 detected melanocytes and their stem cells in skin to confirm that sequential biopsies consistently spanned the lesional border. Importantly, treated biopsies displayed a significant reduction in $CD3^+$ T-cell abundance coinciding with the onset of repigmentation ($^*p = 0.041$). Just prior to repigmentation, a trend toward increased T-cell infiltration was observed, correlating with an apparent increase in the CD4:CD8 ratio, suggesting that suppressive T-cells play a role in repigmentation. FoxP3+ and γδ T-cells can be successfully detected. Thus, perilesional skin biopsies from swine vitiligo lesions responding to $HSP70i_{Q435A}$ treatment provide a unique opportunity to study the process of repigmentation in human-like skin.

CS.09.06 | Effect of non-cultured epidermal cell suspension grafting on the melanocyte environment in vitiligo

Samia M. Esmat[1]; Magda Assaf[2]; Dina Kadry[1]; Ghada El hanafy[1]; Dalia Abdelhalim[1]; Marwa Adly[1]; Marwa El hawary[1]; Heba Saad[3]; Dalia Bassuni[1]

[1]Dermatology Department-Cairo University, Cairo, Egypt; [2]Zagazig University, Zagazig, Egypt, 3. National Research Centre, Cairo, Egypt

Background: Defective matrix metalloproteinases (MMPs) and cadherins in vitiligo are suggested to affect melanocyte motility and adhesiveness respectively. During the process of non-cultured epidermal cells suspension grafting (NCECS), new healthy keratinocytes and melanocytes are introduced to vitiliginous skin together with different maneuvers used for preparation of the recipient site.

Objective: Evaluate effect of non-cultured epidermal cell suspension grafting, after preparation of the recipient site by CO2 laser resurfacing, on MMP2 and E-cadherin expression, and if those changes are related to the achieved repigmentation

Patients and Method: This randomized prospective study included twenty stable, acral vitiligo cases and twenty healthy volunteers as controls. In each case, acral lesion was treated by NCECS grafting after preparation by CO2 ablation. Biopsies were taken from the edge of the lesion before and six months after grafting and stained by H&E and immunohistochemically for E-cadherin and MMP2.

Results: Levels of E-cadherin and MMP2 were significantly lower in perilesional vitiligo skin compared to controls. Repigmentation was obtained in eight of the twenty cases (40%). Increased expression of E-cadherin as well as both epidermal and dermal MMP2 was obtained after treatment. There was a significant correlation between the rise in MMP2 and the percent of achieved repigmentation.

Conclusion: NCES grafting following preparation of the recipient site by CO2 laser ablation increases expression of E-cadherin and epidermal and dermal MMP2. This probably helps the motility and adhesiveness of cultured melanocytes and establishes repigmentation. Whether these changes are secondary to the new grafted epidermal cells, or due to the effect of the preparation of the recipient site is not yet clear.

CS.11.01 | Albinism in Africa and in Europe: Same genetic condition but very different outcome

Almudena Fernández; Lluis Montoliu

CNB-CSIC and CIBERER-ISCIII, Madrid, Spain

Albinism is a rare genetic condition presented with a severe visual deficit and with a variable hypopigmentation phenotype, not always observed. There are at least 20 types of albinism, associated with mutations in at least 20 genes, traditionally categorized as syndromic (Hermansky-Pudlak or Chediak-Higashi) or non-syndromic, and, among the latter, further classified as oculocutaneous albinism (OCA, seven subtypes known so far) or ocular albinism (OA). In Europe, where the hypopigmented phenotype can be handled correctly, with adequate clothes, hats/caps, sunscreen lotions and specific care, the skin sunburns are rarely a problem and, usually, do not become clinically relevant. However, in Africa, where most people with albinism can be explained by mutations in the OCA2 gene, the hypopigmentation is clearly visible and extremely handicapping as well, thus becoming the primary cause of discomfort and safety. In the absence of adequate clothing, hats or sunscreens, the unprotected hypopigmented skin from people with albinism in Africa can easily burn, because of sun exposure, and trigger the appearance and development of skin tumors which, if not removed surgically early enough, they might become malignant, metastasize and eventually determine a totally unnecessary premature death of these persons. Furthermore, what is particularly worrying for people with albinism in some countries in Africa is, much regrettably, that they are targeted as a community, due to aberrant thoughts and beliefs, fueled by witchcraft, where body parts from people with albinism are astonishingly supposed to bring good luck to those purchasing these totally unacceptable human "trophies". We all need to stand for people with albinism, all over the world, but particularly in Africa. We need to fight these horrible practices and ask the corresponding African governments to also combat these crimes and bring the responsible criminals before the Justice. In this presentation, we will compare, using illustrative pictures, the situation of people with albinism in Europe and Africa. Human beings with the same genetic condition but, unfortunately, with a very different outcome.

CS.11.02 | The challenges of albinism in southern Africa – from genetics to public health advocacy

Prashiela Manga[1]; Jennifer G. Kromberg[2]

[1]The Ronald O. Perelman Department of Dermatology, New York University School of Medicine, New York, NY, USA; [2]University of the Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa

Albinism affects about 1 in 4000 people in southern Africa. People with albinism (PWA) face physical, social and psychological challenges including high risk of skin cancer and visual deficits due to ocular hypopigmentation and optic tract maldevelopment. PWA need to avoid the sun, preventing them taking employment that necessitates being outside, while poor vision limits their independence (for example restricting their capacity to drive) and propagates the misconception that PWA have reduced intellectual capacity.

Social challenges include negative self-perception, discrimination and more recently a dramatic rise in murders of PWA. While infanticide has long been reported, witchcraft-related killings have sharply increased, especially in Tanzania. Non-governmental organizations such as Under the Same Sun have raised awareness and some efforts have been made by southern African authorities to address the

threat. In February 2017, a traditional healer was sentenced to life imprisonment in South Africa for the murder of a PWA whose body was used to make traditional medicine for good luck and increasing wealth.

Psychological challenges include delayed mother-affected child bonding, ostracism and difficulty finding marriage partners. Outreach programs, such as those of the Albinism Society of South Africa, play an important role in educating the public, promoting advocacy and social acceptance.

Ensuring that PWA are provided with the knowledge and help to face these challenges is thus critical. A valuable education mechanism for PWA is genetic counselling, which has been provided in Johannesburg since the early 1970s. Identification of the genes involved in albinism has made carrier testing and prenatal diagnosis a reality. To date seven prenatal tests have been performed in Johannesburg, with one fetus found to be affected (parents chose not to terminate the pregnancy). Addressing the needs of PWA albinism therefore ranges from countering ancient myths to education about the latest technologies for diagnosis and potential therapies.

CS.11.03 | Vision response to dopamine replacement therapy in OCA albinism

Michael C. Struck

University of Wisconsin Madison, Madison, WI, USA

Currently, there is neither treatment that can replace the lack of melanin pigment nor repair the structural neuro-developmental deficiency or physiologic deficits that result in the functional visual deficit of albinism. Care of the visually related symptoms of albinism relies on supportive measures.

The molecular biology of OCA types 1–4 gives understanding of a shared common pathophysiology, in that the final pathway is tyrosinase enzyme dysfunction. The tyrosine/melanin cascade is also tied to the production of L-Dopa catecholamine in the retinal tissue.

In our pilot study, we proposed that replacement of the retinal deficiency of dopamine, would improve vision function as a result of improved retinal function in response to replacement of the deficient neurotransmitter. This study had a pretest-post-test design to determine if improvement in vision occurs in response to replacement of deficiency (dopamine). Visual acuity is the primary outcome data. However secondary outcome data including, ERG testing, OCT, contrast sensitivity, VEP, and color sensitivity were additional determinants to confirm vision improvement as a result of improved retinal function.

All patients were treated with oral supplement Levodopa/carbidopa 4 mg/kg/day in three divided doses for 3 months.

Preliminary results of the first 19 patients to complete testing are reported. We found significant improvement in contrast sensitivity ability tested in the dominant eye and both eyes, $p = 0.023$ and $p = 0.016$ respectively. Also, visual evoked potential (VEP) resolution testing of sine wave grading in amblyopic, dominant and binocular tests showed significant improvement $p = <0.001$. No significant improvement was found in visual acuity.

Initial analysis of changes in visual function from physiologic replacement of the retinal deficiency of dopamine are promising. However, preliminary power calculations suggest the recruitment of study subjects is underpowered to determine significance of results in variables other than VEP and contrast.

CS.11.04 | Molecular basis of chemical chaperone therapy for oculocutaneous albinism type 1

Ayako Teramae[1]; Yui Kobayashi[1]; Hiroyuki Kunimoto[2]; Kouichi Nakajima[2]; Tamio Suzuki[3]; Daisuke Tsuruta[1]; Kazuyoshi Fukai[1]

[1]*Department of Dermatology Osaka City University Graduate Shool of Medicine, Osaka, Japan;* [2]*Department of Immunolgy Osaka City University Graduate School of Medicine, Osaka, Japan;* [3]*Department of Dermatology Yamagata University Faculty of Medicine, Yamagata, Japan*

Background: Oculocutaneous albinism (OCA) type1 is characterized by hypopigmentation of the skin, hair and eye. OCA1 is caused by mutations in the tyrosinase gene. Some mutant tyrosinases causing OCA1 have been shown to be retained within the ER, a phenomenon known as ER-retention. Recently, it has been shown that chemical chaperone therapy can be effective for treating ER-retention diseases. Chemical chaperones bind to misfolded protein and form stable complexes, which can be transported to the Golgi apparatus. And then the catalytic activities of mutant enzymes can be rescued.

Aim: To investigate the localization of mutant tyrosinases involved in Japanese OCA1A and examine the efficacy of chemical chaperone therapy. Method: Mutant tyrosinases causing Japanese OCA1A were transduced in HeLa cells using lentivirus vector, and their subcellular localization was investigated by western blotting and immunohistochemical staining. HeLa cells that expressed each mutant tyrosinase were treated with a chemical compound X and tyrosinase activities were evaluated using the MBTH assay.

Results: All mutant tyrosinases (except H211Y) were localized within the ER. Notably, there was a significantly increase in tyrosinase activity with the P431L mutant at a chemical compound X. Discussion: An in-vivo study utilizing this therapeutic approach is currently underway. Conclusion: This study provides promise that OCA1 might be treatable with chemical chaperone therapy, at least for certain types of missense tyrosinase mutations.

CS.11.05 | The NIH oculocutaneous albinism natural history study: The first decade

Bradley Power[1]; Brian P. Brooks[2]; Wadih M. Zein[2]; Carmen Brewer[3]; Christopher Zalewski[3]; Terri Nguyen[4]; Marjan Huizing[1]; William Gahl[1]; David Adams[1]

[1]National Human Genome Research Institute, Bethesda, MD, USA; [2]National Eye Institute, Bethesda, MD, USA; [3]National Institute on Deafness and Other Communication Disorders, Bethesda, MD, USA; [4]National Institutes of Health, Bethesda, MD, USA

Oculocutaneous albinism (OCA) is an autosomal recessive disease that causes a reduction in pigment in hair, skin and eyes of affected individuals. The visual manifestations include reduced visual acuity, nystagmus, strabismus, significant refractive errors, and increased sensitivity to light. The prevalence of OCA varies based on type and region, but most populations have an incidence of approximately 1 in 17,000 births. Most OCA cases are caused by variants in one of two known genes, *TYR* (OCA type 1) and *OCA2* (OCA type 2). Other rarer OCA genes continue to be discovered. The NIH OCA Natural History Study has four primary goals. 1) To clinically and comprehensively characterize known OCA types. 2) To use study participants' cultured melanocytes to study pigment biology, variability in pigment formation related to genotype, and response to proposed treatments. 3) To recruit study participants with hypopigmentation not due to known albinism-causing genes. And, 4) to evaluate methods of quantifying eye pigmentation, skin pigmentation and other clinical parameters that may be usable as outcome measures in future treatment studies. Here we summarize selected findings from the study including clinical assessments of vision, hearing, rehabilitation needs and other investigations associated with the study. These data refine the phenotype, define unknown genotypes, and lay the groundwork for future therapeutic studies and clinical guidelines.

CS.11.06 | Melanin analysis for hair samples from Japanese patients with Hermansky-Pudlak syndrome type 1, 4, 6, and 9

Ken Okamura[1]; Yuta Araki[1]; Yuko Abe[1]; Kazumasa Wakamatsu[2]; Gen Tamiya[3]; Mariko Seishima[4]; Takafumi Umetsu[5]; Atsushi Kato[6]; Masakazu Kawaguchi[1]; Masahiro Hayashi[1]; Yutaka Hozumi[1]; Tamio Suzuki[1]

[1]Department of Dermatology, Yamagata University Faculty of Medicine, Yamagata, Japan; [2]Department of Chemistry, Fujita Health University School of Health Sciences, Toyoake, Japan; [3]Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan; [4]Department of Dermatology, Gifu University Graduate School of Medicine, Gifu, Japan; [5]Department of Pulmonary Medicine and Clinical Immunology, Dokkyo University School of Medicine, Mibu, Japan; [6]Division of Hematology, Tokyo Kyosai Hospital, Tokyo, Japan

Hermansky-Pudlak syndrome (HPS), inherited as autosomal recessive trait, is characterized by oculocutaneous albinism (OCA), bleeding tendency, and ceroid deposition. To date, ten genes have been identified as causative genes for HPS, and eight of the ten genes encode subunits of BLOC (biogenesis of lysosome-related organelles complex)-1 (*HPS7*, *HPS8*, and *HPS9*), BLOC-2 (*HPS3*, *HPS5*, and *HPS6*), or BLOC-3 (*HPS1*, and *HPS4*). Among Japanese, 10% of the OCA patients are HPS1, while other HPS subtypes are extremely rare. In this study, we firstly examined by whole-exome sequencing for the patients who were clinically diagnosed with OCA without no mutation in *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, and *HPS1*. Then, one each of the patients with HPS4, HPS6, and HPS9 were newly identified with three novel mutations in all (c.123T>A, p.Y41X in *HPS4*, c.60_64dupGCGGC, p.L22RfsX33 in *HPS6*, and c.285_286dupTC, p.H96LfsX22 in *HPS9*). Next, electron microscopy and biochemical analysis for the hair from these three patients and a representative patient with HPS1 harboring homozygous IVS5+5G>A mutation in *HPS1* (previously reported) were conducted. Electron microscopically, all the hair samples from patients showed less number, smaller size, and less mature melanosomes as compared with healthy control. The degree of these tendency were more marked in patients with HPS1 and HPS4 than patients with HPS6 and HPS9. Biochemical analysis revealed that total melanin content was significantly reduced and a tendency toward more pheomelanin pigment was found in all the patients, On the other hand, degradation product of benzothiazine-type pheomelanin (4-AHP) was increased in all the patients with HPS. Among samples from patients, HPS6 showed the highest total melanin content, while HPS1 revealed the highest 4-AHP. In this study, we demonstrated the impact of the dysfunction of BLOC-1, 2 and 3 on the trafficking or maturation of melanosomes through the hairs, which are non-invasive samples.

CS.11.07 | Mutations in Chinese Hermansky-Pudlak syndrome patients and the consideration for precise intervention

Aihua Wei[1]; Jing Ma[2]; Yefeng Yuan[3]; Wei Li[2]; Dayong Bai[1]; Daniel F. Cutler[4]

[1]Department of Dermatology, Beijing Tongren Hospital, Capital Medical University, Beijing, China; [2]Center for Medical Genetics, Beijing Children's Hospital, Capital Medical University, Beijing, China; [3]Institute of Genetics & Developmental Biology, Chinese Academy of Sciences, Beijing, China; [4]MRC Laboratory for Molecular Cell Biology, University College of London, London, UK

Hermansky-Pudlak syndrome (HPS) is a rare recessive disorder characterized by hypopigmentation, bleeding diathesis and other symptoms due to multiple defects in lysosome-related organelles. Ten HPS subtypes have been identified with mutations in *HPS1* to *HPS10*. We have screened 100 hypopigmentation genes by Sanger sequencing and next-generation sequencing and identified eight HPS-1, two HPS-3, one HPS-5, and three HPS-6 in Chinese HPS patients with typical ocular or oculocutaneous albinism and the absence of platelet dense granules together with other variable phenotypes. One adult HPS-1 had indications of interstitial pneumonia. Among these mutations, 16 were previously unreported alleles (6 in *HPS1*, 3 in *HPS3*, 2 in *HPS5*, 5 in *HPS6*). Our results demonstrate the feasibility and utility of NGS-based panel diagnostics for HPS. In addition, we found differential von Willebrand factor (VWF) releasing responses to the administration of

desmopressin (DDAVP) in mouse mutants of HPS-1, HPS-6 and HPS-9, suggesting the differential effects to the administration of DDAVP in HPS patients when reversing the bleeding diathesis. Our results have expanded the mutational spectrum of HPS genes in Chinese population, and the precise genotyping of HPS subtypes is a prerequisite for the precise intervention of bleeding or other symptoms.

CS.12.01 | Brn2 is a radioprotector and its absence promotes melanoma initiation

Lionel Larue

Institut Curie, Orsay, France

Melanoma is known as a radioresistant tumor. Melanoma is intratumorally highly heterogeneous due to its genetic instability and plasticity. This heterogeneity may explain, at least in part, the natural neo and acquired resistance of melanoma against therapies. In a two dimensional biological world, cells may switch from "proliferative" to "invasive", and vice versa. Two transcription factors, Mitf and Brn2, may be of great importance in this switch. Brn2 is a POU transcription factor belonging to the Oct family. In vitro studies have shown that Brn2 is transcriptionally controlled by the Lef/β-catenin complex and indirectly controlled by Braf. Moreover, the level of Brn2 mRNA is controlled by miR-211, which is directly induced by Mitf. This switch would be modulated by the transcriptional activity of Brn2 on Mitf, but also on other crucial proteins of the melanocyte lineage such as Pax3. In human melanoma metastasis, it appears that melanoma cells are mainly Brn2-positive or Mitf-positive. However some cells express both or none of these two proteins. Here, we evaluated the importance of Brn2 during the establishment, the renewal and transformation of melanocytes using genetically modified mouse models, human genetics and cell lines. It appears that Brn2 is dispensable during the establishment and the renewal of melanocytes. The specific lack of Brn2 in the melanocyte lineage reveals that this protein is important for melanoma resistance against ionizing irradiation, which consequently results in the disappearance of melanocyte stem cells over time. Moreover, mouse melanoma models relevant for humans were generated, showing that Brn2 plays an important role during melanoma initiation and can be better used to improve melanoma therapies.

CS.12.02 | Genetic and epigenetic factors regulating melanoma initiation and progression

Marcus Bosenberg

Yale, New Haven, CT, USA

Genetically engineered mouse models of melanoma based on genetic changes observed in human melanoma have provided a variety of insights into the initiation and progression of melanoma, including characteristics of melanoma initiation and progression, factors regulating oncogene-induced senescence, and epigenetic regulators of melanoma. Genetically engineered mouse melanoma models developed and characterized in our laboratory will be discussed in relation to the above topics.

CS.12.03 | Identification and phenotypic plasticity of metastatic cells in a mouse model of melanoma

Xiaoshuang Li; Raul Torres; Lidia Kos

Florida International University, Miami, FL, USA

Melanoma is the deadliest form of skin cancer due to its high propensity to metastasize and resistance to current therapies. We have created a spontaneous mouse model of metastatic melanoma (*Dct-Grm1/K5-Edn3*) where metastasis to the lungs is 80% penetrant. The primary tumors of these mice present cellular heterogeneity with cells at varying levels of differentiation. The main goal of this study is to determine the metastatic potential of the primary tumor resident Tyrosinase positive cells and evaluate the dynamic pattern of gene expression as those cells move from the primary tumors to the sites of metastasis. To accomplish this aim we crossed the *Dct-Grm1/K5-Edn3* mice to *CreER*^{T2}/ ROSAmT/mG mice to indelibly label Tyrosinase cell populations within the primary tumor with EGFP by topical application of 4-hydroxytamoxifen at the tumor site. *In vivo* lineage tracing and characterization of GFP-labeled cells was performed in the metastatic lesions. We found that primary tumor derived Tyrosinase positive cells or their progeny can establish successful metastases in the lung. The metastatic cells in close association with the vascular endothelium lose pigmentation and do not express melanocytic markers, however, they mimic endothelial cell properties and gain the expression of platelet endothelial cell adhesion molecule PECAM-1 (also known as CD31) and vascular endothelial (VE)-Cadherin. In the lung metastatic foci, GFP-labeled cells resume pigmentation production and lose endothelial cell markers expression. This *in vivo* lineage tracing system in mouse reveals tumor phenotypic plasticity and will be a powerful model to evaluate and help us understand the etiology and pathogenesis of melanoma metastasis. Further characterization of those more aggressive cells in melanoma will allow for the development of new prognostic tests and novel therapeutic strategies to eliminate metastasis.

CS.12.04 | EPAC-RAP1 axis-mediated switch in the response of primary and metastatic melanoma cells to cyclic AMP signaling

Carlos I. Rodriguez; Edgardo Castro-Pérez; Jaclyn A. Wisinski; Kirthana Prabhakar; Laura M. Block; B J. Longley; Michelle E. Kimple; Vijay Setaluri

University of Wisconsin-Madison, Madison, WI, USA

Cyclic AMP (cAMP) is an important second messenger that regulates a wide range of physiological processes. In mammalian cutaneous

melanocytes, cAMP-mediated signaling pathways activated by G-protein coupled receptor MC1R (melanocortin 1 receptor) play critical roles in melanocyte homeostasis including cell survival, proliferation and pigment synthesis. Impaired cAMP signaling is associated with increased risk of cutaneous melanoma. Whereas mutations in mitogen activated protein kinase (MAPK) pathway components are the most frequent oncogenic drivers of melanoma, the role of cAMP in melanoma is not well understood. Here, using the BRAF(V600E)/PTEN-null mouse model of melanoma, we show that topical application of an adenylate cyclase agonist, forskolin, a cAMP inducer, accelerated melanoma tumor development *in vivo* and stimulated the proliferation of mouse and human primary melanoma cells, but not human metastatic melanoma cells *in vitro*. The differential responses of primary and metastatic melanoma cells was also evident upon pharmacological inhibition of the cAMP effector protein kinase A. Pharmacological inhibition and small interfering RNA-mediated knockdown of other cAMP signaling pathway components showed that EPAC-RAP1 axis, an alternative cAMP signaling pathway, mediates the switch in response of primary and metastatic melanoma cells to cAMP. Evaluation of pERK levels showed that this phenotypic switch was not correlated with changes in MAPK pathway activity. Although cAMP elevation did not alter the sensitivity of metastatic melanoma cells to BRAF(V600E) and MEK inhibitors, the EPAC-RAP1 axis appears to contribute to resistance to MAPK pathway inhibition. Our data suggest a MAPK pathway-independent switch in response to cAMP signaling during melanoma progression.

CS.12.05 | A novel role for CDKN2A loss in melanoma invasion and metastatic dissemination

Hanlin Zeng; A H. Shain; Boris Bastian; Robert L. Judson

University of California, San Francisco, San Francisco, CA, USA

CDKN2A acts as a critical tumor suppressor in melanoma, as evidenced by frequent loss of function mutations and deletion. Loss of *CDKN2A* is believed to permit escape from senescent pre-neoplastic cell populations through relief of a cell cycle block mediated by its two gene products. We performed a comprehensive analysis of *CDKN2A* gene status, mRNA and protein expression levels of p16 and p14, the protein products of the *CDKN2A* locus, in a cohort of melanomas and their adjacent pre-neoplastic lesions and observed that bi-allelic *CDKN2A* loss coincides with a the progression stage when primary melanomas become invasive. In melanoma lines, p16 is a potent barrier to metastasis, independent of its known role inhibiting cell proliferation. We genetically engineered primary human melanocytes to harbor *CDKN2A* deletions and/or BRAF V600E mutation at their endogenous *BRAF* locus. Using this physiologic model for the early phases of neoplastic transformation, we found no evidence for BRAF-induced senescence, rather observing that p16 loss activates a master regulator of melanoma invasion, BRN2, likely through phospho-state specific binding of Rb to MITF. These results demonstrate that one of the most frequently altered genes across human cancers, *CDKN2A*, has an unexpected novel role in inhibiting cellular invasion through direct binding to lineage specific transcription factors and can act as gatekeeper of early metastatic dissemination.

CS.12.06 | The AP-1 transcription factor FOSL1 causes melanocyte reprogramming and transformation

Katja Maurus[2]; Anita Hufnagel[1]; Manfred Schartl[1]; Svenja Meierjohann[1]

[1]Physiological Chemistry, University of Würzburg, Würzburg, Germany; [2]Institute of Pathology, University of Würzburg, Würzburg, Germany

The MAPK pathway is activated in the large majority of melanomas and is the target of several therapeutic approaches. Its activation is the result of oncogenic mutations in BRAF and NRAS and already occurs in premalignant lesions such as nevi. During the course of melanoma development, PTEN inactivation occurs frequently, thereby leading to additional PI3K activation. Although it is a well-established fact that concomitant MAPK and PI3K activation results in melanoma formation, the underlying responsible processes are incompletely understood.

Here, we show that joint MAPK and PI3K activation leads to the stabilization of FOSL1, a member of the AP-1 transcription factor family. While nevi do not express FOSL1, its levels increase with progression. To investigate a possible role of FOSL1 in melanomagenesis, we performed inducible FOSL1 expression in melanocytes. Intriguingly, we found that FOSL1 acts oncogenic and transforms melanocytes, enabling subcutaneous tumor growth *in vivo*. During the process of transformation, FOSL1 reprograms the melanocytes by shifting the balance between MITF and AXL and re-enforcing pro-tumorigenic transcription factors MYC, E2F3 and AP-1. This results in the enhancement of several growth-promoting processes such as ribosome biogenesis and pyrimidine metabolism. Overall, we demonstrate that FOSL1 is a novel reprogramming factor for melanocytes with potent tumor transformation potential.

CS.12.07 | Hepatocellular steatosis induces tumorigenicity of melanoma cells in vitro and hepatic metastasis in an in vivo model

Claus Hellerbrand; Anja Bosserhoff

Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany

Obesity increases the risk of different types of cancer including melanoma and worsens its prognosis. Moreover, more than 90% of obese individuals develop fatty livers (hepatic steatosis) and it is increasingly recognized that this liver condition promotes the development and progression of primary liver cancer. Still, it is surprisingly unknown whether (obesity-induced) hepatic steatosis affects the homing and survival of melanoma cells.

The aim of this study was to analyze influence of hepatic steatosis on metastasis of melanoma cells.

Methods and Results: Primary human hepatocytes were incubated with increasing doses of fatty acids for 24 hr causing dose dependent cellular lipid accumulation. Subsequently, medium was exchanged with fresh medium, and by incubation for another 24 hr conditioned media (CM) from control and steatotic hepatocytes were generated. In Boyden chamber assays, CM from steatotic hepatocytes was significantly more chemoattractive for melanoma cell lines Mel Ju and Mel Im than CM from control hepatocytes. Moreover, incubation with CM from steatotic hepatocytes induced proliferation and JNK-activation of melanoma cells significantly more than CM from control hepatocytes. Next, C57/BL6 mice were fed with a hepatic steatosis inducing high-fat diet (HFD; 30% fat) for 8 weeks; controls received standard chow. Subsequently, an established syngeneic mouse model for hepatic metastasis was applied. Here, murine B16 melanoma cells were injected into the spleen of the mice. Twelve days after tumor cell injection, macroscopic and histological analysis revealed significantly more and larger metastases in the livers of HFD-fed compared to control mice. Furthermore, immunohistochemical analysis showed increased Ki67-expression and JUN-phosphorylation in metastases formed in steatotic livers.

Summary and Conclusions: Hepatocellular steatosis attracts melanoma cells and promotes proliferation of metastasized melanoma cells. Identification of the metastasis-promoting factors secreted by steatotic hepatocytes can build the basis for targeted therapies to prevent or treat hepatic metastases of melanoma.

CS.13.01 | Neuromelanins: Structure, synthesis and role in Parkinson disease

Luigi Zecca[1]; Ioannis Isaias[2]; Clifford Cassidy[3]; Guillermo Horga[3]; David Sulzer[4]; Fabio Zucca[1]

[1]Institute of Biomedical Technologies - CNR, Segrate, Italy; [2]Department of Neurology, University Hospital and Julius-Maximilian-University, Wuerzburg, Germany; [3]Departments of Psychiatry, Columbia University Medical Center, New York, NY, USA; [4]Department of Psychiatry, Neurology and Pharmacology, Columbia University Medical Center, New York, NY, USA

We showed that neuromelanins (NM) are a family of compounds accumulating in human brain, especially in catecholamine neurons of substantia nigra (SN) and locus coeruleus(LC) degenerating in Parkinson's disease (PD). The decrease of NM concentration which we measured in PD can be imaged by magnetic resonance imaging (MRI) and we have clarified the basis for NM contrast mechanism of MRI. The MRI of NM loss is becoming a new tool to confirm PD diagnosis. We observed that NM is contained in special autolysosomes with proteins and lipid bodies. Its synthesis is fuelled by cytosolic catecholamines whose concentration depends on vesicular monoamine transporter-2 expression. We found in NM-containing organelle several lysosomal membrane and matrix proteins, in addition to aggregation, degradation, and autophagy proteins. In NM organelle we observed several lysosomal proteins contained also in melanosome and GPNMB present in melanosome too. We showed that main components of lipid bodies in NM-containing organelle are dolichols with lower content of sphingomyelin, phospholipids and ceramides. We suggested that NM can be protective because it chelates reactive/toxic metals (Fe, Zn, Al, Cr, Mo, Pb and Hg) and form stable non-toxic complexes. Moreover, a protective mechanism is the synthesis of NM which removes reactive/toxic quinones. However, NM can be also toxic in PD when released extracellularly by degenerating neurons. Here NM can activate microglia with production of H_2O_2, NO and pro-inflammatory factors which cause further neurodegeneration and subsequent release of NM and microglia activation. This produces a cycle of neuroinflammation/neurodegeneration contributing to progression of PD. Another neurotoxic process connected to NM is related to accumulation of major histocompatibility class I complex (MHC-I) in NM-containing organelles of SN and LC neurons degenerating in PD. MHC-I binds antigens derived from foreign proteins, presenting them on neuronal membrane, making NM containing neurons a target for $CD8^+$ cytotoxic T-cells.

CS.13.02 | DTNBP1 (Dysbindin-1)—A gene with links to pigmentation and increased risk for schizophrenia

Jun Zhao; Zheng Li

NIMH, NIH, Bethesda, MD, USA

DTNBP1 encodes the dystrobrevin-binding protein-1 (Dysbindin-1), which is well-characterized as a member of the biogenesis of lysosome-related organelles complex-1 (BLOC1). In humans, *DTNBP1* mutations are known to underlie Hermansky-Pudlak Syndrome-7 (*HPS7*) whose symptoms include a reduction of pigmentation in eye and skin and a bleeding diathesis similar to those seen in other forms of HPS. Interestingly, genetic variants of *DTNBP1* are also associated with a slightly enhanced risk for schizophrenia. In fact, *DTNBP1* is normally expressed in neurons in brain regions implicated in schizophrenia, and its expression is reduced in post-mortem brains of schizophrenia patients. It is unclear, however, whether neuronal alterations are the result of a disruption of the BLOC1 complex or whether other mechanisms are involved. Using a variety of electrophysiological and imaging techniques as well as dysbindin-1-mutant (*Dtnbp1*^{sdy}) mice, we here show that Dysbindin-1 regulates mitochondrial dynamics by binding to the Dynamin-like protein-1 (DLP1) and promoting its oligomerization. In turn, disruption of mitochondrial dynamics affects gamma oscillation and hence neuronal network activity. It is conceivable, therefore, that *DTNBP1*-mediated effects on mitochondria may contribute to impaired neuronal network activity in schizophrenia.

CS.13.03 | Why are melanoma and Parkinson's linked?: Role of MC1R

Xiqun Chen

Massachusetts General Hospital, Charlestown, MA, USA

Patients with Parkinson's disease (PD), one of the most common neurodegenerative diseases, generally have reduced risk of developing almost all types of cancer, with one notable exception – melanoma, a malignant tumor of melanin-producing cells in skin. A large number of epidemiological studies have reported that individuals with PD are more likely to develop melanoma, and melanoma patients are reciprocally at higher risk of developing PD. Although well documented since the 1970s, mechanisms underlying this association remain largely unknown. The talk will highlight recent findings by our interdisciplinary collaborative team including leading epidemiologists, melanoma specialists, and cancer geneticists.

CS.13.04 | Biphasic melanocyte development in primary and secondary neurulation involves ADAMTS

Catherine D. Van Raamsdonk

Department of Medical Genetics, University of British Columbia, Vancouver, BC, USA

The caudal end of the vertebrate body is created by the tailbud, a multipotent mesenchyme. Tailbud cells condense and canalize to form the secondary neural tube, which we hypothesize can produce melanocytes, a neural crest cell derivative. Here, we studied melanocyte development in the caudal region of the mouse embryo. We found that the progression of melanoblast development and migration is consistent with a secondary neural tube origin. Segmental nerves in the tail expressed two classical melanocyte markers, Dct-LacZ and Microphthalmia (Mitf), suggesting an unusual relationship between nerves and melanoblasts in secondary neurulation. To understand the specifics of melanocyte development in the tail, we used forward genetics to clone two alleles of the *A disintegrin and metalloprotease with thrombospondin motifs 9 (Adamts9)* gene, *Und3* and *Und4*, which exhibit a ring of hypo-pigmentation in the middle of the tail, but are pigmented normally in the rest of the body. A ring phenotype in these *Adamts9* mutants and previously described in the middle of the trunk in *Adamts20-belted* mutants suggests that ADAMTS metalloproteases are required to coordinate melanoblast growth with a lengthening of the anterior-posterior axis. A specialized role for *Adamts20* and *Adamts9* in the primary and secondary bodies, respectively, emphasizes a biphasic melanocyte development scheme under distinct, yet related proteolytic control.

CS.13.05 | Functional heterogeneity and selective myelination potential of hair follicle stem cells expressing melanocyte stem cell markers

Sandeep S. Joshi[1]; Thomas J. Hornyak[1,2,3]

[1]*Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, USA;* [2]*Department of Dermatology, University of Maryland School of Medicine, Baltimore, MD, USA;* [3]*Research & Development Service, VA Maryland Health Care System, Baltimore, MD, USA*

Melanocyte stem cells (McSCs) are the undifferentiated melanocytic cells of the mammalian hair follicle (HF) responsible for cyclic generation of a large number of differentiated melanocytes during each HF cycle. HF McSCs reside in both the CD34+ bulge and the CD34- secondary hair germ (SHG) regions of the HF during telogen. Using Dct-H2BGFP mice, we can separate bulge/LPP and SHG McSCs using FACS with GFP and anti-CD34 to show that these two subsets of McSCs are functionally distinct. CD34-GFP+ SHG cells express high levels of melanocyte differentiation genes and produce pigmented cells when grown in melanocyte differentiation medium. CD34+GFP+ bulge/LPP cells grow preferentially in neural crest stem cell medium and express markers of other neural crest derivatives such as glia, neurons, and myofibroblasts. To test whether CD34+ McSC subset might possess the ability to differentiate as glia and myelinate neuron in culture, we developed a dorsal root ganglion (DRG) co-culture system. The DRGs isolated from myelin-deficient, *Shiverer* (*shi/shi*) mice(P5) were cultured to develop neurites and CD34+ and CD34- McSCs, were co-cultured with these extended neurites to study their myelination. In co-culture with DRG explants, CD34+GFP+ bulge McSCs express myelin basic protein (Mbp) in a neuronal distribution and form electron-dense, multilayered myelin sheaths surrounding Mbp-deficient *shi/shi* neurites. EM analysis of co-cultures of CD34+ MeSCs revealed evidence of compact myelin in 6/8 culture sections. In contrast, a loose myelin sheath was detected in only 1/8 CD34- MeSC co-culture sections. *In vivo*, the fluorescent dye-labelled CD34+ McSCs transplanted into the vitreal space of 2/2 *shi/shi* eyes and into 2/2 *shi/shi* brains co-express Mbp, whereas, CD34- McSCs do not. Thus, our data suggest that CD34+ bulge McSCs, and their counterparts in human skin, may be useful for myelinating neurons *in vivo*, leading to new therapeutic opportunities for demyelinating diseases and traumatic nerve injury.

CS.13.06 | The Role of PRDM1 in melanoma initiation and progression

Ritsuko Iwanaga[1]; Jessica Hsu[1]; David Orlicky[1]; Yiqun Shellman[1]; Kristin B. Artinger[1]

[1]*University of Colorado Anschutz Medical Campus, Aurora, CO, USA;* [2]*University of Colorado School of Dental Medicine, Aurora, CO, USA*

Tumors that arise from cells of neural crest origin, such as melanocytes, give rise to melanoma, which are highly aggressive due to their ability

to maintain embryonic properties such as migration, proliferation and survival. Melanoma is an aggressive cancer resulting in a 5-year survival rate of only 15-20%. A limited number of patients have a long-lasting response to the recent FDA approved treatment, and this underscores a clear need to develop new treatment strategies. Therefore, it is critical to identify the origin of melanoma and its molecular niche. Melanoma is thought to originate from undifferentiated but activated neural crest progenitor cells, but the precise cell type and location of these cells is still unknown. The PRDM1 protein encodes a transcription factor with histone methyltransferase activity and is required for neural crest progenitor fates. In the absence of *prdm1*, neural crest formation is severely affected resulting in a reduction of all neural crest derivatives, indicating that PRDM1 is critical for melanocyte development. Additionally, we observe reduced expression of PRDM1 in human tumors originating from neural crest lineages, which suggests that PRDM1 may act as a tumor suppressor in neural crest-derived tumors including melanoma. Taken together, we hypothesize that PRDM1, which has an established role in neural crest cell development, may impede melanoma initiation and progression by regulating melanocyte precursor cell homeostasis. Moreover, we hypothesize that when mutated, PRDM1 may accelerate melanoma onset and disease progression. To test this hypothesis, we will take advantage of an established zebrafish melanoma model and neural crest-specific transgenic and mutant models to closely study the role of PRDM1 in melanoma. The zebrafish model allows us to perform these studies in an intact tumor microenvironment; this type of in-depth analysis following cells for an extended period of time cannot be performed in any other model organism.

CS.14.02 | Melanoma epidemiology: Recent trends and future challenges

David Whiteman

QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

Several remarkable trends have been observed recently with respect to the occurrence of cutaneous melanoma. In most Western countries with predominantly fair-skinned populations, melanoma incidence has risen sharply in the past 30 years – far more rapidly than mortality rates. For example, melanoma incidence in the USA and northern Europe has risen by more than 3% year-on-year during the past 30 years – an extraordinary rate of increase almost unique among human cancers. Death rates from melanoma have also increased, but at slower annual rates of increase (USA 0.2% p.a.; northern Europe ~1 - 1.5% p.a.). Statistical models which account for underlying changes in population size and structure suggest that melanoma incidence will continue to rise for at least another decade in the US and Northern Europe, and then likely plateau. In contrast, melanoma rates in Australia peaked in 2005 and have declined significantly since that time. The absolute numbers of people requiring treatment for newly diagnosed melanoma will continue to rise steeply in the next few decades, owing to the very high rates of melanoma occurring in older age groups in all Western countries, and the increasing numbers of people surviving into older age. Several strategies exist to control the melanoma epidemic, including primary prevention and early detection. Both strategies have merit and have been implemented to varying degrees in different populations. Primary prevention aims to reduce the population prevalence of hazardous exposure to sunlight and artificial ultraviolet radiation. Several recent discoveries relating to the effects of UVR on skin cells will have implications for melanoma control strategies, and research is needed to quantify these effects.

CS.14.03 | IRF4: An example of genetic heterogeneity in melanoma incidence and survival

Marianne Berwick; Kriti Mishra

University of New Mexico, Albuquerque, NM, USA

Here we report the effects of one single nucleotide polymorphism (SNP), IRF4, and on melanoma incidence and mortality. The gene product of IRF4 is an interferon regulatory factor involved in the regulation of gene expression in response to interferon and other cytokines. A functional SNP, rs12203592, is located on intron 4 of the IRF4 gene. The T allele reduces the expression of IRF4 and the C allele increases expression in melanocytes. It has been associated with melanoma risk and survival as well as other cancers. The IRF4 gene is also strongly associated with pigmentary features.

Among individuals with melanoma, there was a positive association between the T allele and solar elastosis (UV radiation) and an inverse association between the T allele and navel tissue. Recently, the IRF4 rs12203592 T allele was found to increases the risk of dying from melanoma in populations in Barcelona and Essen.

GEM (Genes, Environment and Melanoma) is a population-based case control study with 9 population centers, having enrolled 3579 individuals from 2000-2003. The population centers are New South Wales and Tasmania, Australia, Piedmont, Italy, Ontario and British Columbia, Canada, and New Jersey, North Carolina, Orange County and Michigan. Standardized methods were used to evaluate pigmentation, genetics and pathology.

We will present new data demonstrating associations of IRF4 with Breslow thickness and AJCC stage of melanoma.

The association of IRF with melanoma may be due to its role in the immune response and emphasizes tumorigenesis and prognosis is a gene-environment interaction. Better understanding of this interaction can potentially impact risk assessment in individual patients and drive the prevention and clinical management of disease.

CS.14.04 | Screening for melanoma

Martin A. Weinstock

V A Medical Center Providence and Brown University, Providence, RI, USA

Melanoma is the leading cause of mortality among skin conditions in the United States, and secondary prevention (early detection) the most promising avenue to cut that death rate by 50% or more

within a short period. To accomplish this task sufficient evidence must be gathered to demonstrate that a secondary prevention program will be effective and that its benefits outweigh any associated harms. The last decade has seen a revolution in this regard. Studies across the globe have suggested efficacy for melanoma screening. However, these have been largely case-control or time series, and the latter have been controversial. Most recently evidence has been published to indicate that the harms associated with this screening can be quite minimal, particularly when attention is paid to developing screening tools designed to minimizing harms while maximizing benefits. Further debate surrounds the standard that should be used to determine the evidentiary threshold for implementing a screening program.

Weinstock et al. *Am J Prev Med* 2007;32:517-52
Berwick et al. JNCI 1996;88:17-23; 2005;97:195-199
Pollitt et al. *Cancer Epidemiol Biomarkers Prev* 2009;18:3018-3023
Aitken et al. *Int J Cancer* 2010;126:450-458
Geller et al. *Cancer* 2009;115:1318-1327
Schneider et al. *J Am Acad Dermatol* 2008;58:741-9
Katalinic, Waldmann, Weinstock, et al. *Cancer* 2012;118:5395-3402
Waldmann, Nolte, Weinstock, et al. *Br J Cancer* 2012:106:970-974
Boniol et al. *BMJ Open* 2015;5:e008158
Katalinic et al. *Dtsch Arztebl Int* 2015;112:629-634
Stang et al. *Cancer* 2016;122:432-437
Stang et al. *Eur J Cancer* 2016;64:83-88.
Shaikh et al. *J Cancer Educ* 2012;27:709-716
Eide et al. J Am Board Fam Med 2013;26:648-657.
Ferris et al. JAMA Oncol 2017 (in press)
Weinstock et al. Cancer 2016;122:3152-3156.
Weinstock MA. Early detection of melanoma. JAMA 2000;284:886-889.
Tsao, Weinstock. JAMA 2016;316:398-400.
https://www.visualdx.com/educational-resources "INFORMED"

CS.14.05 | Epidemiological evaluation of an interaction between sex and UV index in melanoma risk

Feng Liu-Smith, Frank Meyskens
UC Irvine, Irvine, CA, USA

The single most important environmental factor for melanoma is ultraviolet radiation (UV). However, UVR may not impact different gender and age in the same way. Published data from us and others revealed that melanoma incidence rates increased with age in both men and women, but with different patterns. Females showed higher risk at younger age (< 45-54). Recently we collected cancer registry data from various registries where majority populations are Caucasians with light skin color and hair color, and modeled the incidence data with local UV index which reflects the clear day erythema UV dose at noon. Linear regression model indicated that the male incidence rates (age-adjusted) were associated with geographical UVI (rho = 0.61, p =0.003) while the female rates were not (rho = 0.31, p = 0.09). The association level increased with age in men but remained insignificant for women for all age. We further utilize a negative binomial regression model to investigate possible interactions among age, sex and local UVI in melanoma risk. It was not surprised to observe that the interaction between sex and age, or that between age and UVI played significant roles. It was new to us that the interaction between sex and local UVI was also a significant risk factor for melanoma. More interestingly, when we stratify our data by age, we found that sex alone played a significant role for melanoma risk at younger age (< 45-54). Sex itself did not play a significant role at older ages, instead, the interaction between sex and UVI was the significant risk factor for older age. These results suggest that the early onset melanoma may be less impacted by UV and more impacted by gender factor, likely a hormonal effect for early onset melanoma should be considered, and age- and sex-specific prevention methods are suggested.

CS.14.06 | A novel phenotype of ALDH2 polymorphism: Drinking-induced skin pigmentation

Akiko Matsumoto[1]; Toshiya Hara[2]; Chisato Shimanoe[1]; Takefumi Yuzuriha[2]; Chikako Yoshimori[2]; Takeo Muto[2]; Koichi Endo[2]; Megumi Hara[1]; Masayoshi Ichiba[1]; Takeo Konakahara[3]; Ichiro Yajima[6]; Vasilis Vasiliou[4]; Mayumi Fujita[5]

[1]Saga University, Saga, Japan; [2]National Hospital Organization, Hizen Psychiatric Center, Saga, Japan; [3]Department of Pure and Applied Chemistry, Faculty of Science & Technology, Tokyo University of Science (RIKADAI), Noda, Japan; [4]Department of Environmental Health Sciences, Yale School of Public Health, New Haven, CT, USA; [5]Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; [6]Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan

A genetic polymorphism of aldehyde dehydrogenase 2 (ALDH2), *ALDH2*2*, is observed in half of the East Asian population, and impairs acetaldehyde metabolism after alcohol intake. Here, we report an unexpected phenotype of this polymorphism—ethanol-induced skin melanosis—in a mouse model and human subjects. Ten-week-old *Aldh2*-knockout mice were fed a standard hard diet and ethanol solution (3–20%; forced and sustained administration). *Aldh2*-knockout mice did not show obvious melanosis in the internal organs, including the esophagus, after 50-week ethanol administration. However, dose-dependent skin pigmentation occurred in certain parts of the skin, including the paws and tails (n = 111, fed until natural death, p < 0.001). The pigmentation faded upon discontinuation of ethanol drinking. No such phenotype was observed in wild-type mice (n = 68, p = 0.08–0.95; interaction p < 0.001). The pigmentation was mainly localized in the epidermis and partially in the dermis, staining positive for the Fontana–Masson stain and melanin-bleaching, and negative for Berlin blue. Melan A-positive melanocytes were observed in the hair follicles of the paws and tails in both *Aldh2*-knockout and wild-type mice. For human subjects, the skin color of 91 Japanese adults (26 women), aged 49 ± 11 (mean ± SD) years, was analyzed spectrophotometrically between

December 2015 and February 2016. Alcohol consumption, Brinkman index, and sun exposure score (0–27) were collected via questionnaires. The results showed supportive correlations with mouse data by multiple regression analysis. Carriers of the *ALDH2*2* (n = 29) showed drinking-induced pigmentation in their forehead skin (p = 0.01), but no significant pigmentation was observed in other parts of the skin, e.g., upper extremities or abdomen, or in non-carriers (n = 62; p = 0.23; interaction p = 0.01). Thus, a novel phenotype of *ALDH2*2*, i.e., ethanol-induced, reversible melanosis of the forehead skin of *ALDH2*2* carriers, was identified.

CS.15.01 | VITI-SUN: A multicentric prospective study to evaluate sun protection behavior in patients with vitiligo

Elio Kechichian[1]; Bhavnit Bhatia[2]; Iltefat Hamzavi[2]; Jean-Pierre Castelneau[1]; Jean-Marie Meurant[3]; Julien Seneschal[4]; Khaled Ezzedine[1]

[1]*Department of Dermatology, Hôpital Henri Mondor, Créteil, France;* [2]*Department of Dermatology, Henry Ford Health System, Detroit, MI, USA;* [3]*Association Française du Vitiligo, Paris, France;* [4]*Department of Dermatology, Hôpital St-André, Bordeaux, France*

Patients with vitiligo have a reduced protection on depigmented areas. To date, however, little is known about photoprotection habits in patients with vitiligo even though these patients are thought to have a decreased ability to efficiently protect their skin against sun damage. Moreover, exposure to artificial UV is one of the major elements of the treatment of vitiligo even though certain phenotypes of vitiligo could be photoinduced. In this context we are conducting a multicentric prospective study between France and the USA to evaluate sun protection habits in patients with vitiligo. For that purpose a semi-directed questionnaire to investigate the sun protection behavior of vitiligo patients (adults> 18 years of age) have been created. Vitiligo severity will be self-assessed by patients using a recently validated patient reported outcome, the SA-VES.

The main objective of this study is to describe photoprotective behavior in patients with vitiligo.

The secondary objectives are:

- To evaluate the link between photo-exposure and occurrence of vitiligo outbreak
- To evaluate the photoprotection habits according to patients phototype and to the severity of the disease

We plan to include 500 patients with vitiligo including all phototypes. We have currently enrolled more than 200 patients and our results point to different sun protective habits between men and women.

Our study may help to correctly address photoprotection in patients with vitiligo.

CS.15.02 | Validation of ImageJ software for digital measurement of target lesions in vitiligo

Nanja van Geel[1]; Dries Vandendriessche[1]; Elise Vandersichel[1]; Sofie De Schepper[1]; Cecilia A. Prinsen[2]; Reinhart Speeckaert[1]

[1]*Department of Dermatology, Ghent University Hospital, Ghent, Belgium;* [2]*Department of Epidemiology and Biostatistics Amsterdam Public Health research institute, VU University Medical Center, Amsterdam, Netherlands*

Background: The ability to objectively measure surface areas of target lesions in vitiligo is important for localized therapies (e.g. pigment cell transplantation, localised UVB treatment, cream treatment). Different methods are available, varying from simple and widely available techniques (e.g. transparent sheet method) to more sophisticated 3D digital image analyses systems. So far, no gold standard method is available yet.

Objectives: The objective of this study was to assess the reliability, validity and user-friendliness of using ImageJ software to assess the surface areas of target lesions in vitiligo.

Methods: Four different observers with different degrees of experience independently performed 156 measurements on 52 lesions varying in size and location of 10 vitiligo patients. Planimetric measurements using ImageJ were performed on three types of material: classic digital pictures, UV digital pictures and lesions contours on transparent sheets. Results were compared to a 3D simulating measurement instrument and evaluated against predefined hypotheses of what was expected. The inter-rater reliability of the measurements was evaluated. To assess the intra-rater reliability, measurements were repeated by 3 raters on a randomized series of the same material/lesions. Timing and user friendliness were recorded for the different steps.

Results: Analysis of this study is currently ongoing and can be presented during the meeting.

CS.15.03 | Development and validation of the vitiligo extent score for target areas (VESTA): An international collaborative study

Jung Min Bae[1]; Seung-Chul Lee[2]; Ichiro Katayama[3]; Tamio Suzuki[4]; Ki-Ho Kim[5]; Eric Lan[6]; Flora Xiang[7]; Hee Young Kang[8]; Sang Ho Oh[9]; Young Wook Ryoo[10]

[1]*The Catholic University of Korea, Suwon, Korea;* [2]*Chonnam National University School of Medicine, Gwangju, Korea;* [3]*Osaka University, Osaka, Japan;* [4]*Yamagata University Faculty of Medicine, Yamagata, Japan;* [5]*Dong-A University School of Medicine, Busan, Korea;* [6]*Kaohsiung Medical University, Kaohsiung, Taiwan;* [7]*Fudan University, Fudan, China;* [8]*Ajou University School of Medicine, Suwon, Korea;* [9]*Yonsei University College of Medicine, Seoul, Korea;* [10]*Keimyung University, Daegu, Korea*

Background: The convenient and reliable instrument to assess the treatment response of each vitiliginous patch should be introduced,

even though a variety of measurements have been used in different trials. We introduced the Vitiligo Extent Score for Target Areas (VESTA) as a variant of the Vitiligo Extent Score (VES) that has been developed to assess the global vitiligo involvement.

Objectives: To develop and validate the VESTA.

Methods: A total of 55 dermatologists assessed the rates of repigmentation in 17 pairs of images consisting of vitiligo lesions pre- and post-treatment with VESTA and rough estimate, respectively. The same participants rated the same image pairs once more two weeks later. The accuracy and inter- and intra-rater reliability of VESTA were evaluated using concordance correlation coefficient (CCC) and intra-class correlation (ICC).

Results: The VESTA showed higher accuracy (CCC: 0.949, 95% CI 0.942-0.955 versus 0.896, 95% CI 0.883-0.908), higher inter-rater (ICC: 0.944, 95% CI 0.937-0.951 versus 0.943, 95% CI 0.935-0.950) and higher intra-rater (ICC: 0.928, 95% CI 0.876-0.968 versus 0.900, 95% CI 0.831-0.954) reliabilities than the rough estimate.

Conclusions: VESTA would be a promising instrument to assess the treatment response in individual lesions. It could be easily applied as a convenient and reliable tool in clinical trials of vitiligo.

CS.15.04 | RNA sequencing of melanocytes isolated from human vitiligo skin identifies GLI1 induced by narrow band UVB treatment in the hair follicle bulge

Nathaniel B. Goldstein[1]; Maranke I. Koster[1,2]; Kenneth L. Jones[3,4]; Bifeng Gao[5]; Laura G. Hoaglin[1,2]; Steven Robinson[6]; Michael J. Wright[1]; Sujatha Venkataraman[4]; Ilinca Birlea[1]; Abigail Luman[1]; Karoline Lambert[1]; Yiqun Shellman[1]; Mayumi Fujita[1,2,7]; William Robinson[6]; Dennis R. Roop[1,2]; David A. Norris[1,2,7]; Stanca A. Birlea[1,2]

[1]*Department of Dermatology, University of Colorado, Aurora, CO, USA;* [2]*Gates Center for Regenerative Medicine, University of Colorado, Aurora, CO, USA;* [3]*Department of Hematology, University of Colorado, Aurora, CO, USA;* [4]*Department of Pediatrics, University of Colorado, Aurora, CO, USA;* [5]*Sequencing and Microarray Core, University of Colorado, Aurora, CO, USA;* [6]*Division of Oncology, University of Colorado, Aurora, CO, USA;* [7]*Denver Department of Veterans Affairs Medical Center, Denver, CO, USA*

The stimulation of melanocyte precursors in the hair follicle (HF) bulge by narrow band UVB (NBUVB) during vitiligo repigmentation is inadequately understood. To better characterize this process, we developed a method using whole transcriptome RNA sequencing (RNA-seq) to explore the gene signature of cells isolated from the HF bulge and interfollicular epidermis in the NBUVB-treated vitiligo skin. Our strategy consisted of rapid immunostaining, followed by fluorescent laser capture microdissection of the melanocyte precursors from the HF bulge and the mature melanocytes from the epidermal basal layer, using skin from 6 NBUVB-treated vitiligo patients. We then isolated RNA from the melanocyte samples, and performed RNA sequencing and gene expression analysis. Using a candidate gene approach, we examined the RNA-seq expression levels of 189 genes from a published stem cell differentiation panel (nCounter® Virtual Stem Cell Gene Set, Nanostring Technologies). The top differentially expressed genes in the melanocytes isolated from the NBUVB-treated bulge as compared with those from the NBUVB-treated epidermis were *FZD7* and *GLI1* (P<1.0E-03; fold change (FC)>46). These results were validated by qRT-PCR using new laser capture melanocyte samples from NBUVB-treated vitiligo patients (N=7) ($P_{adjusted}$<5.0E-03; FC>40). Next, we examined if NBUVB modulates the expression of *FZD7* and *GLI1* in bulge melanocyte precursors isolated from NBUVB-treated vitiligo skin (N=7), untreated vitiligo skin (N=6) and normal control skin (N=6). We found that only *GLI1* transcript was significantly upregulated in the bulge melanocytes of the NBUVB-treated vitiligo skin as compared with the untreated vitiligo skin ($P_{adjusted}$=2.7E-03; FC=9.2). This result was confirmed by fluorescent immunostaining using an anti-GLI1 antibody, which showed a significantly higher expression in the HF bulge melanocyte precursors of NBUVB-treated vitiligo patients (N=6) compared to untreated vitiligo patients (N=6) (P=4.4E-04; FC=1.5). We have developed functional studies using cell culture models to determine whether GLI1 enhances melanocytes proliferation and migration.

CS.15.05 | New game player of vitiligo: Mast cell activation in vitiligo and rhododendrol-induced leukoderma

Ichiro Katayama; Aya Takahashi; Fei Yang; Atsushi Tanemura; Lingli Yang

Osaka University, Osaka, Japan

Vitiligo is a relatively common depigmented skin disorder associated with epidermal melanocyte disappearance. Recent reports have shown that imbalance of epidermal cytokine expressions plays important role in induction of hypomelanotic lesion formation in vitiligo. In addition to decreased expressions of melanogenic cytokine, ET-1 or SCF, increased expressions of proinflammatory cytokine such as IL6, TNFα, IFN-g or chemokine, CXCL10 in lesional skin have been reported in vitiligo.

We previously reported that IL17A producing Th17 cells infiltrate to the lesional skin of vitiligo and IL17A stimulates both fibroblasts and keratinocytes to generate proinflammatory cytokines such as TNFα, IL1, or IL6, which downregulate MITF activation resulting in depigmentation in vitiligo. More recently, IL-17A (+) mast cells were found to infiltrate into psoriasis skin at higher density than IL-17A (+) T cells, termed mast cell extracellular trap (MET). Similar observation with significantly increased infiltration of tryparse (+) and degranulated mast cells in the basal layer of skin were confirmed in vitiligo and rhododendrol induced leukoderma. In the previous literatures by Tomita et al., and Inoue S et al., histamine was reported to stimulate melanogenesis *in vitro* and contributes UVB induced hypermelanosis through histamine H2 receptor. Cosmetically, hypermelanosis of

the perilesional skin of vitiligo or rhododendrol-induced leukomelanoderma is an important matter to be resolved in addition to depigmentation. Recently, disability of melanocyte adhesion to basal keratinocytes initiates melanocyte disappearance in vitiligo. We observed altered expression of several cell surface molecules in basal keratinocyte of vitiligo (manuscript in preparation), which might affect melanocyte disappearance in a similar manner to E-cadherin story proposed by Wagner et al. in 2015. Local activation of mast cells and their products might contribute melanocyte disappearance through impaired adhesion between lesional melanocytes and basal keratinocyte.

CS.15.06 | Evaluation of serum CXCL-10 in relation to minigraft test in detecting stability of vitiligo

Marwa M. Abdallah; Marwa K. Asaad; Reham M. Hassan

Ain-Shams University, Faculty of Medicine, Cairo, Egypt, Cairo, Egypt

Background: Stability is an essential aspect in the surgical treatment of vitiligo. One or more-year stability has been suggested for better surgery outcome. Stability in vitiligo could not be easily evaluated, due mainly to absence of a reliable serum marker and largely depended on performing a minigraft test. Recently, the chemokine CXCL-10 has emerged as a putative serum marker that correlated with disease activity.

Aim of the work: The aim of our study was to evaluate the serum level of CXCL-10 in patients with ≥1 year stability and compare them with patients with shorter period of stability and to correlate them with minigraft test outcome.

Patients and Methods: Fifteen patients with 6-11 months stable disease (group 1) and fifteen patients with at least 12 months stability (group 2) were subjected to a minigraft test and evaluation of serum level of CXCL-10. Repigmentation was evaluated at 3 months.

Results: Serum level of CXCL-10 was not different between both groups (P>0.05). Minigraft test was positive in 40.0% in those with 6-11 months vs 86.7% in those with ≥1 year stability (p=0.008). The extent of repigmentation at 3 months in patients with longer stability was significantly higher than those with shorter disease stability (P= 0.023). In group 1 but not group 2 a negative minigraft test was associated with higher CXCL-10 level (P=0.008). Moreover, a negative correlation was found between serum CXCL-10 level and extent of repigmentation in group 1 (R= -0.670, P=0.006), but not in group 2 (R= −0.030, P=0.916).

Conclusion: Herein we have shown that ≥1 year stability is essential before attempting surgery. Minigraft test remains to be a reliable tool to detect disease stability. CXCL-10 level may be helpful in patients with shorter disease stability.

CS.15.07 | Blistering skin in vitiligo patients permits analysis of both immune and melanocyte factors that influence disease pathogenesis

Maggi A. Refat[1]; James P. Strassner[1]; Micheal Fresoli[1]; Jillian Richmond[1]; Mehdi Rashighi[2]; Bassel Mahmoud[2]; Dori Goldberg[2]; John E. Harris[1]

[1]University of Massachusetts Medical School, Worcester, MA, USA; [2]University of Massachusetts Memorial Medical Center, Worcester, MA, USA

Vitiligo treatment has been limited by an incomplete understanding of pathogenesis, however recent advances support the development of new, targeted therapies that are expected to enter clinical trials soon. Some therapies target inflammation, which will be most effective in patients with active disease, while others promote melanocyte regeneration, which will be most effective in patients with stable disease. Thus, emerging treatments highlight a need to identify and validate biomarkers of disease activity to efficiently enroll the patients most suited to the trial. We have validated a minimally invasive modified suction blistering technique that allows for assessment of disease activity and treatment responses, in addition to analyzing individual cell types that contribute to disease pathogenesis. CXCL9 protein in the blister fluid achieved the highest sensitivity and specificity for defining active versus stable disease, as well as treatment response. Moreover, suction blistering allowed detailed and quantitative phenotyping of melanocytes and their stem cell lineage using flow cytometry, by which we have successfully identified differentiated melanocytes, melanocyte stem cells, and melanoblasts.

Therefore, suction blistering is a powerful technique through which to measure disease stability, mechanism of repigmentation, and advance our understanding of disease pathogenesis.

CS.15.08 | When to do thyroid screening in vitiligo: The Amsterdam approach

Albert Wolkerstorfer[1]; Marije Kroon[1]; Charlotte Vrijman[2]; Rosalie Luiten[1]

[1]Academic Medical Centre, Netherlands Institute for Pigment Disorders, Amsterdam, Netherlands; [2]Ziekenhuis Groep Twente, Hengelo, Netherlands

Thyroid disease is known to be associated with non-segmental vitiligo. However, the outcomes of prevalence studies on thyroid disease in vitiligo vary widely and prospective screening studies are rare. Consequently, the recommendations vary from regular annual screening in all patients to screening in risk groups only.

Previously, we performed a systematic review and metaanalysis of a total of forty-eight studies published between 1968 and 2012. Thyroid disease, autoimmune thyroid disease and presence of thyroid-specific autoantibodies showed a mean prevalence of, respectively,

15.1%, 14.3% and 20.8% in patients with vitiligo and an RR of, respectively, 1.9, 2.5 and 5.2 (all statistically significant).

Because of this high risk we previously enrolled a total of 434 adults with non-segmental vitiligo for screening. Though, the overall prevalence of thyroid dysfunction in adult patients with non-segmental vitiligo was found to be high, the number of newly diagnosed cases with overt and subclinical thyroid dysfunction in our population was low. Most patients had already been diagnosed by their general practitioner and in half of the patients the thyroid disease started before the onset of vitiligo. Thyroid disease was found predominantly among older women and in subjects with a positive family history of thyroid disease.

In children, we noticed in a similar screening program a different outcome. We previously enrolled a total of 260 children with non-segmental vitiligo for screening and found 16 patients (6.2%) with a new diagnosis of autoimmune thyroiditis with thyroid hormone disturbances.

Therefore, in our department, in children we routinely perform thyroid screening including anti-TPO antibodies while in adults we limit screening to high-risk subpopulations (women with non-segmental vitiligo, above 35 years and with a positive family history of thyroid disease). In case of normal thyroid hormones and normal anti-TPO we do not repeat the screening routinely.

CS.16.03 | ROS1 and ALK fusions are novel therapeutic targets in non-sun exposed subtypes of melanoma

Kasey L. Couts[1]; Danielle Murphy[2]; Jason Christiansen[2]; Jacqueline A. Turner[1]; Judson Bemis[1]; Caroline E. McCoach[1]; Karl D. Lewis[1]; Pratik Multani[2]; Edna Chow-Maneval[2]; William A. Robinson[1]; Robert C. Doebele[1]

[1]University of Colorado Anschutz Medical Campus, Aurora, CO, USA; [2]Ignyta, Inc., San Diego, CA, USA

Kinase gene fusions, such as those found with *ALK*, *NTRK1/2/3*, and *ROS1*, are known oncogenic drivers occurring at a low frequency (2-5%) across various solid tumors, and targeted inhibitors demonstrate substantial clinical activity against these tumors. Fusions of *ALK*, *BRAF*, *NTRK1*, *RAF1*, *RET*, and *ROS1* are frequent events (5-15%) in Spitz nevi and Spitzoid melanomas. Studies on common sun-exposed melanomas found a low occurrence of *BRAF* and *RAF1* fusions, but no fusions were found with other common kinases. Non-sun exposed subtypes of melanoma, including acral lentiginous (ALM) and mucosal melanoma (MM), have lower mutation burden and increased chromosomal instability compared to sun-exposed melanomas. Therefore, we hypothesized kinase fusions may be more frequent in ALM and MM. In this study, we used targeted NGS sequencing to screen 25 non-sun exposed melanomas (22 ALM and 3 MM) for fusions of *ALK*, *NTRK1/2/3* and *ROS1*. We identified fusions in two patient samples (8%). A *GOPC-ROS1* fusion was identified in a 46 year old male with stage IV ALM. The patient was enrolled in the STARTRK-1 phase 1 trial of a TRK/ROS1/ALK inhibitor (entrectinib), which demonstrated an overall response rate of 79% in Phase 1 in patients whose tumors harbored these kinase fusions. The patient experienced a dramatic and durable response (-38% at 3 months and -55% at 11 months, by RECIST 1.1). We also identified an *EML4-ALK* fusion in a 62 year old female with MM. This patient did not require therapy; therefore, we developed pre-clinical models from the patient tumor and showed it to be sensitive to entrectinib. This is the first report of *ROS1* and *ALK* fusions, and their response to targeted therapy, in malignant melanoma. Our findings implicate *ROS1* and *ALK* fusions as actionable therapeutic targets in non-sun exposed melanomas, and these patients may have clinical benefit from entrectinib.

CS.16.04 | Stromal neuregulin-1 modulates the response to MEK inhibitors in WT BRAF/WT NRAS (WT/WT) melanomas

Claudia Capparelli[1]; Timothy J. Purwin[1]; Michael A. Davies[2]; Jeffrey Gershenwald[3]; Andrew E. Aplin[1]

[1]Department of Cancer Biology, Thomas Jefferson University, Philadelphia, PA, USA; [2]Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [3]Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

MEK-ERK1/2 signaling is elevated in the majority of melanomas; however, MEK inhibitors are frequently ineffective in melanoma that are wild type for BRAF and NRAS (WT/WT). Since factors in the tumor microenvironment (TME) often regulate drug resistance, we tested the effects of several growth factors on WT/WT melanoma growth in the presence of MEK inhibitor (MEKi). Notably, neuregulin-1 (NRG1) protected WT/WT melanoma cells from growth inhibition mediated by the MEKi, trametinib. Phospho-proteomic analysis implicated adaptive activation of ErbB2 in trametinib-treated cells and ErbB2 and ErbB3 blocking antibodies prevented the protective effects of NRG1. Apart from a subset of lines that have autocrine production, NRG1 expression was low in WT/WT lines; however, NRG1 was consistently detected in the conditioned medium from fibroblasts and cancer-associated fibroblasts (CAF), which activated ErbB3/ErbB2 signaling in MEK inhibited WT/WT melanoma cells. ErbB3 and ErbB2 neutralizing antibodies blocked the protective effects of fibroblast- and CAF-derived NRG1 in vitro and co-operated with MEK inhibitors to block xenograft growth in vivo. Together our results provide a rationale for the treatment of WT/WT melanomas expressing NRG1 and ErbB3 with the combination of MEK inhibitors and ErbB3/ErbB2 antibodies.

CS.16.05 | Combined inhibition of BET bromodomain proteins and BRAF/MEK kinases as a potential therapy for BRAF mutant melanoma

Manoela Tiago[1]; Timothy Purwin[1]; Michael A. Davis[2]; Andrew Aplin[1,3]

[1]Thomas Jefferson University, Philadelphia, PA, USA; [2]MD Anderson Cancer Center, Houston, TX, USA; [3]Siney Kimmel Cancer Center, Philadelphia, PA, USA

Metastatic melanoma is the most lethal form of skin cancer with increasing incidence and extremely poor prognosis in advanced stages.

Current treatments, such as targeted agents (BRAF/MEK inhibitors), show remarkable clinical efficacy against mutant BRAF melanoma; however, they often become ineffective due to resistance. Adaptive RTK upregulation, along with ERK1/2 pathway reactivation, contributes to poor patient responses to the combination of BRAF and MEK inhibitors (BRAFi/MEKi). Here, utilizing RPPA data analysis and Western Blotting we show that dabrafenib/trametinib treatment adaptively upregulated the RTKs ERBB2, ERBB3, PDGFRbeta, and VEGFR-2, and the transcription factor FOXD3. The bromodomain and extra-terminal (BET) proteins are epigenetic readers that bind to histone marks and regulate "tumor-driving" gene expression. Recent studies have shown that BET inhibitors alter kinase pathways, reduce the expression of RTKs, decrease tumor cell growth and survival. Here, we tested whether the addition of BET inhibitor could improve the cytotoxic effects of BRAFi/MEKi alone on BRAF mutated melanoma cell lines. RPPA analysis also showed the triple treatment (BRAF/MEK/BET inhibition) was able to reverse the upregulation of RTKs and FOXD3 induced by BRAFi/MEKi. The triple treatment was able to increase pro-apoptotic proteins such as Bim and dramatically reduced cell growth in monolayer and in human skin reconstructs compared to BRAFi/MEKi alone. Thus, our data suggests the inhibition of BET bromodomain proteins will improve the efficacy of BRAFi/MEKi in BRAF mutated melanoma.

CS.16.06 | Experimental therapy method on BRAFi and natural compounds

Frank L. Meyskens; Feng Liu-Smith

UC Irvine, Irvine, CA, USA

Recently the FDA-approved mutant BRAF inhibitor (BRAFi) Plx4032 (Vemurafenib) showed unprecedented efficacy. Tragically, tumors rapidly acquired resistance and progressed. The acquired resistance involves activation of NRAS and/or reactivation of ERK1/2 via several pathways. No NRAS-targeted therapy has been developed yet in any cancer types. Combing BRAFi dabrafenib with a MEK1/2 inhibitor (MEKi) Trametinib increased progression-free survival in melanoma patients but resistance still developed. A number of combination treatment approaches have been under development, and apparently needed. In searching for such combination therapy methods, We have screened a number of natural compounds for their synergistic activity with Vemurafenib, and we discovered that a flavonoid luteolin showed substantial synergistic effect with Vemurafenib in all 5 melanoma cell lines examined. Superb tumor inhibitory effect was also observed in xenografted melanoma tumors. The precise synergistic molecular mechanism is under investigation as we found luteolin induced both mitochondrial and cytoplasmic reactive oxygen species, and may also inhibit PI3K/AKT pathway. Luteolin may also directly target NRAS which is the key alternative kinase rewiring hub for Vemurafenib resistance. The long term objective of our work is to develop novel combinatorial treatment approaches that will cure patients with metastatic melanoma.

CS.17.02 | Differences in mutational processes and driver genes between acral, mucosal and cutaneous melanomas

Nicholas Hayward

QIMR Berghofer medical Research Institute, Brisbane, QLD, Australia

Australia has the highest incidence of cutaneous melanoma worldwide, where it is the fourth most common cancer and a leading cause of cancer death in young adults. Late stage metastatic melanoma was until very recently usually rapidly fatal. However, inhibitors of the MAP kinase pathway and of immune checkpoint mechanisms have dramatically extended patient survival, highlighting the importance of identification of therapeutic targets in cancer through molecular characterization. To comprehensively extend the molecular characterization of melanoma, the Australian Melanoma Genome Project (AMGP) was launched in August 2012 with the support of Melanoma Institute Australia, the Australian Government via Bioplatforms Australia, NSW Health and the Cancer Council NSW. The AMGP aims to analyse whole genomes from 500 primary and metastatic melanomas. To date, whole genome sequencing has been completed on matched tumour and blood DNA from 183 patients with cutaneous, acral or mucosal subtypes of melanoma. The landscape of coding and non-coding mutations in cutaneous melanoma showed signatures of ultraviolet radiation induced mutagenesis. In contrast, acral and mucosal melanomas had mutation signatures of unknown aetiology and were dominated by structural changes. TERT promoter mutations were most frequent in cutaneous melanoma, however neither they nor ATRX mutations, associated with alternative lengthening of telomeres, were correlated with greater telomere length. Almost all melanomas had potentially actionable mutations, and most of these were in components of the mitogen-activated protein kinase and phosphoinositol kinase pathways.

CS.17.03 | Melanin pigment and melanoma heterogeneity

Clare G. Fedele[1,2]; Lokugan Silva[1,2]; Ralph Rossi[1]; Mark Shackleton[1,2,3]

[1]The Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; [2]University of Melbourne, Melbourne, VIC, Australia; [3]Alfred Health and Monash University, Melbourne, VIC, Australia

Production of melanin pigment is the archetypal readout of differentiation in normal melanocytes. In histological assessments of melanomas, which arise from melanocytes, we found a high degree of intratumoral heterogeneity (ITH) for pigment production, even in overtly pigmented tumors. Using a novel flow cytometry-based approach to prospectively separate cells according to pigment content from pigmented patient melanomas and cell lines, we found that low-pigment cells are abundant and have enhanced clonogenic potential *in vitro* and tumorigenic potential *in vivo*, compared to high-pigment cells. Moreover, low- but not high-pigment cells generated progeny

that recapitulated the pigment heterogeneity of the original tumor or cell line.

Transcriptome profiling of low- and high-pigment cell subsets suggested drivers of the heritable functional distinctions between them. By gene ontology and Gene Set Enrichment Analysis, high-pigment cells showed increased expression not only of markers of melanocytic differentiation, but also of p53 activation. Furthermore, the non-clonogenic state in melanoma cells was associated with increased oxidative phosphorylation. In contrast, low-pigment cells displayed gene expression patterns associated with ribosome biogenesis and upregulation of Myc-driven transcription. We therefore treated heterogeneously pigmented cell lines with the RNA Polymerase I inhibitor CX5461, which inhibits ribosome biogenesis. CX5461 increased pigmentation and senescence markers while markedly decreasing clonogenicity.

Our data are inconsistent with proposed plastic relationships in melanoma between high- and low-pigment melanoma cells. Rather, we find evidence in pigmented melanomas of relatively flat cellular hierarchies, with low-pigment cells at a typically broad apex of the hierarchy that both self-renew as well as generate high-pigment cells that are irreversibly non-clonogenic. We also find that this may be exploited therapeutically by inhibiting mechanisms such as ribosome biogenesis that apparently sustain the highly clonogenic, low-pigment state

CS.17.04 | Genome-wide DNA methylation analysis in melanocytes and melanomas from the same individual

Susumu Fujiwara[1]; Hiroshi Nagai[1]; Haruki Jinbo[1]; Tomoyo Tanaka[2]; Masukazu Inoie[2]; Chikako Nishigori[1]

[1]Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, Kobe, Japan; [2]R&D Department, Japan Tissue Engineering Co., Ltd, Gamagori, Japan

Various epigenetic changes—such as DNA methylation and histone acetylation—are associated with melanocyte carcinogenesis. Since DNA methylation levels are influenced by environmental factors, melanocytes and melanomas should be isolated from the same patient to fully evaluate changes in DNA methylation status. However, most previous reports use established melanoma cell lines and commercially available melanocytes, likely because of the difficulties in collecting the sufficient number of adult melanocytes for analysis. In this study, we describe a novel melanocytes isolation technique using epidermal sheet cultivation even with less proliferative tissue from older patient. We analyzed not only paired melanoma tissue from four patients, but also analyzed an additional melanocyte sample, one set of primary and metastatic melanoma samples, three commercially available melanocyte cell strains, and four melanoma cell lines. Cluster analysis of DNA methylation data classified freshly isolated melanocytes and melanomas into the same group, whereas the four melanoma cell lines were placed in a distant clade. Moreover, our melanoma cell lines analysis discovered hypermethylation compared to melanocytes on several novel loci (*KRTCAP3*, *AGAP2*, *VGLL4*, *ZNF154*, and *TTC22*), as well as those identified in previous studies (*COL1A2*, *GPX3*); however, the latter two were not observed in fresh melanoma samples, suggesting that prolonged culturing and repeatedly freeze-thawed cell lines might be inappropriate for use in epigenetic analysis. Subsequent studies revealed that *NPM2 (nucleophosmin/nucleoplasmin 2)* was hypermethylated and downregulated in melanoma, which was consistent with previous reports. *NPM2* immunohistopathological reactivity was only observed in one of ten melanoma samples, but nearly all melanocytes samples (85%, 19/22) and benign melanocytic nevi (93%, 14/15) displayed positive staining, suggesting *NPM2* could be used to differentiate melanomas from normal melanocytes or benign disease. Thus, our results highlight the importance of using matched fresh melanocyte and melanoma samples in DNA methylation studies.

CS.17.05 | Whole exome sequencing identifies recurrent R625 mutations in novel drug target and driver, SF3B1 in the largest cohort to date of mucosal melanoma

Jennifer D. Hintzsche; Nicholas T. Gorden; Carol M. Amato; Jihye Kim; Kelsey Wuensch; Kasey Couts; Theresa Medina; Keith Wells; Aik Choon Tan; William Robinson

University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA

Mucosal melanoma makes up 1% of all melanoma diagnoses. This disease lacks a UV-signature and has a poor prognosis due to the absence of common driver mutations found in cutaneous melanoma. To develop a better understanding the molecular landscape of these rare melanomas, we performed Whole Exome Sequencing (WES) of 39 tumors from 19 patients to identify driver mutations. 135 sun-exposed cutaneous melanomas also underwent WES for comparison. The IMPACT WES-analysis pipeline was used to call variants and copy number alterations. Chi-square tests compared the genes mutated between these cohorts, where a p-value of less than 0.05 was considered significant. After comparison between the mucosal and cutaneous cohorts, the mucosal melanoma group had 161 genes that were enriched ($p < 0.05$). *KIT* and *NF1* were mutated in 50% of all samples and were co-mutated in 32% of all samples. The most significantly ($p < 0.001$) mutated gene was *SF3B1* (7/19, 37%), with recurrent mutations at R625H/S/C that were validated by PCR. Previously identified as a driver in uveal melanoma, *SF3B1* has never been identified in vulvovaginal mucosal melanoma. Mutations in other spliceosome pathway genes were also found to be enriched in mucosal melanoma ($p < 0.05$). Recurrent R625 mutations in *SF3B1* as well as the co-mutation of *NF1* and *KIT* have expanded the molecular knowledge of mucosal melanoma. Four genes were found to be alternatively spliced in *SF3B1* mutant samples compared to *SF3B1* wild-type samples. This study also validated that alternative splicing is occurring in *SF3B1* mutant samples in several genes and proposes *SF3B1* as a novel drug target in mucosal melanoma. This study is the

largest WES project of mucosal melanomas to date, and the first to identify and validate *SF3B1* mutations in vulvovaginal mucosal melanoma, as well as provide functional insight into the consequences of these mutations.

CS.17.06 | Precision targeting of epigenomic master regulators in malignant melanoma

Fabian V. Filipp

Systems Biology and Cancer Metabolism, Program for Quantitative Systems Biology, University of California Merced, Merced, CA, USA

Molecular insights from genome and systems biology are influencing how cancer is diagnosed and treated. We critically evaluate big data challenges in precision medicine. The melanoma research community has identified distinct subtypes involving chronic sun-induced damage and the mitogen-activated protein kinase driver pathway. In addition, despite low mutation burden, non-genomic mitogen-activated protein kinase melanoma drivers are found in membrane receptors, metabolism, or epigenetic signaling with the ability to bypass central mitogen-activated protein kinase molecules and activating a similar program of mitogenic effectors. Mutation hotspots, structural modeling, UV-signature, and genomic as well as non-genomic mechanisms of disease initiation and progression are taken into consideration to identify resistance mutations and novel drug targets. A comprehensive precision medicine profile of a malignant melanoma patient illustrates future rational drug targeting strategies. Network analysis emphasizes an important role of epigenetic and metabolic master regulators in oncogenesis. Co-occurrence of driver mutations in signaling, metabolic, and epigenetic factors highlights how cumulative alterations of our genomes and epigenomes progressively lead to uncontrolled cell proliferation. Precision insights have the ability to identify independent molecular pathways suitable for drug targeting. Synergistic treatment combinations of orthogonal modalities including immunotherapy, mitogen-activated protein kinase inhibitors, epigenetic inhibitors, and metabolic inhibitors have the potential to overcome immune evasion, side effects, and drug resistance.

CS.18.01 | Melanocytes sense blue-light and regulate the pigmentation through the opsin 3

Claire Regazzetti[1]; Laura Sormani[1]; Delphine Debayle[1]; Françoise Bernerd[2]; Meri Tulic[1]; Gian Marco De Donatis[1]; Bérengère Chignon-Sicard[1]; Stephane Rocchi[1]; Thierry Passeron[1]

[1]*Université de Nice Sophia-Antipolis, Nice, France;* [2]*L'OREAL Research and Innovation, Aulnay sous Bois, France*

The shorter wavelengths of the visible light (from 400 to 465 nm) have been recently reported to induce a long lasting hyperpigmentation but only in melano-competent individuals.

Here, we provide evidence demonstrating that opsin 3 (OPN3) is the key sensor in melanocytes responsible for hyperpigmentation induced by the shorter wavelengths of the visible light. The melanogenesis induced through OPN3 is calcium dependent and further activates CAMKII followed by CREB, ERK, and p38 leading to the phosphorylation of MITF and ultimately to the increase of the melanogenesis enzymes: tyrosinase and dopachrome tautomerase (DCT). Furthermore, blue light induces the formation of a protein complex that we demonstrated to be formed by tyrosinase and DCT. This complex is mainly formed in dark-skinned melanocytes and induces a sustained tyrosinase activity, thus explaining the long-lasting hyperpigmentation that is observed only in skin type III and higher after blue light irradiation. OPN3 thus functions as the sensor for visible light pigmentation. OPN3 and the tyrosinase complex induced after its activation appear as new potential targets for regulating melanogenesis but also to protect dark skins against blue light in physiological conditions and in pigmentary disorders.

CS.18.03 | Comparison of 311-nm Ti:Sapphire laser vs. 308-nm excimer laser treatment for vitiligo: A prospective randomized controlled non-inferiority trial

Jung Min Bae; Sung Hye Eun; Hanna Lee; Ji Hae Lee; Gyong Moon Kim

The Catholic University of Korea, Suwon, Korea

Background: The 308-nm excimer laser (EL) has been widely used for localized vitiligo. Recently, the 311-nm Ti:Sapphire laser (TSL) was developed following the peak spectrum of NBUVB (311 nm).
Objectives: To compare the efficacy and safety of TSL vs. EL in the treatment of vitiligo.
Methods: A randomized controlled non-inferiority trial based on split-body was performed. The paired symmetric vitiliginous lesions were randomized to TSL or EL treatment groups. All lesions were treated twice weekly for a total of 12-week period. The degree of repigmentation was assessed as % from baseline by using a computer program every 4 weeks, and the non-inferiority margin was set at 10%.
Results: A total of 20 patients, aged 23 to 79 years, were enrolled. Seventy-four paired lesions were assigned to EL group (n = 37) or TSL group (n = 37). The mean difference between two groups (EL minus TSL) was -2.862%, and the 95% confidence interval (-6.531% to 0.807%) was lower than the non-inferiority margin (10%). No serious adverse effect was observed in either group.
Conclusion: The present study demonstrated that 311-nm TSL was as effective as 308-nm EL in the treatment of vitiligo. TSL treatment was tolerable with similar profiles of adverse effects of excimer laser treatment, and has some advantages including deeper penetration by the 311-nm wavelength and the use of a solid medium with reduced maintenance costs.

CS.18.04 | Fluticasone propionate 0.05% cream improves repigmentation in narrow-band UVB phototherapy of non-segmental vitiligo: A randomized controlled trial

Albert Wolkerstorfer; Marije Kroon; Rosalie Luiten

Academic Medical Centre, Netherlands Institute for Pigment Disorders, Amsterdam, Netherlands

Background: Topical anti-inflammatory drugs and UVB phototherapy are safe and effective as monotherapy in vitiligo. However, no evidence from RCT's is available on the efficacy of combining topical corticosteroids with 311 nm narrow-band UVB (NB-UVB) therapy.

Objective: To compare the efficacy of NB-UVB with NB-UVB plus fluticasone propionate 0.05% cream (FP) in patients with non-segmental vitiligo.

Methods: Consecutive patients aged 18 years or older were eligible. In each patient one representative target lesion, with a diameter of at least two cm, was selected on the trunk or upper limbs. Fifty patients were randomized to NB-UVB therapy twice weekly for 12 months, alone or in combination with topical FP. FP was applied, only on the target lesion, once daily during the first three months and in a scheme of three days per week during the remaining nine months. The primary outcome was the percentage of repigmentation of the target lesion after 12 months of therapy and at three months follow-up, judged by two blinded dermatologists on digital photographs. Secondary outcomes were the patient's global assessment and Skindex-29 scores.

Results: At 12 months and three months follow-up, there was a significant difference in repigmentation between the two groups in favour of the combination treatment. Twelve months: NB-UVB: median 18.8% IQR:1.9-27.5, NB-UVB+FP: median 45.0% IQR:10.0-72.5, ($p<0.05$). Three months follow-up: NB-UVB:18.8% (1.9-28.8), NB-UVB+FP:35.0% (10.0-75.0). In total there were seven (14.9%) patients who did not show any repigmentation after 12 months of therapy. No side effects were observed.

Conclusions: The combination of NB-UVB and FP provides better repigmentation than NB-UVB alone. The use of FP was safe in the long-term treatment of non-segmental vitiligo.

CS.18.05 | Laser treatment of congenital melanocytic nevi: A systematic review

Janny E. Lommerts[1,2]; Celine Eggen[3]; Esther Van Zuuren[4]; Jacqueline Limpens[5]; Suzanne Pasmans[3]; Albert Wolkerstorfer[1,2]

[1]*Department of Dermatology, Academic Medical Centre, Amsterdam, Netherlands;* [2]*Netherlands Institute for Pigment Disorders, Amsterdam, Netherlands;* [3]*Department of Dermatology, Eramus Medical Center, Rotterdam, Netherlands;* [4]*Department of Dermatology, Leiden University Medical Center, Leiden, Netherlands;* [5]*Medical Library, Academic Medical Centre, Amsterdam, Netherlands*

Background: Congenital melanocytic nevi (CMN) are melanocytic lesions, which are present at birth or appear in the first few weeks of life. Recent studies show a lower risk on malignant melanoma than previously estimated. As a result, the treatment paradigm in CMN shifted to less invasive treatment options such as laser treatment. In this study, we systematically reviewed the efficacy and safety of laser therapy for CMN.

Methods: We searched and screened articles in the electronic databases of MEDLINE, EMBASE, CENTRAL and PubMed. The quality of evidence (rated with the GRADE approach) and quality of the individual clinical trials was performed by 2 assessors. Data-extraction was based on the extraction of the results of pre-specified outcome measures.

Results: We found 24 eligible studies (3 non-randomized controlled studies and 21 case series), which included a total of 434 patients. The quality of the majority of the individual studies was poor and the quality of the evidence of each outcome was rated as very low. In total, 20 different combinations of lasers were assessed. The treatment with a Q-switched laser was the most frequently used and large to giant CMN were mainly treated with ablative lasers. Lasers in CMN showed rather good results for clearing of hyperpigmentation on the short term. However, the outcome measures varied widely and high incidences of scarring, repigmentation and complications were reported. No malignant change was seen.

Conclusion: Most studies report short-term improvement of CMN after laser therapy. No high quality evidence is available on long-term efficacy and safety. Therefore, the benefit of lasers for the therapy for CMN remains unclear. This systematic review emphasizes the need for well-conducted and well-reported prospective studies on laser therapy in CMN.

CS.18.06 | The synergistic effect of minimal amounts of long-wavelength ultraviolet a1 and visible light on pigmentation

Indermeet Kohli[1]; Suteeraporn Chaowattanapanit[1]; Tasneem F. Mohammad[1]; Cynthia L. Nicholson[1]; Nikoforos Kollias[2]; Henry W. Lim[1]; Iltefat H. Hamzavi[1]

[1]*Henry Ford Health System, Detroit, MI, USA;* [2]*Independent, Boston, MA, USA*

Background: Visible light induces dark and persistent pigmentation in addition to erythema, thermal damage, and free radical production. Pigmentation phases include immediate pigment darkening (IPD), persistent pigment darkening (PPD), and delayed tanning (DT) that occur immediately, between 2-24 hours, and 5-7 days after irradiation respectively. FDA approved sunscreen testing protocols require the use of solar simulators with spectral output in ultraviolet (UV) domain only. However, sunlight consists of visible light (VL) and infrared radiation in addition to UV. The purpose of this study is to evaluate the relative contributions of VL and UVA on cutaneous pigmentation.

Methods: Ten subjects with Fitzpatrick skin phototype IV-VI were recruited. On day 0, subjects were irradiated on either side of their back with 480 J/cm^2 of VL using two different light sources; one containing

pure VL, the other containing VL with less than 1% UVA1. Evaluations were performed immediately, at 24 hours, 7 days, and 14 days after irradiation. Assessment methods included investigator's global assessment, clinical photography, and spectroscopy.

Results: IPD, PPD and DT were observed with both light sources. However, the intensity of the pigmentation was approximately two times greater when subjects were irradiated with the light source containing less than 1% UVA1 along with VL compared to that with pure VL.

Conclusions: The degree of pigmentation induced by VL is significantly enhanced by the presence of trace amount of UVA1; suggesting synergy between the two. Current organic sunscreens do not offer protection from this part of the solar spectrum. Non-micronized inorganic sunscreens do, but are cosmetically unacceptable. The findings emphasize the need to modify the spectral output of solar simulators for product testing. The development of sunscreens and other means of photoprotection against VL is necessary to protect against its biologic effects as well as prevent the exacerbation of photodermatoses.

CS.19.01 | Targeting the developmental melanocyte lineage in melanoma

Liz Patton

University of Edinburgh, Edinburgh, UK

One of the major challenges for melanoma treatment and therapeutic design is intratumour heterogeneity. The source of melanoma heterogeneity is not well understood, but neural crest and melanocyte developmental lineage genes are likely to be important in establishing these cell types. We use live-imaging of the zebrafish melanocyte developmental lineage as the basis for phenotypic chemical screens for new therapeutic targets and drug-leads in melanoma. Screening for targets of the melanocyte lineage is highly relevant to melanoma because melanocytes are the melanoma cell of origin, and genes that specify the melanocyte stem cells and the lineage during embryogenesis are the same genes that play fundamental roles in cancer. Using our zebrafish screening approach, we have identified two new pathways in the melanocyte lineage. First, we have identified an ALDH subpopulation in melanocyte development that is targeted by 5-nitrofuran antibiotics. Importantly, we find that ALDH enzymes bioactivate 5-nitrofuran antibiotics to target $ALDH^{High}$ cancer stem cell subpopulations in human melanoma. Second, we have discovered a PRL3 phosphatase dependent function in the melanocyte stem cell lineage. Unexpectedly, we have discovered that PRL3 regulates transcriptional machinery components to maintain melanocytes in an undifferentiated state. PRL3 is overexpressed in many metastatic cancers, and our work provides a new mechanism through which PRL3 phosphatase might contribute to undifferentiated cell populations in melanoma. Taken together, our work shows that understanding and targeting the melanocyte lineage is directly relevant to melanoma, and reveals therapeutically targetable processes that might elucidate pathways for cancer therapy.

CS.19.02 | GDF6-induced BMP signaling reawakens a neural crest identity in melanoma to prevent differentiation and cell death

Arvind Venkatesan[1]; Rajesh Vyas[1]; Alec Gramann[1]; Karen Dresser[1]; Sharvari Gujja[1]; Sanchita Bhatnagar[1]; Sagar Chhangawala[2]; Camilla B. Gomes[3]; Hualin S. Xi[1]; Christine Lian[3]; Yariv Houvras[2]; Yvonne Edwards[1]; April Deng[1]; Michael Green[1,4]; Craig Ceol[1]

[1]University of Massachusetts Medical School, Worcester, MA, USA; [2]Weill Cornell Medical College, New York, NY, USA; [3]Brigham and Women's Hospital, Boston, MA, USA; [4]Howard Hughes Medical Institute, Worcester, MA, USA

It has been known for some time that melanoma cells express neural crest markers, but the importance of this expression and the means of activating these markers have been poorly understood. Recently, studies in zebrafish have shown that neural crest gene expression is found in the cell of origin of melanomas, indicating that a fundamental change in cell identity occurs at the earliest stages of tumor onset. Whether this change facilitates tumor progression has not yet been established. In comparative oncogenomic studies to uncover new melanoma genes, we identified the recurrently amplified BMP ligand gene *GDF6*. GDF6 protein is not expressed in normal melanocytes but is present in nearly 80% of melanomas, where its level of expression correlates with patient survival. In knockdown and gain-of-function studies, we found that *GDF6* promotes melanoma initiation and outgrowth. Expression analyses found that *GDF6*-induced BMP signaling maintains a trunk neural crest identity in melanomas. In maintaining this identity, *GDF6* induces SMAD1/5/8 phosphorylation, and phospho-SMAD1/5/8 directly bind to and repress transcription of *MITF* to prevent differentiation of melanoma cells. Our study uncovers an important role for *GDF6* and BMP signaling in reawakening an embryonic cell identity to promote melanoma progression and provides new opportunities for targeted therapy of GDF6-positive cancers.

CS.19.04 | Genetic basis of normal and transformed pigment cells in the xiphophorus and medaka model

Janine Regneri[1]; Barbara Klotz[1]; Brigitta Wilde[1]; Hisashi Hashimoto[2]; Amaury Herpin[3]; Manfred Schartl[1]

[1]Physiological Chemistry, University of Wuerzburg, Wuerzburg, Germany; [2]Nagoya University, Nagoya, Japan; [3]INRA, Fish Physiology and Genomics Institute (INRA-LPGP), Rennes, France

Genes involved in normal pigment cell development and pigment pattern formation can also critically impact the pathological processes of pigment cell tumor formation. On the other hand, genes identified by their role in melanoma may have a function in the non-transformed pigment cells. To obtain a better understanding of the reciprocal action of pigment cell development genes we are using the *Xiphophorus* and medaka fish models.

Xiphophorus fish develop like many other poeciliid fish peculiar spot patterns made up of giant melanocytes, so-called macromelanophores. These macromelanophores show features of senescent pigment cells, comparable to human nevus cells. We show that the cell-cycle inhibitor Cdkn2ab mediates a senescence-like state of *Xiphophorus* pigment cells. Moreover, ectopic overexpression of *cdkn2ab* in *Xiphophorus* melanoma cells led to a growth arrest in-vitro. Transgenic medaka expressing the *Xiphophorus* melanoma-inducing oncogene *xmrk* under control of the *mitfa* promoter develop xanthoerythrophoromas and melanomas. Coexpression of *cdkn2ab* under the *mitfa* promoter in double-transgenic medaka fish resulted in total suppression of pigment cell tumor formation. This tumor suppressor effect of *cdkn2ab* was even visible in triple mutants that carried in addition a loss-of-function mutation in *tp53*. Conversely, CRISPR/Cas9 generated knock-outs showed a much higher malignancy of *xmrk* caused melanoma. Unexpectedly, overexpression of *cdkn2ab* led to a 25% increase of normal melanocytes in wildtype pigmented fish.

Mutation of *sox5* has recently been shown to be causative for the ml3 phenotype in medaka. These mutants show an overabundance of leucophores and absence of xanthophores, while melanophores appear to be unaffected. When the *mitf:xmrk* transgene was introduced into a *sox5* mutant background these fish did not develop any type of pigment cell tumors. The suppression of melanoma indicates a different action of *sox5* in normal and neoplastically transformed melanocytes.

CS.19.05 | A Zebrafish model of NF1-mutant melanomas that lack activating mutations of BRAF or NRAS

Shuning He[1]; Marc R. Mansour[4]; Hillary M. Layden[1]; Scott J. Rodig[2]; E. Elizabeth Patton[3]; A. Thomas Look[1]

[1]*Dana-Farber Cancer Institute, Boston, MA, USA;* [2]*Brigham and Women's Hospital, Boston, MA, USA;* [3]*University of Edinburgh, Edinburgh, UK;* [4]*University College London, London, UK*

Cutaneous melanoma is the most lethal type of skin cancer, with ~80,000 newly diagnosed melanoma and ~10,000 melanoma-associated deaths per year in the US. Thus, there is a need for improved understanding of the molecular pathogenesis and more effective targeted therapies for this devastating disease. The recent work of The Cancer Genome Atlas Network has defined melanoma as an RTK/RAS-driven solid tumor that can be classified into four genomic subtypes: *BRAF*-mutant, *RAS*-mutant, *NF1*-mutant, and triple-wild-type. This landmark study highlighted the important role of the previously understudied *NF1* tumor suppressor in melanoma pathogenesis, especially for the 9% of melanoma patients who have acquired inactivating *NF1*-mutations, but lack hotspot mutations that activate *BRAF* or *RAS*. To date, animal models have not been developed for the *NF1*-mutant subtype of melanoma, which has significantly impaired the development of novel therapeutic strategies for this subtype. Here we report the first zebrafish model for *NF1*-mutant melanoma, which we generated by combining the loss of *nf1* with loss of both *pten* and *p53*. The resultant compound mutant zebrafish develop aggressive melanomas from the age of 7 weeks and the tumor penetrance is 80% by the age of 18 weeks. We demonstrate further that these high-risk zebrafish melanomas were exclusive of hotspot mutations of *braf* and *nras*. Sustained inhibition of both MEK and PI3K suppressed tumor progression in vivo, whereas inhibition of MEK or PI3K alone was insufficient to suppress the growth of these tumors. Surprisingly, single agent therapy with rapamycin, an MTOR inhibitor, proved even better for short- and long-term suppression of tumor cell growth in *nf1/pten*-mutant melanomas. Thus our model appears ideal for the testing of drugs that will prove uniquely active for the significant subset of *NF1*-mutant, *BRAF/NRAS*-wildtype human melanomas.

CS.20.01 | The common ATM Ser49Cys variant is functionally defective for DNA damage response signalling

Caroline Atkinson[1]; Aideen McInerney-Leo[2]; Martina Proctor[1]; Catherine Lanagan[1]; Alexander Stevenson[1]; Stephen Ainger[3]; Richard A. Sturm[3]; Emma Duncan[2]; Brian Gabrielli[1]

[1]*Mater Research Institute- The University of Queensland, Brisbane, QLD, Australia;* [2]*Queensland University of Technology, Brisbane, QLD, Australia;* [3]*The University of Queensland Diamantina Institute, Brisbane, QLD, Australia*

Cell cycle checkpoints are responsible for co-ordinating cell cycle and repair responses to such DNA damage. An apical component of the cell cycle checkpoint and DNA damage repair response is the ataxia telangiectasia mutated (ATM) protein. ATM signals DNA damage checkpoint arrest and repair of double strand breaks by activation of downstream substrates such as CHK2 and p53. Mutations in ATM result in the human genetic disorder ataxia telangiectasia which is characterised by genomic instability, radiation sensitivity and cancer predisposition. The majority of cancer associated ATM mutations have produced truncated proteins and complete loss of function. A variant of ATM present in 1% of the European population is ATM Ser49Cys, and this variant has been associated with a five-fold increased risk of melanoma development. However, the functional consequence of the ATM Ser49Cys alteration is yet to be defined. Here, patient-derived lymphoblastoid and melanoblast lines containing the ATM Ser49Cys variant have been assessed for their ATM signalling following treatment with ionizing or UV radiation. We demonstrate that this ATM variant is functionally defective for only its p53 signalling response, and this is a direct consequence of the Ser49Cys change. We also present evidence that this defect also contributes to defective response to oncogene induced senescence, and this may be a basis of its preferential association with melanoma. We have previously identified a novel mechanisms that is responsible for defective ATM response in a high proportion of melanomas. Together, these data indicate that more subtle defects in ATM-dependent responses to DNA damage and stress than complete loss of function,

are significant contributors to melanoma development. These defects may also be a potential target to selectively destroy melanomas and possibly other cancers with similar defects, and synthetic lethality screening has identified a number of classes of genes that represent useful targets.

CS.20.02 | Overall survival with nivolumab (NIVO) and ipilimumab ((IPI) combination therapy in a phase III trial of advanced melanoma (CheckMate 067)

James Larkin[1]; Vanna Chiarion-Sileni[2]; Rene Gonzalez[3]; Piotr Rutkowski[4]; Jean-Jacques Grob[5]; C. Lance Cowey[6]; Christopher D. Lao[7]; Dirk Schadendorf[8]; Pier Francesco Ferrucci[9]; Michael Smylie[10]; Reinhard Dummer[11]; Andrew Hill[12]; John Haanen[13]; Michele Maio[14]; Grant McArthur[15]; Dana Walker[16]; Linda Rollin[16]; Christine Horak[16]; F. Stephen Hodi[17]; Jedd D. Wolchok[18]

[1]Royal Marsden Hospital, London, UK; [2]Oncology Institute of Veneto IRCCS, Padua, Italy; [3]University of Colorado Cancer Center, Denver, CO, USA; [4]Maria Sklodowska-Curie Memorial Cancer Center & Institute of Oncology, Warsaw, Poland; [5]Hospital de la Timone, Marseille, France; [6]Texas Oncology-Baylor Charles A. Sammons Cancer Center, Dallas, TX, USA; [7]University of Michigan, Ann Arbor, MI, USA; [8]Department of Dermatology, University of Essen, Essen, Germany; [9]European Institute of Oncology, Milan, Italy; [10]Cross Cancer Institute, Edmonton, AB, Canada; [11]Universitäts Spital, Zurich, Switzerland; [12]Tasman Oncology Research, Southport, QLD, Australia; [13]Netherlands Cancer Institute, Amsterdam, Netherlands; [14]University Hospital of Siena, Siena, Italy; [15]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; [16]Bristol-Myers Squibb, Princeton, NJ, USA; [17]Dana-Farber Cancer Institute, Boston, MA, USA; [18]Memorial Sloan-Kettering Cancer Center and Weill Cornell Medical College, New York, NY, USA

Background: NIVO plus IPI improved progression-free survival (PFS) and objective response rate vs IPI alone in the phase II CheckMate 069 and phase III CheckMate 067 trials of advanced melanoma. Here, we report overall survival (OS) results from CheckMate 067 (NCT01844505).

Methods: Treatment-naïve patients (pts) (N=945) were randomized 1:1:1 to NIVO 1 mg/kg + IPI 3 mg/kg Q3W x 4 followed by NIVO 3 mg/kg Q2W, NIVO 3 mg/kg Q2W + placebo, or IPI 3 mg/kg Q3W x 4 + placebo, until progression or unacceptable toxicity. Pts were stratified by PD-L1 status (<5% vs ≥5%), *BRAF* status, and M-stage. Co-primary endpoints were PFS and OS in the NIVO-containing arms vs IPI.

Results: At a minimum follow-up of 28 months, median OS has not been reached in the NIVO+IPI or NIVO groups, and was 20.0 months for IPI (hazard ratio [HR]: NIVO+IPI vs IPI, 0.55; NIVO vs IPI, 0.63; P<0.0001). In descriptive analyses, the relative risk of death in the NIVO+IPI group was reduced by 12% vs NIVO (HR=0.88); 2-year OS rates were 64%, 59%, and 45% for NIVO+IPI, NIVO, and IPI, respectively. Consistent OS results favoring NIVO+IPI over NIVO were observed across subgroups, including M1c and LDH.

In pts with tumor PD-L1 expression ≥5%, median OS appeared comparable between NIVO+IPI and NIVO. Median duration of response has not been reached with NIVO+IPI and was 31.1 months for NIVO and 18.2 months for IPI. The safety profile remained similar to the initial report, with grade 3/4 treatment-related AEs in the NIVO+IPI, NIVO, and IPI groups of 58%, 21%, and 28%, respectively.

Conclusions: Both NIVO+IPI and NIVO significantly improved OS vs IPI alone. In descriptive analyses, NIVO+IPI appeared to provide favorable survival outcomes over NIVO alone.

This study was funded by Bristol-Myers Squibb. Medical writing assistance was provided by StemScientific.

CS.20.05 | T cell receptors help define the cytokine pattern and responsiveness of host CD8 T cells

Chris Ankney[1,3]; Jonathan M. Eby[1]; Steven W. Henning[1]; Daniel Dilling[2]; Michael I. Nishimura[3,4]; I. C. Le Poole[3,5]; Timothy P. Riley[6]; Brian M. Baker[6]; Chrystal Paulos[7]

[1]Loyola University Chicago, Maywood, IL, USA; [2]Department of Medicine, Loyola University Chicago, Maywood, IL, USA; [3]Oncology Research Institute, Maywood, IL, USA; [4]Department of Surgery, Loyola University Chicago, Maywood, IL, USA; [5]Department of Pathology, Loyola University Chicago, Maywood, IL, USA; [6]Department of Chemistry and Biochemistry and the Harper Cancer Research Institute, University of Notre Dame, South Bend, IN, USA; [7]Department of Surgery, Medical University of South Carolina, Charleston, SC, USA

Adoptive transfer of T cell receptor transgenic T cells is an approach currently in clinical trials for melanoma treatment. We compared the properties of T cell receptors reactive with a gp100-derivative peptide to identify a candidate with optimal reactivity. Receptors T4H2, R6C12 from peripheral blood of peptide-vaccinated melanoma patients and SILv44 from vitiligo patient skin (SILv44) were sub cloned into retro-and lentiviral vectors, separating both TCR subunits by a viral slippage sequence. TCRs were introduced into human CD8+ T cells. Transgenic T cells were combined 2:1 with HLA-A2+ melanoma cells or T2 cells pulsed at 5 μM and 5 nM peptide. Responses were measured by intracellular cytokine staining of IFN-γ and IL-17A. Expression of CD107a served as a measure of cytotoxicity, and CCR6 was evaluated to confirm a true Tc17 phenotype. TCR transduced T cells (2x10^6, applied twice) were used to treat immunodeficient mice challenged with 888A2 human melanoma cells. Transgenic T cells displayed similar T cell receptor expression and IFN-γ in response to gp100+ target cells, but SILv44+ T cells expressed 1.73 fold greater IL-17A levels. Minimal expression by PMA/ionomycin stimulated cells indicates IL-17A release is TCR dependent. Ninety-six % of IL-17A+, transgenic T cells co-express CCR6, further supporting a Tc17 phenotype, yet IL-17 secreting cells largely (70%) co-expressed IFN-γ suggesting that this is a plastic population of T cells. IL17+ SILv44 transgenic T cells also displayed greater CD107a expression toward gp100+ targets (MFI of 764) compared to T4H2+ (688) or R6C12+ (509) T cells, correlating with superior ability of SILv44+ T cells to contain tumors in mice. Superior performance of SILv44 transgenic T cells could not be deduced from their IFN-γ secretion pattern. These findings have important implications for the selection of clinically relevant T cell receptors for use in melanoma immunotherapy.

CS.21.01 | Mouse models of pigmentation

Ian J. Jackson

MRC Human Genetics Unit, Edinburgh, UK

We have analysed many spontaneous and induced mutant and transgenic mouse models for pigmentation genes over several decades. Notably we defined the genes mutated in the *brown* and *slaty* mutants as the tyrosinase-related genes *Tyrp1* and *Dct*. We have produced mouse models of normal and red-hair associated human *MC1R*. We generated the widely used *Dct:lacZ* mouse line which identifies melanoblasts, melanocytes and melanocyte stem cells. This enabled us to show that mutants in the KIT receptor tyrosinase kinase (the classic "dominant white spotting" mutations) were not defective in melanoblast migration but in proliferation and survival. More recently we have used fluorescent reporter to track migrating melanoblasts in live cultures of embryonic skin from which we derived a mathematical model of generation of the melanocyte population. We measured migration of *Kit* mutant melanocytes and demonstrated again that migration was unaffected. Our model was able to replicate the white spot seen in *Kit* mutant mice by reducing proliferation whilst maintaining migration.

CS.21.02 | Critical role of WNT signaling in follicular melanocyte stem cells in adult skin

Qi Sun; Mayumi Ito

NYU School of Medicine, New York, NY, USA

Melanocyte stem cells (McSCs) reside in the hair follicle bulge/secondary hair germ niche where they are essential for hair pigmentation and have the potential to also regulate epidermal pigmentation. A better understanding of the molecular mechanisms that govern these stem cells holds broad implications in pigmentation disorders including hair graying, vitiligo and melanoma. We show that Wnt signaling is temporarily activated in McSCs at the onset of hair follicle regeneration and is necessary for their differentiation. Nonetheless, lineage tracing of Wnt-active differentiated McSCs demonstrate that McSCs can revert back to undifferentiated McSCs following withdrawal of Wnt signal activation. This suggests that McSC differentiation driven by Wnt signaling can be reversible, and temporal Wnt activation in McSCs does not deprive their self-renewing capacity. This ability of McSCs to oscillate between the differentiated and undifferentiated/stem cell states is not prevented when they differentiate into mature melanocytes after UVB irradiation. In aged mice, this process is compromised due to the failure to cease Wnt signaling, leading to ectopic McSC differentiation and a failure to return to their undifferentiated state, ultimately resulting in their loss. Upon induction of melanoma forming mutations, McSCs exhibit the potential to form melanoma. The tumorigenic potential of McSCs is regulated by Wnt signaling. Our results show the critical function of Wnt signaling in governing behavior of McSCs in adult skin during normal tissue homeostasis and melanoma.

CS.21.03 | The occurrence of lentigines and hair graying in one disorder with aberrant differentiation as the pathological mechanism

Karoline Lambert; Adam Almeida; Nate Goldstein; Stanca Birlea; Nabanita Murjherjee; Maranke Koster; Kristin Artinger; Kristin Artinger; Theresa Pacheco; Yiqun Shellman

University of Colorado AMC, Aurora, CO, USA

Lentigines are a common form of hyperpigmentation in human skin. Mutations activating the RAS/RAF/MAPK pathway are common in some familial lentigines syndromes, and hyper-proliferation has been proposed as a potential pathological mechanism. We have identified a heterozygous missense substitution (S519N) within the poorly understood SASH1 gene that is causative for a unique inherited lentigines disorder. To define the mechanisms of pigment cell abnormalities in this disorder, we have further examined the histological and clinical phenotypes, tested the effects of the variant and knockdown of SASH1 with *in vitro* studies, and have developed a patient-specific induced-pluripotent stem cell (iPSC) model. Histologically, skin biopsies from affected individuals showed increased numbers of melanocytes; however, these biopsies displayed no obvious increases in proliferating melanocytes. *In vitro*, transfection and stable transduction experiments with melanocyte-derived cells showed that SASH1 did not alter MAPK signaling and the mutation in SASH1 did not increase proliferation or survival. Conversely, knockdown of SASH1 in melanocyte-lineage cells induced growth arrest and altered cell morphology in a manner consistent with more differentiated melanocytes. Further clinical examination identified that affected individuals displayed premature hair graying as young adults, and strongly supported that aberrant differentiation is at least part of the pathological mechanism. Lastly, we have successfully established multiple iPSC clones from two affected individuals and generated functional melanocytes from these clones. We are using this model to examine the molecular mechanism further. In summary, we report a unique human pigmentation disorder with both skin and hair pigmentation abnormalities; our data suggest aberrant differentiation of precursor melanocytes as the potential etiology. Thus, our results indicated that SASH1-mediated lentigines may represent a new type of hyperpigmentation disorders. Future research of SASH1 could lead to new insights into melanocyte differentiation and the etiology of pigmentation disorders.

CS.21.04 | MITF and hair graying; a direct link to innate immunity

Melissa L. Harris[1]; Temesgen Fufa[2]; Sandeep Joshi[3]; Denise Larson[2]; Arturo Incao[2]; Derek Gildea[2]; Niraj Trivedi[2]; Joseph Palmer[1]; Tom Hornyak[3]; William Pavan[2]

[1]*Department of Biology, University of Alabama, Birmingham, Birmingham, AL, USA;* [2]*National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA;* [3]*Department of Biochemistry and Molecular Biology, University of Maryland, School of Medicine, Baltimore, MD, USA*

Melanocyte stem cells (McSCs) and mouse models of hair graying serve as useful tools to uncover mechanisms involved stem cell self-renewal and the maintenance of regenerative tissues. Interested in assessing genetic variants of hair graying, we identified *Mitf*$^{mi\text{-}vga9/+}$ as a mediator of premature McSC differentiation. Based on transcriptome and molecular analysis we report a novel role for MITF in the regulation of innate immune gene expression. We also demonstrate that viral mimic (poly I:C) is sufficient to expose genetic susceptibility to hair graying. These observations point to a critical, intrinsic suppressor of innate immunity within melanocytes and the consequences of its dysregulation, both of which may have particular implications for the autoimmune, hypopigmentation disorder, vitiligo.

CS.21.05 | Epilation induces hair and skin hyperpigmentation by upregulating endogenous EDN3 expression in mice

Huirong Li; Lilv Fan; Shanpu Zhu; Ling Hou

Laboratory of Developmental Cell Biology and Disease, Center for Vision Science Research, Wenzhou Medical University, Wenzhou, China

Mammalian melanocytes determine the pigmentation patterns of skin and hair and protect the organism from UV radiation, one of the risk factors for cutaneous cancers such as melanoma. During development, they are generated from neural crest-derived precursors (melanoblasts) that migrate along characteristic pathways to various destinations including hair follicles and epidermis or dermis. The precursors also migrate into the bulge region of developing hair follicles where they persist as self-renewing melanocyte stem cells (McSCs) and regenerate melanocytes during the adult physiological hair cycle. In addition, in response to various types of injury, such as excisional wounding or UVB irradiation, McSCs can also regenerate mature melanocytes for hair and skin pigmentation. However, how McSCs respond to injury remains largely unknown. Here we show that after epilation of mice, McSCs regenerate follicular and epidermal melanocytes, resulting in skin and hair hyperpigmentation. We further show that epilation leads to endogenous EDN3 upregulation in the dermal papilla, the secondary hair germ cells, and the epidermis. Genetic and pharmacological disruption of G-protein coupled receptor EDNRB in vivo significantly blocks the effect of epilation on follicular and epidermal melanocyte regeneration as well as skin and hair hyperpigmentation. Taken together, these results indicate that epilation induces McSCs activation through EDN3/EDNRB signaling and in turn leads to skin and hair hyperpigmentation. The findings suggest that EDN/EDNRB signaling may serve as a potential therapeutic target to promote repigmentation in hypopigmentation disorders.

CS.21.06 | Rat coat color mutations: Their introduction and availability from the national bio resource project for the rat

Takashi Kuramoto

Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Kyoto, Japan

The rat may be the first animal to have been domesticated mainly for scientific purposes. To date, various coat color mutations have been identified, including classical mutations such as albino (*c*), pink-eyed dilution (*p*), non-agouti (*a*), and piebald. The piebald mutation is usually called "hooded (*h*)". Hooded rats have a unique pattern in which the entire ventral surface is white, whereas dorsal pigmentation is limited to the head and shoulders (the "hood") and a mid-dorsal stripe extending back to the tip of the tail. The hooded phenotype is caused by insertion of an endogenous retrovirus element into the intragenic region of the *Kit* gene. To extend rat genetic resources, I introduced coat color mutations from fancy rats into the laboratory, including mink, grey, Pearl, and Downunder. The genes responsible have been mapped to particular regions of chromosomes, but have not been identified at the molecular level. Most rat coat color mutations have been deposited in the National BioResource Project for the Rat (NBRP-Rat) in Japan, the largest rat resource center in the world, and are available from the project. We anticipate that rat coat color mutations will contribute to pigment cell research.

CS.22.05 | Ashy dermatosis, long term follow-up and quality of life data

Marcel Bekkenk; Myrna Bakker; Marieke Berghuis; Albert Wolkerstorfer

Academic Medical Center, Amsterdam, Netherlands

Background: Ashy dermatosis or erythema dyschromicum perstans (EDP) is an uncommon skin disorder characterized by asymptomatic ashy-gray maculae. Opinions vary whether EDP is a variant of lichen planus (lichen planus pigmentosus, LPP) or a distinct entity. The course is often described as chronic and the disease leaves permanent discoloration. However, the exact course and the impact of EDP on Health Related Quality of Life (HRQL) are unknown.

Methods: A retrospective, questionnaire based analysis including HRQL (Skindex-29) of patients with EDP/LPP was performed. All patients in our clinic with EDP/LPP from 1994 to 2013 with available baseline photo's and histology were re-evaluated. Apart from the data

from the questionnaire an independent dermatologist compared the clinical appearance (when still present) of the skin with the appearance at diagnosis.

Results: A total of 38 patients were included in this study. Morphological changes were observed in 70.9% of patients. differences in color, size and/or number of lesions were observed in 70.9% of patients. In 58,1% EDP was considered stable, 32,2% improved and 9.7% deteriorated. In 4 patients EDP completely disappeared. Based on prognosis, groups were too small to retrospectively identify subgroups or prognostic parameters.

The mean scale scores for symptoms (26.0), emotions (43.6) and functioning (22.7) indicate no, severe and mild influence on HRQL respectively. The emotional domain scored significantly higher compared to 'symptoms' ($p<0.01$) and 'functioning' ($p<0.01$).

Conclusion: EDP/LPP is a heterogeneous disorder with high variation in clinical presentation. Although in most patients morphological changes in clinical presentation were observed over time, the overall representation of the disorder in majority of patients remained stable. No specific subgroups or parameters could be identified. EDP/LPP has an adverse impact on HRQL, with the greatest impact on the emotional domain.

CS.23.01 | Strategies for the prevention or treatment of hyperpigmentary disorders

Hideya Ando[1]; Satoshi Yoshimoto[1]; Moemi Yoshida[1]; Yoshiaki Ohagi[1]; Hiroki Yanagi[1]; Masamitsu Ichihashi[2]

[1]*Department of Applied Chemistry and Biotechnology, Okayama University of Science, Okayama, Japan;* [2]*SAISEI MIRAI Clinic, Kobe, Japan*

Excess production of melanin and/or its abnormal distribution can cause irregular hyperpigmentation of the skin, leading to various hyperpigmentary disorders such as melasma and age spots. To date, various skin lightening agents that prevent or improve hyperpigmentary disorders have been developed. Many of these inhibit the activity of tyrosinase, an enzyme required for melanin synthesis, for example, by competitive or non-competitive inhibition of its catalytic activity, by inhibiting its maturation or by accelerating its degradation. It has also been reported that several paracrine factors derived from keratinocytes and from fibroblasts regulate melanin synthesis in melanocytes. Regarding a different aspect from the regulation of melanogenesis, the transfer of melanosomes from melanocytes to keratinocytes or fibroblasts is also potentially involved in the appearance of hyperpigmentary disorders. We recently found that dermal fibroblasts actively internalize melanosomes released from melanocytes. In fact, some reports have demonstrated that fibroblasts containing melanosome aggregates exist in the dermal area of melasma and age spots. The inhibition of melanosome incorporation by dermal fibroblasts and/or the removal of melanosomes from the dermis would be effective strategies for the prevention or treatment of hyperpigmentary disorders.

CS.23.02 | How to understand melasma for the effective treatment

Kyoungchan Park; Hyeryung Choi; Jungwon Shin

Bundang Seoul National University Hospital, Seongnam, Korea

Melasma is a common acquired hypermelanosis that affects sun exposed areas of the skin with frequent facial involvement. Its histologic manifestations are evident in the epidermis, extracellular matrix, and dermis. In addition to epidermal pigmentation, pathologic findings of melasma include extracellular matrix abnormality especially solar elastosis. The disrupted basement membrane has been described with variable incidences. In the dermis, an increase in vascularity and an increase in the number of mast cells were observed, indicating that dermal factors have critical roles in the pathogenesis of melasma, despite the fact that melasma is characterized by epidermal pigmentation. In addition, melanogenesis occurs through multistep mechanisms in melanosomes. For the effective treatment of melasma, these complex mechanisms need to be controlled. I'd like to discuss histologic characteristics of melasma with consideration to their implication for melasma treatment. In addition, complex signaling mechanism will be discussed to reduce melanogenesis in melanosomes. By combining these approaches, ideal treatment of melasma will be possible (This study was supported by a grant from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea, grant number: HN14C0094).

CS.23.04 | Similarities and differences in gene expression between various facial hyperpigmented spots

Tomohiro Hakozaki[1]; Steven Zhao[1]; Bradley Jarrold[1]; Timothy Laughlin[1]; Jay Tiesman[1]; Debora Whittenbarger[2]; Beth Jewellmotz[2]; James Nordlund[3]

[1]*The Procter & Gamble Company, Mason, OH, USA;* [2]*The Procter & Gamble Company, Cincinnati, OH, USA;* [3]*Professor Emeritus, University of Cincinnati College of Medicine, Cincinnati, OH, USA*

Facial hyperpigmented spots are common issues across all ethnicities. There are several different types of hyperpigmented lesions defined by appearance, age of onset or histological features. However, currently their similarities or differences at molecular level have not been fully elucidated. To develop a comprehensive molecular understanding of facial spot types, we undertook a groundbreaking study to compare gene expression profiles across 5 different facial spot types.

77 Northern Asian females of varying decent (Chinese, Korean, Taiwanese, Japanese) diagnosed to possess solar lentigo (SL), freckles, melasma, pigmented seborrheic keratosis (SK) and/or post-inflammatory hyperpigmentation (PIH) including resolving acne, were enrolled in the study (age range 20-70). Following facial imaging, 2 mm full thickness biopsies were taken from 1) spot areas around cheek;

2) adjacent homogeneous skin tone non-spot area as sun-exposed control; 3) behind the ear as non-sun-exposed control. Biopsies were processed for histology and laser capture microdissection (LCM) fractionation into basal epidermis, suprabasal epidermis and dermis compartments. Spot types were confirmed through histological examination by dermatopathologists yielding SL=37, melasma=12, freckles=12, SK=23 and PIH=27. Total RNA was extracted from each LCM compartment and transcriptomics profiling was conducted using Affymetrix HG-U219 gene arrays.

Bioinformatics cluster analysis revealed significant differences in gene expression between different spot types in all compartments. Theme analysis unveiled unique biological themes by spot types as well as interesting commonalities. For instance, pigmentation was up-regulated at basal layer across all spot types except SK as expected, while we also observed upregulation of energy production and cholesterol storage themes in suprabasal layer for all spot types, suggesting common alterations in keratinocyte function in spot areas. These results indicate a wide range of processes related to skin function are affected in spot areas, thus more in depth understanding of these commonalities and differences are important for effective treatments.

CS.23.05 | Protein nanocages for cutaneous drug delivery

Sathya Moorthy Bhaskar; Sierin Lim

Nanyang Technological University, Singapore, Singapore

The skin protects the body from UV-induced DNA damage by the sun exposure through the pigment, melanin produced by the melanocytes. This pigment is sometimes over-expressed leading to pigmentation disorders such as melasma. Current treatment involves using tyrosinase inhibitors and lasers, leads to complications such as depigmentation, irritation, and dermatitis, with only 50% patient response. This is mainly due the inability of the delivery system to penetrate the stratum corneum layer of the skin and its non-specificity to the melanocytes. This project is aimed at engineering E2 protein nanocage for enhanced penetration into the stratum corneum layer of the epidermis and targeting/penetrating the melanocytes for the delivery of therapeutics. Genetic fusion of SPACE (Skin Penetrating And Cell Entering) peptide to the E2 nanocage helps its transduction through the stratum corneum layer, *in vivo* and to the interior of the melanocytes *in vitro*. Further modification of the E2 protein cage with targeting ligands can facilitate its uptake in melanocytes through the corresponding cell membrane receptors. Multiple modifications could also be imparted to the E2 protein cages without affecting its self-assembly, thereby aiding both penetration and targeting functions for drug delivery. Successful delivery of the engineered protein cages can aid the formulation of novel protein-based drug releasing molecules to be applied to the skin, which can be biocompatible with efficient pharmacokinetics.

CS.23.06 | Arginase-2, a miR-1299 target, enhances pigmentation in melasma by reducing melanosome degradation via senescence-induced autophagy inhibition

Ai-Young Lee[1]; Nan-Hyung Kim[1]; Soo-Hyun Choi[1]; Tae-Ryong Lee[2]

[1]Department of Dermatology, Dongguk University School of Medicine, Goyang-si, Gyeonggi-do, Korea; [2]Bioscience Institute, AmorePacific Corporation R&D Center, Yongin, Gyeonggi-do, Korea

Expression profiles suggested a significant concomitant downregulation of miR-1299 and upregulation of arginase-2 (ARG2) in hyperpigmented skin of melasma patients. The inverse relationship between miR-1299 and ARG2 was identified in primary cultured human keratinocytes and melanocytes, denoting ARG2 as a target of miR-1299. Because of opposite regulation of tyrosinase and PMEL17 protein expression by miR-1299, the role of ARG2 in pigmentation was examined in keratinocytes with or without ARG2 overexpression cocultured with normal melanocytes. Results suggested a positive regulation of ARG2 in keratinocytes on tyrosinase and PMEL17 protein expression. However, ARG2 did not increase mRNA levels of tyrosinase or PMEL17 or melanosome transfer. On the other hand, ARG2 in keratinocytes reduced autophagy and stimulated senescence. Autophagy was also impaired in keratinocytes with late passages. Collectively, these results suggest that ARG2 in keratinocytes could reduce autophagy by stimulating cellular senescence, resulting in skin pigmentation via reduced degradation of transferred melanosomes.

CS.24.01 | Advances in clinical skin imaging of melanoma and pigmentary skin disorders using multiphoton microscopy

Mihaela Balu[1]; Griffin Lentsch[1]; Inga Saknite[1,2]; Joshua N. Williams[1]; Anand Ganesan[3]; Christopher B. Zachary[3]; Kristen M. Kelly[3]; Bruce J. Tromberg[1]

[1]Beckman Laser Institute/UC Irvine, Irvine, CA, USA; [2]Biophotonics Laboratory, Institute of Atomic Physics and Spectroscopy, University of Latvia, Riga, Latvia; [3]Department of Dermatology, University of California, Irvine, Irvine, CA, USA

Multiphoton microscopy (MPM) is a laser-scanning optical imaging technique that relies on nonlinear light-matter interactions such as two-photon excited fluorescence (TPEF) and second harmonic generation (SHG) to achieve 3D images of living tissues with label-free molecular contrast and sub-micron resolution. In skin, the main sources of fluorescence are reduced nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), keratin, melanin, and elastin fibers, whereas SHG is used to visualize collagen fibers in the dermis. The MPM technology has been recently advanced into clinical setting by the development of a compact, portable MPM tomograph (MPTflex, JenLab, Germany). We have been using this device in clinical studies for several applications to evaluate the potential of MPM to diagnose

and guide therapy for various skin conditions. This presentation will summarize our current advances in clinical research studies on *in-vivo* MPM imaging of pigmented skin lesions including melanoma as well as of pigmentary skin disorders, such as vitiligo. Our qualitative and quantitative study on imaging pigmented skin lesions (common, dysplastic nevi and melanoma) includes MPM images acquired from 40 patients prior to biopsy and demonstrates that MPM has the ability to identify histological features and distinguish melanoma from benign nevi. In the pilot study for vitiligo imaging, we sought to determine whether MPM was capable to map the route of migrating melanocytes along the hair follicles and to visualize vitiligo re-pigmentation. We imaged migration of melanocytes from the hair to the epidermis in 8 patients undergoing micrograft transplantation and narrow band UVB light therapy.

These promising results, along with further technological developments to allow fast imaging of larger tissue areas, can set the ground for expanded studies to evaluate the MPM as an aid for improving clinical skin diagnosis, guiding therapy and understanding treatment effects.

CS.24.02 | The role of clinicians, consumers and artificial intelligence in the diagnosis of skin lesions

H. Peter Soyer

Dermatology Research Centre, Diamantina Institute, Faculty of Medicine, The University of Queensland, Brisbane, QLD, Australia; Department of Dermatology, Princess Alexandra Hospital, Brisbane, QLD, Australia

Skin cancer is common and costly in Australia. Even small improvements in melanoma detection could make a large difference to patients' health outcomes and the health care system. Mobile dermatoscopes with Apps allow people to take clear pictures of spots or moles they are concerned about, and send to a doctor. Up until recently melanoma detection Apps marketed directly to people concerned about skin cancer had low sensitivity, but exciting advances in the field of image recognition have made the prospect of a highly sensitive automatic analyses system a reality in the near future. We are planning to test the accuracy of mobile dermoscopy with and without automatic algorithms (artificial intelligence) in a clinically relevant setting with people at high risk of melanoma.

Findings from this study will be used to determine what role artificial intelligence algorithms should play in melanoma detection, and if appropriate, will be used to further plan for their implementation in clinical practice. We propose these technologies could empower consumers to be more involved in their health care, and this form of self-monitoring could complement annual 3D imaging in a targeted, high risk screening and surveillance program. By bringing together these sophisticated technologies, we anticipate significant advances in personalised screening for the early detection of melanoma. Consumer engagement will play a relevant role for better outcomes in health policy, will guide health services research and will be integral embedding melanoma control into the wider community.

CS.24.04 | Continued evaluation of a 31-gene expression profile to predict metastasis in an expanded cohort of 782 cutaneous melanoma patients

Robert W. Cook; Kyle R. Covington; Federico A. Monzon

Castle Biosciences, Inc., Friendswood, TX, USA

Background: We have previously described a validated 31-gene expression profile (GEP) that predicts metastatic risk in a cohort of cutaneous melanoma (CM) cases, providing a binary outcome of Class 1 (low risk of metastasis) or Class 2 (high risk). Here we evaluate the prognostic capabilities of the GEP independently and in combination with sentinel lymph node (SLN) status in a cumulative cohort of CM patients.

Methods: 782 primary tumors were analyzed as part of an IRB-approved archival tissue study. qPCR analysis assessed expression of the 31-gene signature and predictive modeling was performed to classify tumors as either Class 1 or Class 2. In addition to binary Class, predetermined cut-points of normal (1A) and reduced (1B) confidence Class 1, and reduced (2A) and normal (2B) confidence Class 2 groups were evaluated. Recurrence-free (RFS), distant metastasis-free (DMFS), overall (OS), and melanoma-specific survival (MSS) were assessed using Kaplan-Meier analysis and Cox regression.

Results: Kaplan-Meier analysis showed significant separation of low vs. high risk groups for all endpoints using SLN status and GEP Class ($p<0.0001$). Cox multivariate regression indicated that Breslow thickness, SLN status and GEP Class were significant predictors of RFS, DMFS, OS and MSS risk ($p<0.05$ for all). In the SLN-negative group, 59 of 82 (72%) cases with a recurrence, 40 of 56 (71%) with distant metastases, 42 of 67 (63%) deaths, and 12 of 15 (80%) deaths due to melanoma were called high-risk Class 2. Accuracy of distant metastasis prediction by GEP showed 76% sensitivity, 67% specificity, 39% positive and 91% negative predictive values compared to 63%, 70%, 47% and 82%, respectively, for SLN.

Conclusions: Data from this expanded cohort indicate that the 31-GEP test for prognosis can enhance clinical risk assessment beyond current methods of prognostication. Results achieved in this multicenter study are consistent with previous reports of GEP performance.

CS.24.05 | Development of a bright-field RNA in situ hybridization assay for diagnosis of atypical melanocytic nevi and malignant melanoma

Xiao-Jun Ma[1]; Catharine L. Kauffman[2]; David Stout[2]; Xingyong Wu[1]; Yuling Luo[1]; Paul Shitabata[3]

[1]*Advanced Cell Diagnostics Inc., A Bio-Techne Brand, Newark, CA, USA;* [2]*Georgetown Dermpath, Washington CA, DC, USA;* [3]*Dermatopathology Institute, Torrance, CA, USA*

Accurately distinguishing benign melanocytic lesions from malignant melanoma remains one of the most difficult challenges in diagnostic

pathology. In an effort to address this unmet clinical need, we developed a bright-field RNA in situ hybridization (ISH) assay to detect six RNA biomarkers for accurately classifying melanomas and nevi.

Based on microarray analysis and literature review, we selected and evaluated 23 genes in a training set of 149 samples (92 melanomas and 57 nevi) using the RNAscope ISH technology, which led to the development of a six-gene RNA ISH assay for distinguishing between melanomas and benign nevi. The final assay was evaluated in an independent cohort of 171 cases consisting of a broad spectrum of nevi (n=116) and melanomas (n=55), and it demonstrated 89% sensitivity (95% CI 78%-96%) and 92% specificity (95% CI 85%-97%).

This slide-based assay utilizes real-world diagnostic specimens of melanocytic lesions, and the results can be viewed under a standard light microscope without special equipment. It may serve as a powerful molecular adjunct in the diagnosis of early stage melanoma, especially in the setting of severely atypical nevi.

CS.24.06 | Baseline peripheral blood ratios are associated with microscopic metastases of cutaneous melanoma to the sentinel lymph node

Alyss V. Robinson[1]; Claire Keeble[1]; Maria Marples[1,2]; Howard Peach[1]; Donald Dewar[1]; Ryckie G. Wade[1,3]

[1]Faculty of Medicine and Health, Leeds, UK; [2]Leeds Cancer Centre, St James' University Hospital, Leeds, UK; [3]Department of Plastic and Reconstructive Surgery, Leeds General Infirmary, Leeds, UK

Background: In peripheral blood, the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and lymphocyte-monocyte ratio (LMR) change in response to malignancy. These biomarkers are associated with metastasis in a number of malignancies, but have not been assessed in early stage cutaneous melanoma. We aimed to determine whether baseline NLR, PLR and LMR are associated with metastasis of cutaneous melanoma to the sentinel lymph node.

Methods: This cohort study describes a consecutive series of patients who underwent wide excision and sentinel lymph node biopsy (SLNB) for cutaneous melanoma over 10 years. Binary logistic regression was used to identify variables associated with sentinel lymph node involvement according to clinical features. Adjusted odds ratios (OR) with 95% confidence intervals (CI) are presented.

Results: One thousand two hundred and fifty-two SLNBs were performed, of which bloods were available for 569. Of these, 147 (25.8%) SLNBs contained metastatic melanoma. At baseline, 254 (44.6%) were AJCC stage I, 280 (49.1%) were stage II and 29 (5.1%) were stage III. Increasing Breslow thickness, mitotic rate and maximum diameter; presence of ulceration, angiolymphatic invasion and microsatellites; and absence of tumour infiltrating lymphocytes were all associated with a positive SLNB. An increased NLR was associated with age and regression in the primary tumour. After adjusting for these variables, a baseline NLR >1.9, PLR >180 and LMR <4.3 were significantly associated with microscopic metastasis of melanoma in the sentinel node (HR 1.82 [95% CI 1.05, 3.17], 1.79 [1.02, 3.15] and 1.79 [1.06, 2.99], p <0.05 for NLR, PLR and LMR respectively).

Conclusion: The adjusted baseline peripheral blood NLR, PLR and LMR appear to be associated with metastases of cutaneous melanoma to the sentinel lymph node. Further investigation is required to validate this association, and explore the use of these biomarkers as risk stratification tools in early disease.

CS.25.01 | SMAD signaling promotes melanoma metastasis independently of phenotype switching

Lukas Sommer

University of Zurich, Zurich, Switzerland

Malignant progression of melanoma is thought to require the dynamic shifting of neoplastic cells between proliferative and invasive phenotypes. Contrary to this conventional "phenotype switching" model, we now show that augmented SMAD-dependent signaling in melanoma leads to emergence of malignant cells simultaneously displaying proliferative and invasive properties. Specifically, conditional deletion of *Smad4*, which abrogates canonical SMAD signaling, prevented tumor initiation, proliferation and metastasis formation *in vivo*, pointing to the requirement of both pro-proliferative and pro-invasive TGF-b superfamily factors for metastatic melanoma formation. Ligand screening identified BMP7 to promote melanoma cell proliferation even in the presence of pro-invasive TGF-b factors. However, an invasive phenotype was induced in melanoma cells upon inactivation of the inhibitory SMAD factor SMAD7, surprisingly without counteracting melanoma cell proliferation. Consequently, conditional *Smad7* deletion *in vivo* sustained melanoma growth and at the same time promoted massive metastasis formation. Together with clinical data analysis, our findings indicate that modulation of SMAD7 levels triggers malignant transformation of melanoma by overcoming phenotype switching.

CS.25.03 | BRD9 (Bromodomain Containing Protein 9) Plays Roles in Melanogenesis and Melanoma Proliferation

Tupa Basuroy; Ivana L. delaSerna

The University of Toledo College of Medicine and Life Sciences, Toledo, OH, USA

Melanocytes are cells in the epidermis that produce the pigment melanin and protect skin against damage from ultraviolet radiation. Malignant melanoma, the most dangerous form of skin cancer, develops from the transformation of melanocytes. SWI/SNF chromatin remodeling complexes interact with master regulators of melanocyte differentiation and melanoma oncogenes to regulate the expression of genes important for melanogenesis and melanoma proliferation.

Heterogeneous SWI/SNF complexes that contain either BRG1 or BRM as the catalytic subunit and an assortment of associated factors (BAFs) have been identified. BRG1 and BRM as well as some BAFs have bromodomains (BrDs) which bind to acetylated lysine residues in histone tails. Little is known about the role of bromodomain proteins in regulating SWI/SNF function. Small molecules that specifically inhibit the association of these BrD-containing proteins with chromatin can be used as tools to interrogate BrD function and may have therapeutic potential. I-BRD9 is a chemical inhibitor specific for BRD9, a newly identified BrD-containing component of SWI/SNF complexes that have BRG1 as the catalytic subunit. We found that BRD9 is highly expressed in melanocytes and melanoma cell lines. Co-immunoprecipitation studies indicated that BRD9 and BRG1 physically interact in melanoma cells. To test the hypothesis that BRD9 has a function in melanogenesis and melanoma proliferation, we treated melanocytes with chemical inhibitors of BRD9. Chemical inhibition of BRD9 resulted in decreased melanin synthesis and expression of genes that regulate melanocyte function. Decreased expression of genes that regulate melanin synthesis was associated with altered chromatin structure at regulatory sites. Depletion of BRD9 by siRNA had similar effects on gene expression as chemical inhibition. Furthermore, inhibition of BRD9 compromised proliferation and colony survival. In combination, our data indicate that Brd9 has an important role in regulating melanogenesis and melanoma proliferation and that chemical inhibition may be useful for treating melasma and melanoma.

CS.25.04 | Lack of MITF affects morphology, proliferation and migration of human SKMEL28 melanoma cells

Remina Dilixiati; Ilse Gerritsen; Katrin Moller; Margret H. Ogmunsdottir; Eirikur Steingrimsson

Department of Biochemistry and Molecular Biology, Biomedical Center, Faculty of Medicine, University of Iceland, Reykjavik, Iceland

Microphthalmia-associated transcription factor (MITF) acts as a master regulator of melanocyte development and differentiation, and is known as a melanoma oncogene. A rheostat model has been proposed for MITF function, stating that the level of MITF expression dictates the phenotypes and behaviors of melanoma cells. According to this model, high levels of MITF favor proliferation and differentiation whereas low levels of MITF promote a stem cell-like phenotype. Through this MITF is a key regulator of cellular heterogeneity in melanoma. To further characterize the role of MITF in melanoma cells, we generated SKmel28 cells that lack MITF using the CRISPR technology and then compared cellular morphology, behavior and gene expression to its wild type counterpart. BrdU and MTT assays showed that the MITF CRISPR knock out cell lines have a reduced proliferation rate compared to the parental line. Flow cytometry and microscopy analysis revealed that MITF null cells are increased in size and appear more granular. The migration and invasion ability of MITF null cells was found to be significantly reduced as validated by transwell migration and invasion assays. By RNA sequencing, we found that differentially expressed genes overlap extensively with the gene signatures of $MITF^{high}$ and $MITF^{low}$ melanoma tumors reported in the Cancer Genome Atlas. GO term analysis showed that differentially expressed genes were associated with extracellular matrix, cell adhesion and pigmentation. Taken together, our results indicate that MITF null cells are less migratory and proliferative due to reduced expression of proliferation-associated genes and overexpression of stem cell marker genes. Currently, we are testing the metastatic potential of these cell lines *in vivo*, and we are looking for specific gene sets responsible for the observed phenotype.

CS.25.05 | MITF, TFEB and TFE3 in melanoma – regulation and interaction

Josue Ballesteros Alvarez[1]; Asgeir O. Arnthorsson[1]; Ludwig Karl[1]; Kimberley Anderson[1]; Katrin Moller[1]; Vivian Pogenberg[2]; Matthias Wilmanns[2]; Margret H. Ogmundsdottir[1]; Eririkur Steingrimsson[1]

[1]Department of Biochemistry and Molecular Biology, Biomedical Center, Faculty of Medicine, University of Iceland, Reykjavik, Iceland; [2]European Molecular Biology Laboratory, Hamburg Unit, Hamburg, Germany

The MITF, TFEB, TFE3 and TFEC (MiT-TFE) proteins belong to a larger family of basic helix-loop-helix leucine zipper transcription factors that are able to bind to E and M boxes. MITF is crucial for melanocyte development and has been called a lineage specific oncogene in melanoma whereas its relatives, TFEB and TFE3, are involved in the biogenesis and function of lysosomes and autophagosomes, regulating cellular clearance pathways.

We have investigated the interaction, cross-regulatory relationship and nuclear localization of MITF, TFE3 and TFEB in melanoma cells. Like MITF, the TFEB and TFE3 genes are expressed in melanoma cells and tissues. Using co-immunoprecipitation studies, we demonstrate that MITF, TFEB and TFE3 interact in melanoma cells forming heterodimers. However, they are not able to interact with other members of the bHLH family such as MAX. We have identified a three amino acid region that is responsible for dimerization specificity. Through mutagenesis, we are constructing a version of MITF that only forms homodimers and will determine its effects on gene expression. Reporter gene and ChIP assays show that the three factors are able to bind and activate expression of genes involved in autophagy, as well as in lysosomal and melanosomal biogenesis. Interestingly, some genes are exclusively regulated by one of the factors.

The relationship between MITF, TFEB and TFE3 is complex and involves regulation of gene expression, protein-protein interactions and complementary functions. It is important to unravel further this relationship in melanoma in order to better understand the cross-regulatory mechanisms. This requires the characterization of common and unique targets of these factors and their ability to form homo- and heterodimers.

CS.25.06 | Transcriptional co-activators YAP1 and TAZ have both shared and unique pathways driving melanoma

Jason W. Lui[1,2]; Kelsey Ogomori[2]; Adam Labadorf[1]; Deborah Lang[1]

[1]Boston University, Boston, MA, USA; [2]University of Chicago, Chicago, IL, USA

Melanoma is the most fatal of all skin cancers, mainly due to the high degree of metastasis. Transcriptional co-activators and paralogs YAP1 (Yes associated protein 1) and TAZ (WW domain-containing transcription regulator protein 1) are implicated in both metastasis and tumor growth. Due to their function in development and other cancer types, it is logical that these factors promote melanoma; however their role in this disease is mostly unknown. It is presumed that YAP1 and TAZ have redundant functions but findings from developmental biology studies runs counter to this assumption. We discovered that both YAP1 and TAZ are expressed in a panel of melanoma cell lines. Inhibition of YAP1 and/or TAZ in melanoma cell lines (A375 and mel537) lead to measurable but not overlapping changes in cellular growth, cell morphology, and gene expression. Non-biased RNA-sequencing analysis screens uncovered a pattern of overlapping YAP1 and TAZ targets, as well as genes specific for each factor. Meta-analysis of patient samples demonstrated that several of these genetic targets are overexpressed in melanoma and may be functional drivers of the disease. Our findings suggest that YAP1 and TAZ are not redundant factors in melanoma and drive cancer progression through both YAP1 and TAZ specific genes.

CS.25.07 | Map kinase pathway inhibitor responses and resistance mechanisms in melanomas with BRAF fusions

Jacqueline A. Turner; Kasey L. Couts; Judson Bemis; Stacey M. Bagby; Allison J. Applegate; Jennifer D. Hintzsche; Carol Amato; Kelsey Wuensch; Keith Wells; Theresa Medina; Yiqun G. Shellman; Matthew J. Rioth; John J. Tentler; Aik-Choon Tan; William A. Robinson

University of Colorado Denver School of Medicine, Aurora, CO, USA

Melanomas frequently have oncogenic driver mutations in genes such as *BRAF*, *NRAS*, *KIT*, *GNAQ*, *GNA11*, and *NF1*; however, a subset of melanomas lack mutations in these genes (pan-negative melanomas). Recently described *BRAF* kinase fusions are potential oncogenic drivers in a subset of pan-negative melanomas but the response of melanomas with *BRAF* fusions to targeted therapy has not been well characterized. In this study, we generated cell lines and patient-derived xenograft (PDX) models from two pan-negative melanoma tumors with activating *BRAF* fusions, *AGK-BRAF* and *ARMC10-BRAF*. We treated the cell lines and PDX models with two MAP kinase pathway inhibitors, trametinib (MEK1/2) and SCH772984 (ERK1/2). We demonstrate *AGK-BRAF* and *ARMC10-BRAF* are sensitive to the inhibitors *in vitro* and *in vivo*. Although *AGK-BRAF* and *ARMC10-BRAF* cells respond similarly *in vitro*, *AGK-BRAF* tumors demonstrated less response to inhibitors compared to *ARMC10-BRAF* tumors *in vivo*. We also treated the PDX tumors to resistance and observed that *AGK-BRAF* tumors quickly became resistant to trametinib, while *ARMC10-BRAF* tumors exhibited a durable response and took longer to become resistant. We performed total RNA sequencing of the sensitive and resistant tumors and found resistant *ARMC10-BRAF* tumors had reactivation of the MAP kinase pathway whereas resistant *AGK-BRAF* tumors had elevated levels of *IL-6*, *IL-1β*, *IL-1βR*, and other inflammation associated genes. This suggests that there are different mechanisms of resistance and that inflammation and the microenvironment may play an important role in treatment response and resistance in the *AGK-BRAF* fusion model. This is the first report of potential resistance mechanisms in *BRAF* fusion models. Altogether, these findings suggest that *BRAF* fusions are effective therapeutic targets in melanomas, but *in vivo* inhibitor responses and resistance mechanisms are variable and may depend on the microenvironment.

CS.26.03 | The pattern of birthmarks suggests an unknown population of melanoblasts

Veronica A. Kinsler[1,2]; Lionel Larue[3,4]

[1]University College London (UCL) Institute of Child Health, London, UK; [2]Paediatric Dermatology, Great Ormond St Hospital for Children, London, UK; [3]Institut Curie, Orsay, France; [4]Université Paris-Sud, Paris, France

Elegant and systematic laboratory work has mapped out two neural crest-derived pathways of melanocyte precursor migration, the dorsolateral pathway and the dorsoventral Schwann cell pathway. With these in mind, we reappraised the patterns of congenital pigmentary disorders in humans, and identified three recurrent patterns, which are consistent across genetically-different diseases. Importantly, only two of these are seen in diseases known to affect melanocytes directly, and are therefore cell-autonomous patterns. The third pattern, hypo- or hyper-pigmented fine and whorled Blaschko's lines, although recurrent, is proposed to be melanocyte non-cell-autonomous. The two melanocyte-cell-autonomous patterns we term segmental and non-segmental. While the segmental pattern correlates well with the two, known neural crest-derived migration patterns from animal studies, the melanocyte precursors responsible for the non-segmental pattern have not been identified previously. This novel population appears to originate around the time of gastrulation within the mesoderm, and migrates bilaterally and symmetrically at all levels. These new insights from congenital pigmentary disorders can lead to a better understanding of acquired pigmentary diseases such as vitiligo, and, potentially for melanoma.

CS.26.04 | Final CMN colour is significantly associated with normal skin pigmentation, not with immediate postnatal CMN colour - implications for early superficial removal

Satyamaanasa Polubothu[1,2]; Lara Al-Olabi[1]; Lea Solman-Koustic[2]; Anna Thomas[1]; Veronica Kinsler[1,2]

[1]University College London, London, UK; [2]Great Ormond Street Children's Hospital, London, UK

Congenital Melanocytic Naevi (CMN) can lighten spontaneously over time, sometimes markedly. Final colour is an important outcome after superficial removal techniques, however this is usually compared to photographs from the immediate post-natal period, and does not take into account spontaneous lightening. We sought to establish the natural colour history of CMN postnatally, and explore phenotypic or genotypic predictors of colour change.

112 patients with CMN (41 male) had a mean and median follow up of 5.3 and 4.2 years. Deep phenotyping was undertaken in all, *MC1R* genotyping in 53 children and *NRAS* genotyping from affected tissue in 72 children. 82% had multiple CMN at birth, and 68% were positive for *NRAS* codon 61 mutations (p.Q61K, Q61R or Q61H). Serial photographs taken in a professional hospital setting were analysed systematically by a single observer. Within a patient, the same areas of CMN and normal background skin were measured over time using Adobe Photoshop Elements, with averaging of multiple measurements of L*a*b* colour space values at each time point. Changes in CMN colour space values over time were modelled using multiple logistic regression, against phenotypic and genotypic variables.

Lightening of CMN was strongly significantly associated with background patient skin colour ($p<0.001$) and with germline genotype of *MC1R* variant alleles ($p<0.001$), implying that melanin production and therefore colour of CMN in childhood is related to the germline pigmentary phenotype. Importantly, no significant association between CMN final colour and the immediate postnatal colour (in the first 3 months of life), a pigmentary period known to be temporary and influenced by maternal hormonal levels. No relationship was found to *NRAS* genotype, or projected adult size of CMN. This study has important implications for advising patients on the likelihood of spontaneous lightening, and for the comparison of colour after superficial removal to post-natal photographs.

CS.26.05 | Dermoscopy of small and medium congenital melanocytic nevi in infants and children

Elodie Bou Assi; Bertille Bonniaud; Maxime Luu; Blandine Bel; Géraldine Jeudy; Pierre Vabres

CHU Dijon Bourgogne, Dijon, France

Background: Although dermoscopic features of congenital melanocytic nevi (CMN) have been described, few studies have addressed infants and children exclusively.

Objective: To correlate dermoscopic features of CMN in infants and children with age, shape and anatomical site.

Design: Retrospective observational study from collected digital photography and dermoscopy images of CMN during a 9-year period.

Setting: Outpatient dermatology clinic at a university hospital in France.

Participants: A total of 121 children totalizing 131 small and medium-size CMN (37 in infants and 94 in children under 15 years) were studied.

Main Outcomes and Measures: Frequency of dermoscopic structures and patterns with regard to age, sex, shape or anatomical site. Association was studied using logistic regression.

Results: Most CMN were of medium size (72.5%). Bolognia's sign was frequent (12.2%). Globules were the most frequent dermoscopic structure (82.4%), mainly in a diffuse distribution (64.1%). Likewise, a predominantly globular pattern was present in a majority (71.0%) of CMN. In reticular CMN (19.1%), we identified a specific trabecular subtype. Additional structures specific to CMN were observed: target network, target globules, hypertrichosis. Dermoscopic patterns varied mainly with age, since infants harbored quasi-exclusively globular nevi (94.3%), but also with anatomical location. CMN on the upper body were more frequently of the globular type (odds ratio (OR) = 3.95, 95% confidence interval (CI) 1.40 – 11.10, p =0,009). Reticular CMN were also predominantly found on dorsal regions whereas globular CMN predominated on ventral regions (OR = 5.83, 95% CI 1.90 – 17.84, p = 0,002). No association with projected adult size or shape was found.

Conclusions and Relevance: Pattern differences in ventral and dorsal locations may be related to different embryonic pathways of nevogenesis. Age differences in CMN patterns also suggest that they evolve with time

CS.27.02 | MITF regulates dynamic melanoma heterogeneity

Loredana Spoerri[1]; Crystal A. Tonnessen[1]; Kimberley A. Beaumont[2]; David S. Hill[2]; Sheena M. Daignault[1]; Farzana Ahmed[1]; Wolfgang Weninger[2]; Nikolas K. Haass[1]

[1]University of Queensland, Woolloongabba, QLD, Australia; [2]Centenary Institute, Sydney, NSW, Australia

Differential tumor cell behavior caused by environmental conditions, termed dynamic heterogeneity, is a prime source for drug resistance. As distinct proliferative and invasive capabilities reflect variable drug sensitivities, identifying and characterizing these different responses is crucial to design effective therapies. We utilize real-time cell cycle imaging (FUCCI) in 3D *in vitro* and *in vivo* to study melanoma heterogeneity.

Mouse xenograft tumors generated from cell lines with high microphthalmia-associated transcription factor (MITF) level displayed a homogeneous distribution of cycling cells throughout. In contrast, tumors generated from cell lines with low MITF levels were composed

of clusters of cycling cells and clusters of G1-arrested cells. The proliferating areas were in close proximity to blood vessels, presumably characterized by oxygen/nutrient availability. Indeed, knock-down of MITF in MITF-high melanoma cells resulted in the same clustered phenotype presented in xenografts generated from MITF-low melanoma cells. Melanoma spheroids recapitulated the *in vivo* cycling behavior, considering that here oxygen and nutrients are supplied by diffusion. MITF was undetectable within the hypoxic G1-arrested spheroid core, indicating hypoxia-induced MITF downregulation. Modulation of MITF expression impacted spheroid architecture and size, with overexpression giving rise to less compacted structures and *vice versa*. We show that MITF protects from cell cycle arrest induced by oxygen/nutrient deprivation. High MITF levels prevent cell cycle arrest by reducing the cell-intrinsic propensity to arrest in response to low oxygen/nutrient and concurrently by allowing sufficient supply of oxygen/nutrients to cells. The latter is achieved through decreased cell-cell adhesion resulting in the generation of looser, 'spongier' tumors that may allow more efficient oxygen/nutrient diffusion.

Taken together, MITF is a potent regulator of dynamic heterogeneity, which in turn impacts on drug sensitivity.

CS.27.04 | S897E-EphA2 drives an amoeboid melanoma phenotype that metastasizes to the brain

Inna Smalley; Chao Zhang; Ritin Sharma; Michael F. Emmons; Jane Messina; John M. Koomen; Keiran S. Smalley

H Lee Moffitt Cancer Center, Tampa, FL, USA

Acquired BRAF inhibitor resistance is often associated with the adoption of a poorly defined, aggressive, and invasive phenotype that can be driven through ligand-independent EphA2 signaling. In the current work, we use comprehensive mass spectrometry based proteomics to map the signaling associated with this phenotype. Preliminary analyses have identified an unexpected upregulation of pathways associated with a mesenchymal-to-amoeboid transition (MAT). Functional studies showed the adoption of the amoeboid phenotype to be associated with increased melanoma cell invasion that was mediated through an increased association between EphA2 and cdc42, an inhibition of Rac1 and the downregulation of NEDD9. The MAT phenotype could be recapitulated through introduction of activated cdc42 and through siRNA silencing of NEDD9. Analysis of isogenic pairs of BRAF mutant melanoma cell lines showed the adoption of an amoeboid phenotype to be frequently associated with acquired BRAF inhibitor resistance. We next performed intracardiac injections to determine the patterns of metastasis and found a preferential homing of the amoeboid cells to the brain, but not other organs (including the lung and liver). An analysis of brain metastasis specimens from patients failing BRAF and BRAF-MEK inhibitor therapy showed strong staining for the amoeboid phenotype marker EphA2. In summary, we have demonstrated for the first time that BRAF inhibitor resistance is associated with the adoption of an amoeboid phenotype that may increase the metastatic seeding of melanoma cells to the brain.

CS.27.05 | WISP1 stimulates melanoma cell invasion and tumor metastasis by promoting epithelial–mesenchymal transition (EMT)

Wentao Deng[1,2]; Audry Fernandez[2]; Sarah L. McLaughlin[2,3]; David J. Klinke II[1,2,4]

[1]Department of Microbiology, Immunology and Cell Biology, West Virginia University, Morgantown, WV, USA; [2]WVU Cancer Institute, West Virginia University, Morgantown, WV, USA; [3]Animal Models and Imaging Facility, West Virginia University, Morgantown, WV, USA; [4]Department of Chemical and Biomedical Engineering, West Virginia University, Morgantown, WV, USA

Human melanoma is the most dangerous skin cancer because of its propensity to metastasize. Melanoma cell invasion and metastasis is coordinated by soluble signals present within the tumor microenvironment. Among the signaling factors is WNT1 inducible signaling pathway protein 1 (WISP1), a secreted matricellular protein that is elevated in a variety of cancers. Previously, we observed that Wisp1 represses anti-tumor immunity in a B16 mouse melanoma *in vitro* model and is secreted in response to β-catenin–mediated transcription activation after the disruption of adherens junctions. A review of public-accessible databases and published research suggested that, in human melanoma patient samples, both WISP1 protein and mRNA are elevated, in addition, WISP1 expression is associated with tumor metastasis and EMT gene expression. In this work, we knocked out the Wisp1 gene in mouse melanoma B16 cells and found that Wisp1 disruption repressed wound healing, migration and invasion of the tumor cells. More experiments with mouse and human metastatic melanoma lines using conditional medium from mouse fibroblast NIH3T3 cells with Wisp1 knockout or over-expression further supported a functional scenario of Wisp1 to promote tumor cell invasion. Experimental metastasis assays using C57BL/6Ncrl and NOD-Scid Gamma (NSG) mice showed that the knockout of Wisp1 in B16F10 cells completely eliminate tumor lung and brain metastasis, suggesting an *in vivo* functional role of Wisp1 to promote tumor metastasis. Mechanistically, we found that B16 invaded cells in the invasion assays possess the classic gene expression characteristic for EMT including Snail activation and E-cadherin repression. With Wisp1 knockout, those EMT markers went to opposite directions and were rescued by either recombinant Wisp1 protein or conditional medium overexpressing Wisp1. All these results supported a model that Wisp1 in melanoma microenvironment stimulates tumor cell invasion and metastasis through EMT promotion.

CS.27.06 | The non-cell autonomous role of Edn3/Ednrb signaling during melanoma lung metastasis formation

Juliano Freitas; Martina Cavallini; Jesus Lopez; Joseph Palmer; Lidia Kos

Florida International University, Miami, FL, USA

Metastasis is the ultimate cause of death in 90% of patients with cancer. Melanoma is a highly metastatic cancer which preferentially establishes secondary lesions in the lungs. Human melanoma initiation is mainly driven by activation of oncogenic protein BRaf and deletion of PTEN gene and has recently been modeled in mouse cell lines. The cytokine Endothelin 3 (Edn3) and its receptor Endothelin receptor b (Ednrb) have been implicated in melanoma metastasis. In tumors like breast, ovarian and colon cancers Endothelin signaling is activated on immune cells within the tumor microenvironment. Yet, it is not known whether stromal cells in the melanoma microenvironment respond to Endothelin during tumor progression. In order to establish whether Endothelin plays a non-cell autonomous role during melanoma metastasis formation we injected 3 different $BRaf^{V600E/+}$;$PTEN^{-/-}$ murine melanoma cell lines (D4M, YUMM1.1 and YUMM1.7) into Edn3 over-expressing transgenic *(K5-Edn3)* and control mice. The populations of cells expressing Ednrb in the primary tumor as well metastatic cells in the lungs were monitored by immunofluorescence and flow cytometry. YUMM1.1-derived tumors in *K5-Edn3* mice were larger than those in control mice and metastasized to the lungs. Although none of the three cell lines express Ednrb *in vitro*, immune cells in the YUMM1.1-derived tumors in *K5-Edn3* mice expressed Ednrb suggesting they responded to the Edn3 present in the tumor microenvironment. These data suggest that Edn3/Ednrb signaling plays a non-cell autonomous role in melanoma progression and the metastatic potential of a $BRaf^{V600E/+}$;$PTEN^{-/-}$ murine melanoma cell line.

PS.07.01 | Melanocyte stem cells in eccrine sweat glands: A potential origin of acral melanoma

Emi K. Nishimura

Tokyo Medical and Dental University, Tokyo, Japan

Determination of the origin for early-stage cancer remains a challenging issue. Melanoma is an aggressive cancer of the melanocyte lineage. Early melanoma cells are often found in the epidermis around sweat ducts of human volar skin. However, the niche for melanoma precursors has not been determined yet. We previously identified melanocyte stem cells in hair follicles as a reservoir of melanocytes for hair follicle pigmentation as well as for skin pigmentation. We recently found that the secretory portion (SP) of eccrine sweat glands provide an anatomical niche for melanocyte-melanoma precursor cells, which also have melanocyte stem cell characteristics. We found that McSCs in sweat glands renew themselves in response to genomic stress, while those in hair follicles rather commit to differentiation under genomic stress. FISH analysis of human acral melanoma revealed that melanoblasts with significant CyclinD1 gene amplification reside in the SP of particular sweat gland(s). These findings suggest that the sweat gland niche facilitates self-renewal of melanocyte-melanoma precursors as the potential origin of human acral melanoma.

PS.08.03 | Translational research in vitiligo: Launching a new era of targeted treatments

John E. Harris

University of Massachusetts Medical School, Worcester, MA, USA

Vitiligo is an autoimmune disease that results in white spots on the skin. Decades of translational research has revealed that the disease is a result of melanocyte destruction by CD8+ T cells, and this is heavily influenced by genetic susceptibility of the patient. We found that autoimmunity in vitiligo is driven primarily by T cell-derived IFN- and its target chemokines, CXCL9 and CXCL10, which together create a positive feedback loop that maintains T cell recruitment and localization to the epidermis where melanocytes reside. Sampling of vitiligo patient skin through the induction of suction blisters identified CD8+ T cell number and chemokine protein as sensitive and specific biomarkers of disease activity and early treatment responses. Preclinical and clinical studies reveal that targeting this cytokine pathway results in both prevention and reversal of disease, highlighting a new, targeted treatment strategy for patients with vitiligo. Keratinocytes are the primary source of chemokines in both mice and humans with vitiligo, supporting topical treatments that target IFN- signaling, which has been confirmed in a small clinical study reporting the efficacy of topical ruxolitinib in facial vitiligo. This has led to the first multicenter clinical trial to test a targeted immunotherapeutic in vitiligo patients. Finally, additional pathways that synergize with IFN- to promote disease maintenance through autoimmune memory in the skin offer other avenues for targeted immunotherapy, providing a "diverse portfolio" of treatment options that create hope for tens of millions of patients who suffer from it.

PS.08.04 | Mechanism of action of 4-substituted phenols to induce vitiligo and their potential as anti-melanoma agents

Arthur Kammeyer[1]; Karina J. Willemsen[1]; Nico P. Smit[2]; Rosalie M. Luiten[1]

[1]Dermatology, AMC, University of Amsterdam, Amsterdam, Netherlands; [2]Clinical Chemistry, Leiden University Medical Center, Leiden, Netherlands

Monobenzone is a 4-substituted phenol that interacts with the enzyme tyrosinase in pigmented cells and can induce vitiligo and antimelanoma immunity. 4-methoxyphenol and 4-tertiarybutylphenol have skin depigmenting effects but their ability to induce antimelanoma immunity

is unknown. We here investigated a series of nine structurally-related 4-substituted phenols in vitro for their depigmenting action and evaluated their potential as melanoma immunotherapy drugs. The phenols were tested for tyrosinase inhibition, toxicity against pigmented cells or keratinocytes, quinone formation and immunizing ability. Tyrosinase inhibition was analyzed by spectrometry using the substrate L-DOPA or 3-methylbenzothiazolinone (MBTH) that eliminates false positive absorbance from converted phenols. Depletion of glutathione (GSH) was used as surrogate marker for reactive quinone formation and binding to protein thiol groups, which can cause immunogenic hapten formation. Immunization was tested by stimulating T-cells with dendritic cells (DC) loaded with melanoma cells that were pre-exposed to phenol or not, and analyzing T-cell activation by flow cytometry. Most phenols displayed both tyrosinase inhibition and quinone formation, although the levels did not correlate. Phenols with most immunizing ability also demonstrated tyrosinase inhibition and quinone formation, whereas their toxicity did not correlate to immunizing potential. Most phenols specifically induced CD8+ T-cell responses against pigmented cells, and not against keratinocytes, except for 4-tertiarybutylphenol also inducing aspecific immunity. This indicates that the induced T-cell response is melanocyte/melanoma antigen specific. T-cells stimulated with DC loaded with phenol-exposed melanoma cells, also targeted unexposed melanoma cells. This suggests that the T-cell response may act both locally against exposed skin melanocytes or melanoma cells and against melanocytes or melanoma cells at distant sites that were not exposed, e.g. systemic spread of depigmentation or melanoma regression. In conclusion, we identified the biochemical and immunologic mechanism of action of 4-substituted phenols underlying their depigmenting and vitiligo-inducing effects, which may be applicable for melanoma immunotherapy.

PS.08.05 | IL-37 is highly expressed in T cells in melanoma patients and directly suppresses CD4+ T cell activation

Douglas G. Osborne; Joanie Domenico; Charles A. Dinarello; Mayumi Fujita

University of Colorado, Denver, CO, USA

IL-1 family member IL-37 inhibits both innate and adaptive immune responses. Although the role of IL-37 has been elucidated in various inflammatory models, its role in adaptive immune cells has remained elusive. We have demonstrated that IL-37 participates in peripheral tolerance through the generation of semi-mature tolerogenic dendritic cells (DCs) in antigen-specific adaptive immune responses. However, IL-37's role in T cells and T cell-mediated immune responses is mostly unknown. In addition to DCs, regulatory T cells (Tregs) are known to play essential roles in peripheral tolerance. Peripheral tolerance is one of the hallmarks of cancer. In this study, we found that while IL-37 expression was very low in healthy individuals, the level was increased in melanoma patients. IL-37 level was elevated in multiple immune cell types, specifically in T cells. Analysis of T cell subsets revealed that Tregs expressed more IL-37 than other T cell subsets in melanoma patients. Using transgenic mice expressing IL-37 and peripheral blood T cells from human donors, we found that both exogenous (secreted) and endogenous (expressed) IL-37 regulated CD4+ T cell function and differentiation. Specifically, IL-37 enhanced suppressive function of Tregs, by increasing Foxp3 and IL-10 expression, and directly inhibited CD4+ T cell activation and proliferation *in vitro* and *in vivo*. Our findings shed light on the possible role of IL-37 in the inhibition of T cell-mediated immune responses in melanoma.

PS.08.06 | Vitiligo-like lesions occurring in patients receiving anti-programmed cell death-1 therapies

Katia Boniface[1]; Clément Jacquemin[1]; Jerôme Rambert[2]; Léa Dousset[3]; Alain Taieb[3]; Caroline Dutriaux[3]; Sorilla Prey[3]; Julien Seneschal[1,3]

[1]INSERM U1035 BMGIC, ATIP-AVENIR Immuno-Dermatology, University of Bordeaux, Bordeaux, France; [2]INSERM U1035, AQUIDERM, University of Bordeaux, Bordeaux, France, 3. Department of Dermatology, University of Bordeaux, Bordeaux, France

Immunotherapies targeting programmed cell death 1 (PD-1), a major checkpoint in the effector phase of cytotoxic T cells, have shown remarkable clinical results in the treatment of cancers, such as metastatic melanoma. Nonetheless, these anti-PD-1 therapies are associated with development of immune-related adverse effects. Among them, the occurrence of vitiligoid lesions is of particular interest in the context of melanoma, because this side effect seems associated with increased survival. Therefore, we sought to characterize clinically and biologically vitiligoid lesions occurring under anti-PD-1 therapies in patients with metastatic tumors. Eight patients receiving anti-PD-1 therapies with features of vitiligo-like lesions seen in our department were recruited. Clinical features and photographs were analyzed. For some patients, skin and blood samples were obtained. Results were compared with the vitiligo group. All patients developed lesions localized on photoexposed areas with a specific depigmentation pattern consisting of multiple flecked lesions without Koebner phenomenon. In contrast to vitiligo, patients receiving anti-PD-1 therapies who developed vitiligo-like lesions did not report any personal or family histories of vitiligo, thyroiditis, or other autoimmune disorders. Analysis of blood and skin samples revealed increased C-X-C motif ligand 10 levels in serum of patients developing vitiligo-like lesions, associated with skin infiltration of CD8-T T-cells expressing C-X-C motif receptor 3 and producing elevated levels of interferon-g and tumor necrosis factor-alpha. In conclusion, clinical and biological patterns of vitiligo-like lesions in patients receiving anti-PD-1 differ from vitiligo, suggesting a different mechanism leading to the loss of melanocytes.

CS.29.01 | Rab22A interacts with BLOC-1 and -2 and regulates the formation of recycling endosomes

Saurabh Sakhya[1]; Cedric Delevoye[2]; Graça Raposo[2]; Subba Rao Gangi Setty[1]

[1]Indian Institute of Science, Bangalore, KA, India; [2]Institut Curie, Paris, France

Melanosomes, a class of lysosome-related organelles (LROs) produced by melanocytes, which protect the skin against ionizing radiation. Defects in the formation or cargo transport steps to melanosomes result in oculocutaneous albinism, a clinical phenotype commonly observed in Hermansky-Pudlak syndrome (HPS). Biogenesis of melanosome requires the transport of melanin synthesizing enzymes from distinct subdomains of early/recycling endosomes to maturing melanosomes. Previous studies reported that this process is dependent on HPS-associated complexes BLOC-1, -2 and AP-3. Moreover, these endosomal domains contain several Rab proteins such as Rab4, Rab5, Rab11, Rab14 and Rab22. Here, we investigated the role of Rab22-associated recycling endosomes in the pigment granule formation. Further, we studied the regulation between Rab22 and BLOC-1/-2 complexes for the transport of cargo during melanosome maturation. These studies highlighted the Rab22's function in the formation of recycling tubular structures and melanosome biogenesis.

CS.29.02 | Physiopathology of human pigmentation: The biogenesis of pigment granules and intercellular communication in the skin

Graça Raposo

Institut Curie CNRS UMR144, Paris, France

Our major goals are to unravel the cellular and molecular mechanisms involved in the biogenesis, secretion and functions of two intracellular organelles: a Lysosome Related Organelle (LRO), the melanosome; and exosomes which are endosomal-secreted vesicles. Using the skin epidermis as a model system, we are deciphering how these two players control the pigmentation status of the skin in health and disease. Melanosomes are cell type-specific organelles generated by epidermal melanocytes in which melanin pigments are synthesized and stored. Through a combination of light and electron microscopy, molecular biology and biochemistry, our studies provide a conceptual framework underlying melanosome formation and transfer to neighboring skin keratinocytes. Our work highlights novel trafficking pathways and machineries that, are essential to maintain the pigmented phenotype and photoprotect the skin against ionizing radiations. Our studies pinpoint potential targets to manipulate pigmentation while shedding light on the pathogenesis of a genetic lysosomal disorder, the Hermansky-Pudlak Syndrome. Unexpectedly, investigating the biogenesis of melanosomes also allowed us to elucidate the mechanisms of formation of physiological amyloids which exhibit several analogies with pathological amyloid fibrils that accumulate in neurodegenerative diseases. Recent findings highlight how pigmentation is regulated through melanocyte-keratinocyte interactions. These studies have started to enlighten characteristics of their close intercellular contacts but also a novel mode of communication between keratinocytes and melanocytes via secreted exosomes that host proteins and miRNAs that are likely to contribute in the modulation of pigmentation.

These studies, while pinpointing fundamental mechanisms of human pigmentation also aim to further uncover cellular and molecular basis of melanoma through studies on genes variants coding for trafficking proteins that control pigmentation. Novel insights will hopefully open new paths to the development of strategies to regulate pigmentation in health and disease.

CS.29.03 | Two-pore channel 2 (TPC2) regulates the biogenesis and function of the melanosome

Santiago M. Di Pietro; Andrea L. Ambrosio

Colorado State University, Fort Collins, CO, USA

Melanin is responsible for pigmentation of skin and hair, and is synthesized in a specialized organelle, the melanosome, in melanocytes. A genome-wide association study revealed the *TPCN2* gene is strongly linked to pigmentation variations. *TPCN2* encodes the Two-Pore Channel 2 (TPC2) protein, a cation channel. Nevertheless, how TPC2 regulates pigmentation remained unknown. Here, we show TPC2 is expressed in melanocytes and localizes to the melanosome limiting membrane and, to a lesser extent, to endolysosomal compartments by confocal fluorescence and immunogold electron microscopy. Immunomagnetic isolation of TPC2-containing organelles confirmed its co-residence with melanosomal markers. *TPCN2* knockout by means of CRISPR/*Cas9* gene editing elicited a dramatic increase in pigment content in MNT-1 melanocytic cells. This effect was rescued by transient expression of TPC2-GFP. Consistently, siRNA-mediated knockdown of TPC2 also caused a substantial increase in melanin content in both MNT-1 cells and primary human melanocytes. Using a newly developed genetically encoded pH sensor targeted to melanosomes, we determined the melanosome lumen in TPC2-KO MNT-1 cells and primary melanocytes subjected to TPC2 knockdown is less acidic than in control cells. Fluorescence and electron microscopy analysis revealed TPC2-KO MNT-1 cells have significantly larger melanosomes than control cells, but the number of organelles is unchanged. TPC2 likely regulates melanosomes pH and size by mediating Ca^{2+} release from the organelle, which is decreased in TPC2-KO MNT-1 cells as determined with the Ca^{2+} sensor Tyrosinase-GCaMP6. Thus, our data shows TPC2 regulates pigmentation through two fundamental determinants of melanosome function, pH and size.

CS.29.04 | Systematic analysis of melanosomes in skin of different color phenotypes reveals melanocore cluster reservoirs in keratinocytes

Ilse Hurbain[1,2]; Peggy Sextius[3]; Graça Raposo[1,2]; Christine Duval[3]

[1]Institut Curie, PSL Research University, CNRS, UMR 144, Paris, France; [2]Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR144, Paris, France, 3. L'Oréal Research and Innovation, Aulnay sous Bois, France

Among factors that determine human skin color, the fate of melanosomes in keratinocytes remains elusive. This study aimed at thoroughly investigating the characteristics of melanosomes in different human skin types classified according to their Individual Typological Angle (ITA), an objective colorimetric measure of skin color. Electron microscopy of highly and moderately pigmented skin (HPS, MPS) revealed that the majority of melanosomes (50-60%) are located predominantly in the basal layer but some are also detected in the suprabasal layers of the epidermis (up to the stratum corneum). In lightly pigmented skin (LPS), melanosomes are less numerous and mostly concentrated in the basal layer (>80% of melanosomes). In HPS, melanosomes are found mainly isolated in the basal keratinocytes. In contrast, in MPS and LPS, the majority appear grouped as clusters of melanocores (melanosomes devoided of limiting membrane)."En bloc" contrast optimization revealed that clusters are surrounded by an outer single membrane. Within clusters, melanosomes are smaller than those kept isolated, interestingly however small melanocores clusters reach approximately the size of isolated large melanosomes.

Immunogold labelling showed that clusters of melanocores present, similarly to melanosomes in melanocytes, features of non-acidic lysosome-related organelles (lysosomal membrane proteins, CD63, pH higher than in lysosomes) but they do not contain lysosomal hydrolases and LC3, suggesting that they do not correspond to autophagosomes. Moreover, tomographic 3D reconstruction revealed tight contacts with organelles (e.g. endoplasmic reticulum, mitochondria) which thus may have a key role in the biogenesis or dynamic maintenance of clusters.

Overall, these observations suggest that melanocore clusters in keratinocytes do not correspond to degradative organelles but represent reservoirs or (photo) protective structures required for melanosome integrity and functionality. These results open new avenues to understand the basis of skin pigmentation in different skin color phenotypes and to unveil natural photoprotection mechanisms.

CS.29.05 | Melanin resides in mildly acidic and degradative compartments and resists degradation within keratinocytes

Maria S. Correia; Hugo Moreiras; Francisco J. Pereira; Matilde R. Neto; Tiago C. Festas; Abul K. Tarafder; José S. Ramalho; Miguel C. Seabra; Duarte C. Barral

CEDOC, NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal

Melanin determines skin color and protects skin cells against ultraviolet (UV) radiation-induced DNA damage. Melanin is produced and stored in melanosomes, within melanocytes and is then transferred to keratinocytes. The uptake and processing of melanin in keratinocytes and the transport to the supra-nuclear region, where melanosomes form a parasol that protects the nuclei of keratinocytes are poorly understood, despite representing a critical process in skin pigmentation and protection against UV radiation.

In this study, we analyzed the role of endocytic Rab GTPases in melanin uptake by XB2 keratinocytes. We developed a novel uptake assay using melanocores, *i.e.* melanin plus the proteinaceous core, secreted by melanocytes. We found that the silencing of the early endocytic regulator Rab5b, but not the late endocytic regulators Rab7a or Rab9a, significantly impairs melanocore uptake by XB2 keratinocytes. In further characterizing the intracellular fate of melanin, we observed that melanin resides in compartments that are positive for early and late endocytic markers. Surprisingly, we found that melanin does not localize to either highly degradative or acidic organelles, as assessed by LysoTracker and DQ-BSA staining, despite the abundance of these types of organelles within keratinocytes. Therefore, our results suggest that melanin is stored in specialized endocytic compartments within keratinocytes that are not highly acidic or degradative, which allow melanin to resist degradation for long periods.

CS.29.06 | Myosin VI and actin dynamics control membrane recycling from melanosomes: a step required for their maturation and function

Lea Ripoll[1]; Xavier Heiligenstein[1]; Lia Domingues[1]; Ilse Hurbain[1]; Megan K. Dennis[2]; Evelyne Coudrier[1]; Anne Houdusse[1]; Michael S. Marks[2]; Graça Raposo[1]; Cédric Delevoye[1]

[1]Institut Curie, Paris, France; [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA

Melanocytes produce a lysosome related-organelle (LRO), called the melanosome, which is specialized in the synthesis and storage of the pigment melanin. In the epidermis, pigmented melanosomes are then transferred to keratinocytes to color and photo-protect the skin. The biogenesis and maintenance of a functional and pigmented melanosome rely on multiple intracellular trafficking pathways that bring and recycle components in and out the melanosome. Genetic diseases characterized by hypopigmentation, such as the Hermansky-Pudlak Syndrome, result from mutations in trafficking complexes controlling these pathways.

Among them, the Biogenesis of LRO Complex-1 (BLOC-1) modulates the endosomal dynamic of the actin cytoskeleton and cooperates with the kinesin KIF13A to generate recycling endosomal transport carriers. These carriers deliver melanogenic enzymes and transporters to melanosomes, effecting their maturation and pigmentation (*Delevoye et al., Current Biology, 2016*). The fusion of these carriers with melanosomes requires the SNARE, VAMP7, that is then recycled in separate tubular carriers emerging from melanosomes in a process requiring BLOC-3 (*Dennis et al., The Journal of Cell Biology, 2016*).

Using especially cutting-edge correlative light and electron microscopy and electron tomography, we show that the constriction, scission and release of these tubular intermediates require the myosin VI motor and actin dynamics on the melanosome membrane. Ultimately, the release of these tubules and recycling of the associated components are required for melanosome maturation, secretion and subsequent transfer to keratinocytes.

Our studies provide novel insight into how molecular motors cooperate with cytoskeletal elements to reshape membranes required for both the biogenesis and function of melanosomes.

CS.29.07 | Calcium influx in human melanocytes via TRPM1 triggers melanosome transfer: Differences in responses to UVA or UVB irradiation

Tiechi Lei

Department of Dermatology, Renmin Hospital of Wuhan University, Wuhan, China

The transfer of melanosomes from melanocytes to neighboring keratinocytes is critical to protect the skin from the deleterious effects of ultraviolet A and ultraviolet B irradiation, however, the initial factor(s) that stimulates melanosome transfer remains unclear. Here we demonstrate that the induction of retinal-dependent Ca2 + influx in melanocytes by UVA or UVB irradiation contributes to the stimulation of melanosome transfer and that TRPM1 is involved in that process. Calcium imaging also shows that the intracellular calcium profile of UVA-exposed melanocytes differs greatly from that of UVB-exposed melanocytes in the timing-phase, indicating that the distinct time-phases of Ca2 + influx enable melanosome transfer to be driven in the process of solar UV-induced skin tanning. In addition, flow cytometry analysis indicates that voriconazole, a triazole antifungal agent, significantly inhibits melanosome transfer in co-cultures following UVA or UVB exposure. These findings suggest a previously unrecognized mechanism that modulates melanosome transfer in response to UVA or UVB and show that TRPM1 blockers are a new category of skin whitening agents capable of inhibiting melanosome transfer. Acknowledgements: This work was supported by grants from the National Natural Science Foundation of China (NSFC Grants 81371717 and 81573028).

CS.30.01 | Analysis of the cross-talk between mechanically activated c-kit and integrin-dependent adhesion in the environmental niche

Zuleika Calderin-Sollet; Bernhard Wehrle-Haller

University of Geneva, Department of Cell Physiology and Metabolism, Geneva, Switzerland

Kit-Ligand (KitL) and its receptor c-kit are critical for the survival of melanocytes, germ cells, hematopoietic stem cells and mastocytes. C-kit gene amplification or kinase activating mutations can cause melanoma and various other forms of cancer. Despite initially successful treatments of melanoma, which expresses tyrosine kinase inhibitor (TKIs; e.g. imatinib)-sensitive c-kit mutations, melanoma eventually progress and persists in the tissue microenvironment. Although the TKI activity on the isolated c-kit kinase is well studied, it is not understood how the interaction of the tumour cells with the micro-environmental niche is modified or altered by TKI treatments. Within this niche, anchorage is mediated by membrane-bound KitL/c-kit and extracellular matrix (ECM)/integrin interactions, which synergize to allow the anchorage of normal as well as malignant cells to the niche. Here, we developed an experimental system to analyse KitL/c-kit-dependent cell-niche interactions. We found that immobilized, but not soluble forms of-KitL induced wildtype c-kit-dependent synergies with integrin receptors to mediate spreading on ECM. Imatinib inhibited soluble KitL-mediated spreading, but failed to block spreading by immobilized-KitL. On the other hand, dasatinib prevented spreading in response to soluble, as well as immobilized KitL. Interestingly, immobilized-KitL-mediated spreading on ECM was blocked by oncogenic c-kit mutations in the juxtamembrane and activation loop, which could not be altered by the addition of TKI's. Our data demonstrate complex roles for c-kit signalling for survival/proliferation, as well as niche-anchorage of c-kit expressing cells, which is perturbed by oncogenic mutations of c-kit. While TKI's appear to correct the proliferation function of oncogenic forms of c-kit, unfortunately, however, altered ECM-spreading and niche-anchorage responses of oncogenic c-kit mutations are currently not targeted by TKI's used in the clinic. Our data highlight the need to better understand the cross-talk between c-kit and integrin-dependent adhesion/signalling for the development of new therapies to treat melanomas

CS.30.02 | Melanoma miRNA trafficking controls tumor primary niche formation

Shani Dror[1]; Laureen Sander[2]; Carmit Levy[1]

[1]*Tel Aviv University, Israel, Tel Aviv, Israel;* [2]*DKFZ, Heidelberg, Germany*

Melanoma originates in the epidermis and becomes metastatic after invasion into the dermis. Prior interactions between melanoma cells and dermis are poorly studied. Here, we show that melanoma cells directly affect the formation of the dermal tumor niche by microRNA trafficking before invasion. Melanocytes, cells of melanoma origin, are specialized in releasing pigment vesicles, termed melanosomes. In melanoma in situ, we found melanosome markers in distal fibroblasts before melanoma invasion. The melanosomes carry microRNAs into primary fibroblasts triggering changes, including increased proliferation, migration and pro-inflammatory gene expression, all known features of cancer-associated fibroblasts (CAFs). Specifically, melanosomal microRNA-211 directly targets *IGF2R* and leads to MAPK signaling activation, which reciprocally encourages melanoma growth. Melanosome release inhibitor prevented CAF formation. Since the first interaction of melanoma cells with blood vessels occurs in the dermis, our data suggest an opportunity to block melanoma invasion by preventing the formation of the dermal tumor niche.

CS.30.03 | Kit signaling seems to work redundantly in melanocytes

Takahiro Kunisada; Nobuhiko Taguchi; Hitomi Aoki

Department of Tissue and Orgen Dev, Gifu University Grad. Sch. of Med., Gifu, Japan

Looking at white coat color phenotype *Kit*$^{W/W}$ mice, Kit signaling must be indispensable and could not be compensated by the other signaling pathway at least in a certain phase of the melanocyte development. While Kit is highly expressed in developing brain, *Kit*$^{W/W}$ brain develops quite normally, indicating that Kit is just expressed unfunctionally or Kit function is compensated by the other signaling molecules in developing brain. We recently found severe brain hypoplasia in *Sox1-Cre*; *Kit*$^{2lox/+}$ mutant mice in which *Kit* loss of function mutation was induced during early brain development. This strongly indicates that Kit is actually functioning during normal brain development and could be compensated by other signaling molecules in *Kit*$^{W/W}$ mice. Developmental defect of the brain in *Sox1-Cre*; *Kit*$^{2lox/+}$ mice was supposed to be exposed by a sudden reduction of Kit signaling not quickly compensated by the redundant molecules in embryonic neural stem/precursor cells. We also observed the increase of white spotting in *Tyr-Cre*; *Kit*$^{2lox/+}$ mice in comparison with *Kit*$^{W/+}$ mice, suggesting that sudden loss of Kit signals in developing melanocytes is also deleterious for melanocytes. In case *Kit* is solely responsible for melanocyte development, the extent of *Tyr-Cre; Kit*$^{2lox/+}$ white spotting must be less than or equal to that of *Kit*$^{W/+}$ mice. Identification of the relevant molecule(s) compensating Kit is necessary to reveal the mechanisms of a redundancy of Kit signaling in melanocytes.

CS.30.04 | Role of Brn2 in melanocyte lineage renewal after genotoxic stress

Madeleine Le Coz; Marie Schonau; Lionel Larue

Institut Curie, Orsay, France

Melanocytes (Mc) are melanin producing cells in charge of skin pigmentation and protection against UV. Mc are maintained in physiological context in mouse skin from a pool of melanocyte stem cells (McSC) localized in hair follicle niche called the bulge. In physiological context, quiescent McSC transiently proliferates to generate progenitors called transient amplifying cells (TAC) that will actively proliferate in order to produce new differentiated melanin producing Mc. Furthermore, the loss of Mc renewal during normal aging or pathological aging, such as gamma irradiation, leads to a hair graying phenotype. In physiopathological context, Mc may transform in melanoma (Ma). Ma are heterogeneous tumors at the cellular level due to an alternative process of proliferation and quiescence called « phenotypic-switch » in which two transcription factors are involved and regulate mutually their expression, MITF and BRN2. Thus, in a same tumor, BRN2-positive/MITF-negative cells are low proliferative / « quiescent-like » cells whereas MITF-positive/BRN2-negative cells are highly proliferative. Our results show that after specific BRN2 knock-out in the melanocyte lineage in mouse, the lineage formation is not affected as the pups are black at birth. Moreover, the absence of BRN2 in mouse Mc in normal aging, or accelerated aging by successive depilation, does not affect hair pigmentation. However, exposition of Mc-BRN2^{KO} mice to ionizing radiations leads to a precocious hair greying phenotype compared to littermates controls. These results suggest a role of BRN2 in resistance to ionizing radiations in the melanocyte lineage. Thereby, deciphering molecular and cellular mechanisms by which absence of BRN2 leads to loss of Mc after irradiation will help us to better understand the melanocyte lineage renewal in physiological context and in physiopathological context in order to improve Ma treatments.

CS.30.05 | Myosin-X is required for efficient melanoblast migration and melanoma initiation/metastasis

Hiroshi Tokuo; Lynne M. Coluccio

Department of Physiology & Biophysics, Boston University School of Medicine, Boston, MA, USA

Myosin-X (Myo10), an actin-associated molecular motor, has a clear role in filopodia induction and cell migration in vitro, but its role in vivo in mammals is not well understood. Here, we investigate the role of Myo10 in melanocyte lineage and in melanoma cells. We found that Myo10 knockout (Myo10$^{-/-}$) mice exhibit white spots on their belly caused by reduced melanoblast migration. The molecular mechanism of melanoblast migration is believed to be highly correlated with that of melanoma metastasis. Thus, first we knocked down Myo10 in B16F1 mouse melanoma cell lines and found decreased lung colonization after tail-vein injection. Next, we utilized available mice with conditional melanocyte-specific expression of BRAF^{V600E} (constitutive active: CA) combined with Pten tumor suppressor gene silencing (Tyr::CreER; Braf^{CA}; Pten$^{lox/lox}$), which model the genetic profile of human melanoma, and crossed them with Myo10$^{-/-}$ mice. Surprisingly, whereas control Tyr::CreER; Braf^{CA}; Pten$^{lox/lox}$ mice developed melanoma with 100% penetrance and lymph node metastasis, Tyr::CreER; Braf^{CA}; Pten$^{lox/lox}$; Myo10$^{-/-}$ mice exhibited both reduced melanoma development and metastasis. To assess the role of Myo10 in melanoma-caused death in these mice, we investigated the survival duration in Myo10$^{+/+}$, Myo10$^{+/-}$ and Myo10$^{-/-}$ mice under the genetic background of Tyr::CreER; Braf^{CA}; Pten$^{lox/lox}$. Mice were sacrificed when tumors reached a maximum diameter of 2 cm or when they developed ulcers. The median survival time of mice was extended by hemizygous deletion of Myo10 by ~100% (from 32.5 to 65 days) and homozygous deletion of Myo10 by ~160% (from 32.5 to 85 days) in mice with locally administrated 4-hydroxytamoxifen. These findings provide the first genetic evidence for the involvement of Myo10 not only in melanoblast migration but also in melanoma development and metastasis.

CS.30.06 | Contribution of multiple MITF gene family members to RPE development in zebrafish

James A. Lister; Samantha Spencer

Virginia Commonwealth University, Richmond, VA, USA

The microphthalmia-associated transcription factor (MITF) is known to be required for development of the retinal pigment epithelium (RPE) of the eye in mammals and birds. However in zebrafish, null mutations in the *MITF* ortholog *microphthalmia-associated transcription factor a* (*mitfa)* lack all neural crest-derived melanocytes but display normal development of the RPE. Zebrafish possess a second *MITF* ortholog, *mitfb*, but we have previously shown that *mitfa;mitfb* double mutants also undergo apparently normal RPE development. The purpose of this study was to determine if another member of the zebrafish MITF/MiT family, *tfec*, which is expressed strongly in the presumptive RPE, may function in its development, either independently or in conjunction with *mitfa* and *mitfb*. *tfec* loss of function was first generated by antisense morpholino oligonucleotides targeting splice junctions and subsequently by TALENs and CRISPR/Cas9. Stable *tfec* CRISPR lines were established and bred to *mitfa* and *mitfb* mutants to generate double and triple mutants. Single *tfec* morphants/mutants display delayed melanization of the RPE, with *tfec* mutants showing a more dramatic effect. In comparison to *tfec* single mutants, *tfec;mitfa* double mutants have more severe defects in pigmentation and in ocular morphogenesis, with variable colobomata not observed in the *tfec* single mutants. Finally, eye development appears to be most severely compromised in *mitfa;mitfb* triple mutants. Expression of a subset of RPE markers show alterations as a result of manipulations of *MITF* gene family activity. In summary, knockdown/knockout of *tfec* indicates that it is vital for the development of the RPE. Together our data show that these three MiT factors make overlapping but differential contributions to RPE and eye development, with *tfec* surprisingly being the most important of the three. These findings highlight important differences in the genetics and evolution of RPE development between fish and mammals.

CS.31.01 | Photomodulation effect of light-emitting diode on the characteristics of human melanocytes

Li Chen; Min Jiang; Leihong Flora Xiang

Department of Dermatology, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China

Melasma is a common hyperpigmentation disease on the face. Light-emitting diode (LED) photomodulation (585 nm) reported to be effective for the treatment of melasma. LED devices have been shown to stimulate fibroblast activity and hasten wound healing, but the effects on melanocytes has not been confirmed. To evaluate the effects of LED photomodulation (585 nm) on melanogenesis, human melanocytes were irradiated with LED light. After 4 days irradiation, cell viability and death were analyzed by the CCK-8 assay and flow cytometry. Melanin contents and TYR activity were measured by a Spectramax spectrophotometer. The expression of tyrosinase (TYR), tyrosinase-related protein 1 (TRP1), microphthalmia-associated transcription factor (MITF) were evaluated by RT-PCR and western blot. After radiation with LED 585 nm, the cell viability & death by the CCK-8 assay and flow cytometry showed no apparent difference between irradiated group and control group. Meanwhile, the melanin content in human melanocytes decreased gradually when they were irradiated with LED 585 nm from 5J/cm^2 to 20 J/cm^2. Inhibition was accompanied by reduced expression of TYR, TRP1 and MITF assessed by western blotting and RT-PCR. These results demonstrated that LED photomodulation 585 nm suppresses melanin production in vitro, suggesting that LED 585 nm may play an important role in inhibition of epidermal melanogenesis. This may provide new insights into the efficacy of LED photomodulation in the treatment of hyperpigmentation disease.

CS.31.02 | Whitening agents: Basics and therapeutics

Yoko Funasaka

Department of Dermatology, Nippon Medical School, Tokyo, Japan

Facial hyperpigmentation such as solar lentigo and melasma is a big concern for people and these conditions are mainly due to epidermal hyperpigmentation. The target of whitening agent is the regulation of melanin synthesis and melanosome transfer, via directly acting on melanocytes, or indirectly through surrounding cells such as keratinocytes, fibroblasts, and melanocytes. The overall mechanisms of whitening agents including rhododenol will be reviewed.

CS.31.03 | Isobutylamido thiazolyl resorcinol a new powerful inhibitor of human tyrosinase

Tobias Mann[1]; Wolfram Gerwat[1]; Horst Wenck[1]; Klaus-Heinrich Röhm[2]; Ludger Kolbe[1]

[1]*Beiersdorf R&D, Applied Skin Research, Hamburg, Germany*; [2]*Philipps University, Institute of Physiological Chemistry, Marburg, Germany*

Melasma, actinic lentigines and post-inflammatory hyperpigmentation are major cosmetic concerns. Various strategies have been proposed to reduce unwanted melanin production in human skin. The most prominent target for inhibitors of hyperpigmentation is tyrosinase, the key enzyme of melanin production. In addition, inhibitors of melanocyte activity, compounds interfering with tyrosinase expression, and agents reducing melanosome transfer to keratinocytes were also described as effective drugs for the treatment of hyperpigmentation. Still, due to their immediate and reversible mode of action, selective tyrosinase inhibitors are considered not only most effective but also very safe reducers of hyperpigmentation.

However, most known tyrosinase inhibitors lack clinical efficacy because they were selected based on their ability to inhibit mushroom tyrosinase. To overcome this problem, we expressed human tyrosinase in a mammalian cell system and used the recombinant enzyme to screen a library of more than 50,000 chemically diverse compounds for inhibition of human tyrosinase. Screening hits were analyzed and developed to the final active ingredient by classical hit-to-lead-to candidate chemistry.

Isobutylamido thiazolyl resorcinol (W630) was identified as a very powerful inhibitor of human tyrosinase. Compared to other well-known inhibitors of human skin pigmentation, W630 was by far the most active with an IC_{50} of 1 μM. This high potential to inhibit melanin production was confirmed using melanoDerm™ skin models. In this assay, W630 with an IC_{50} of 0.9 μM, was much more effective than 4-butylresorcinol, the next best compound with an IC_{50} of 13.5 μM. Further analysis of the mode of action and *in silico* modeling of ligand binding to homology models of human tyrosinase revealed that W630 is a strictly competitive inhibitor of the human enzyme and only marginally inhibits mushroom tyrosinase (IC_{50} = 108 μM). *In vivo* studies showed a strong reduction in age spot visibility after treatment with topical formulations containing W630.

CS.31.04 | Interactions between melanocytes and neighboring cells: The contribution of fibroblasts to the ethyl linoleate-induced inhibition of melanogenesis

Mariko Yokota[1]; Satoshi Yoshimoto[2]; Moemi Yoshida[2]; Shoichi Yahagi[1]; Hideya Ando[2]

[1]NIKKOL GROUP Cosmos technical center Co., Ltd., Tokyo, Japan; [2]Department of Applied Chemistry and Biotechnology, Okayama University of Science, Okayama, Japan

Interactions between melanocytes and neighboring cells such as keratinocytes and fibroblasts play important roles in regulating human skin color. Previously, it was reported that several paracrine factors derived from neighboring cells regulate melanin synthesis in melanocytes. However, the regulatory mechanisms of interactive communications among these various types of cells are still unclear. The purpose of this study was to elucidate whether the presence of fibroblasts affects melanogenesis using a co-culture system of fibroblasts and melanocytes and a reconstructed 3D epidermis containing melanocytes. Our results showed that the presence of fibroblasts decreased melanin synthesis in co-cultured melanocytes and in melanocytes in the 3D epidermis. Further, we found that the fibroblast-induced inhibitory effect of melanogenesis was diminished when aged fibroblasts were used compared to young fibroblasts and this was confirmed by the altered of tyrosinase levels determined by Western blotting analysis. We then evaluated the effect of ethyl linoleate, a derivative of linoleic acid known as a skin lightening agent that degrades tyrosinase via the ubiquitin proteasome system, on melanin synthesis using the co-culture system composed of melanocytes and fibroblasts. The results revealed that ethyl linoleate remarkably decreased melanogenesis in melanocytes in the presence of fibroblasts, while that inhibitory effect was limited in the absence of fibroblasts. These results suggest that interactions between melanocytes and fibroblasts contribute to the inhibitory effect of ethyl linoleate on melanogenesis.

CS.31.05 | QuantiGene plex analysis for optimized screening and development of skin whitening active ingredients

Ping Wang[1]; Hongbin Dai[1]; Juanjuan Chen[1]; Peggy Sextius[2]; Richard Betts[1]; Jie Qiu[1]

[1]L'Oréal Research and Innovation, Shanghai, China; [2]L'Oréal Research and Innovation, Aulnay-sous-Bois, France

In vitro skin modelling presents considerable challenges for evaluating the potential whitening effects of active ingredients. Existing cellular 2D model approaches utilizing colorimetry or direct melanin quantification have the advantage of high throughput, but are limited to screening ingredients that act via the inhibition of melanogenesis, and may be confounded by coloured natural extracts.

To overcome these issues and complement existing evaluation approaches, we adapted the QuantiGene Plex analysis system for use with a keratinocyte/melanocyte co-culture model, enabling the rapid and accurate quantification of gene targets associated with whitening pathways. Exposure of the co-culture model to UV irradiation induced a broad modulation of genes associated with melanogenesis, melanin transfer, anti-oxidation, exfoliation and inflammation as determined by QuantiGene Plex assay, resulting in a panel of 37 gene targets.

Initial validation with reference compounds Macelignan, Sulforaphane and Retinoic acid showed consistent results as reported by literature, which confirmed the sensitivity of the system to ingredient-induced modulation of gene expression. Known whitening agents Vitamin C and 4-n-Butylresorcinol demonstrated positive responses within our platform, as did Vitamin E, despite having a mechanism of action independent of melanogenesis. Paeonia Lactiflora Root Extract, a whitening ingredient that possesses natural color, similarly demonstrated results consistent with its known efficacy and also provided insight into its potential whitening mechanism.

Our data demonstrates that a co-culture model combined with the QuantiGene Plex analysis system can provide a valuable complement to existing evaluation approaches by identifying agents that act via diverse whitening-associated gene pathways, which is unaffected by active ingredient color, and also provide information on potential mechanisms of action.

CS.31.06 | Glucosamine may abrogate SCF+EDN1 stimulated melanogenesis via a decrease in MITF expression due to O-GlcNAcylation-affected transcriptional activity of CREB

Genji Imokawa[1]; Shuko Terazawa[2]; Takao Niwano[1]; Hiroaki Nakajima[3]

[1]*Center for Bioscience Research & Education, Utsunomiya University, Tochigi, Japan;* [2]*Research Institute for Biological Functions, Chubu University, Aichi, Japan;* [3]*School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan*

We have already reported that glucosamine (GlcN) distinctly abrogates the stimulated pigmentation in stem cell factor + endothelin-1 (SE)-treated human epidermal equivalents. In this study, we characterized the molecular mechanisms involved in the GlcN-induced anti-melanogenic effects in normal human melanocytes (NHMs). The SE-stimulated gene (12 hr/2 hr) and protein (24 hr/4 hr) expression of melanocyte-specific proteins/MITF at the indicated times of SE post-stimulation were significantly abrogated by 72 hr pre-treatment with GlcN (1 mg/ml). GlcN pre-treatment for 72 hr and 24 hr significantly reduced the protein levels of CREB (15 min, 2 hr)/total MITF (2 hr) and total MITF only (2 hr), respectively, at the indicated times of SE post-stimulation compared with non-treated stimulation control. Treatment with GlcN for 24 hr distinctly increased the O-linked N-acetylglucosaminylation (O-GlcNAcylation) levels of cellular proteins which were completely depleted and distinctly increased by alloxan (an inhibitor of O-linked N-acetylglucosaminyltransferase) and by PuGNAc (an inhibitor of O-N-acetylglucosaminase), respectively. Western blotting following immunoprecipitation analysis revealed that while CREB but not ERK was O-GlcNAcylated even in the absence of GlcN, GlcN treatment for 24 hr markedly increased the O-GlcNAcylation levels of CREB. Pre-treatment with PuGNAc for 24 hr and 72 hr significantly reduced the protein levels of total MITF but not CREB at 2 hr post-SE stimulation. The decreased protein levels of CREB induced by 72 hr GlcN treatment were obviously abrogated by the co-addition of alloxan or the proteosomal degradation inhibitor MG132. These findings suggest that the anti-melanogenic effect elicited by GlcN may be mediated via a decreased expression of MITF due to the attenuated transcriptional activity of CREB based upon its increased O-GlcNAcylation and/or additional degradation.

CS.32.01 | Oncogenic reprogramming of human primary melanocytes into potent tumor initiation cells

Fang Li; Chuan-Yuan Li

Duke University Medical Center, Durham, NC, USA

Cancer stem cells have been implicated in melanoma development and treatment. However, their origin and biological properties are still not well understood. Here we report that a cocktail of four genetic factors — myc, ras, dominant negative p53, and Oct-4 — could induce oncogenic transformation of primary human melanocytes rapidly and efficiently. The transformed cells showed unlimited self-renewal ability with robust expression of telomerase activities. In addition, they expressed typical cancer stem cell markers such as CD133, CD166, and ALDH. They also exhibited extraordinary strong tumor-initiating ability — transplantation of as few as 100 of the transformed cells into nude mice induced xenograft tumor formation. We believe the induced melanoma stem cells will be useful in studying molecular mechanisms involved in melanomagenesis and melanoma treatment.

CS.32.03 | New compounds triggering endoplasmic reticulum stress exert anti-melanoma effects and overcome BRAF inhibitor resistance

Stéphane Rocchi

INSERM U1065, team 1, Nice, France

Melanoma has a high capability of invasion and rapid metastasis to other organs. Even if recently encouraging results were obtained with inhibitors of BRAF, these responses remain transitory and the melanoma acquires in all the cases, a drug resistance and the metastases develop again. Another therapies which reactivates the immunity response , were recently developed but give an objective response in only 15 to 30 % of patients. Thus, it appears necessary to develop new drug candidates for specific treatment of melanoma. Using structure/activity relationship studies, we developed candidates (Thiazole Benzenesulfonamides) exhibiting a strong death-promoting effects in melanoma cells with HA15 as the lead compound of this series. Interestingly, HA15 is active molecule on all melanoma cells independently of mutational status and on melanoma cells freshly isolated from patients sensitive or resistant to BRAF inhibitors. HA15 exhibited also a strong efficacy in xenograft mice models performed with melanoma cells sensitive and resistant to BRAF inhibitors without any sign of toxicity in mice. We next performed pan-genomic, proteomic and biochemical studies to decipher the signaling pathway, the mechanism of action and the target of the best candidates. We identified BIP, an endoplasmic reticulum protein, as the specific target of our compound. We demonstrated clearly that the interaction between our compound and BIP increases Endoplasmic Reticulum Stress and leads to melanoma cell death by concomitant induction autophagy and apoptosis mechanisms. BIP overexpression in various cancers is described, it is thus not surprising that this molecule was also found to be active against other liquid and solid tumors. Taken together, our data suggest that our molecule has an important impact on inhibition of melanoma growth by targeting ER stress, and may therefore be developed for treatment of patients with melanoma in particular and other cancers in general.

CS.32.04 | A potential role of NLRP1 in resistance to drug therapies (Temozolomide, Vemurafenib and Trametinib) in human melanoma

Zili Zhai[1]; Erika Guevara[1]; Mayumi Fujita[1,2]

[1]University of Colorado Denver SOM, Aurora, CO, USA; [2]Denver Veterans Affairs Medical Center, Denver, CO, USA

Despite advances in the development of new targeted drugs, limited efficacy and subsequent resistance make metastatic melanoma a challenging disease. We have demonstrated that the NLR sensor NLRP1 promotes melanoma tumorigenesis via activating IL-1-mediated inflammation and inhibiting apoptosis. Here, we investigated whether these biological properties of NLRP1 could confer the development of drug resistance in human melanoma cells. Pharmacological-relevant doses of temozolomide, vemurafenib, and trametinib significantly increased NLRP1 expression in metastatic melanoma cells. However, NLRP1 upregulation was not coupled with NLRP inflammasome activation because IL-1β synthesis and secretion were variable after drug treatment. To understand the role of upregulated NLRP1 in drug resistance, melanoma cells were grown in medium containing an increasing dose of drugs for generating resistant cells. Temozolomide-resistant 1205Lu and HS294T, vemurafenib-resistant A375 and 1205Lu, and trametinib-resistant SK-mel-28 and HS294T cells also showed upregulated NLRP1. It has been reported that drug therapies induce ER stress-mediated apoptosis in melanoma cells. ER stress response involves the IRE1a-XBP-1, PERK-eIFa-ATF4, and ATF6 pathways, in which ATF4 is a potential transcription regulator of NLRP1. We have found an increased expression of ATF4 and NLRP1 in drug-treated parental cells and drug-resistant cells. Knocking down ATF4 significantly reduced NLRP1 expression in drug-treated parental cells and drug-resistant cells. Furthermore, knocking down ATF4 and NLRP1 partially reversed the resistance of melanoma cells. These data suggest a novel role of upregulated NLRP1 in melanoma drug resistance, which is independent of NLRP inflammasome activation. This NLRP1's role may be associated with its intrinsic anti-apoptosis function through inhibiting pro-apoptotic caspases. The role of NLRP1 and its upstream ATF4 in inhibiting ER-stress- mediated cell death and developing melanoma resistance is under active investigation.

CS.32.05 | Serine 729 facilitates homodimerization and substrate affinity of BRAF splice variants

Michael J. Vido[1,2]; Kaitlyn Le[1,2]; Edward J. Hartsough[1,2]; Andrew E. Aplin[1,2]

[1]Department of Cancer Biology, Thomas Jefferson University, Philadelphia, PA, USA; [2]Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA

BRAF V600E melanomas are highly sensitive to RAF inhibitors but the expression of aberrantly spliced BRAF V600E (BRAF V600E ΔEx) isoforms are associated with resistance in 13%–30% of progressing patients. In contrast to full-length BRAF V600E, BRAF V600E ΔEx exhibit enhanced dimerization and, in the presence of RAF inhibitor therapy, continue to signal via ERK1/2; however, little is known regarding their mechanisms of regulation and resistance. The 14-3-3 protein binding sites, serine 365 (S365) and serine 729 (S729), play a complex role in regulating the dimerization and activity of RAF isoforms. All reported BRAF V600E ΔEx lose S365 but retain S729; therefore, we analyzed the role S729 plays in BRAF V600E ΔEx mediated resistance. Mutation of S729 to alanine does not alter BRAF V600E ΔEx basal activity but renders BRAF V600E ΔEx sensitive to RAF inhibitor as measured by reduced ERK1/2 phosphorylation and cell growth in vitro and in vivo using an ERK reporter system. This effect was significantly greater than that measured when BRAF V600E ΔEx was expressed with a dimerization blocking mutation. Increased sensitivity to RAF inhibitor with the S729 mutation was associated with decreased BRAF V600E ΔEx dimerization and binding to MEK1/2. We observed that BRAF V600E ΔEx associated with MEK1/2 to a greater degree than full-length BRAF V600E. Future experiments will test whether enhanced MEK1/2 association is required for RAF inhibitor resistance. These data suggest that S729 is required for BRAF V600E ΔEx mediated resistance and affects both BRAF dimerization and substrate affinity.

CS.32.06 | Targeting MCL-1 and BCL-2 to overcome resistance to current therapy in melanoma

Nabanita Mukherjee[1]; Adam Almeida[1]; Yan Lu[1]; Madison Rogers[1]; Karoline Lambert[1]; William A. Robinson[2]; Allison J. Applegate[2]; Carol M. Amato[2]; Andrew Strosnider[1]; Sarah Slaven[1]; Yiqun G. Shellman[1]; David A. Norris[1,3]

[1]University of Colorado, Denver/Anschutz Medical Campus, Aurora, CO, USA; [2]Division of Medical Oncology, Anschutz Medical Campus, Aurora, CO, USA; [3]Department of Veterans Affairs Medical Center, Aurora, CO, USA

Current melanoma treatments are limited by relapse and the lack of therapeutic options for BRAF wild-type patients who do not respond to immunotherapy. Many clinicians and researchers focus on killing resistant subpopulations, such as Melanoma Initiating Cells (MICs) to prevent relapse. Anti-apoptotic proteins MCL-1 and BCL-2 are emerging as high-priority therapeutic targets, due to their roles in tumor progression and drug resistance. Here, we tested if targeting both MCL-1 and BCL-2 together can be an effective strategy to de-bulk tumors and eliminate MICs. We examined the effects on cell lines and multiple tumor samples of patients relapsed from current treatments, with a diverse genetic background (with or without the common BRAF, NRAS or NF1 mutations). We targeted MCL-1 with two different approaches: indirectly by inducing NOXA (an inhibitory binding partner of MCL-1) with GSI-I or directly with a specific small molecule MCL-1 inhibitor (A1210477). We first used GSI-I in combination with ABT-737 (BCL-2, BCL- XL, and BCL-W inhibitor). The combination: 1) reduced cell viability and induced apoptosis of the non-MICs; 2) disrupted primary spheres, 3) decreased the ALDH+ cells, and 4) inhibited

the self-renewability of the MICs ($p < .05$) . Mechanistic studies using CRISPR-Cas9 technology show that cell death is dependent on NOXA, but not on BIM. Using a low-cell-number mouse xenograft model, we demonstrated that the combination significantly reduced the tumor initiating ability of MICs from relapsed patient sample ($p < .05$). Similar results were observed with the combination of A1210477 plus clinically relevant ABT-199 (BCL-2 inhibitor) and ABT-263 (clinically compatible version of ABT-737). These combinations also disrupted primary spheres and inhibited the self-renewability of MICs. Taken together, our data suggest that targeting MCL-1 directly or indirectly, along with BCL-2 inhibition is a promising strategy to address treatment relapse and for BRAF wild-type patients who do not respond to immunotherapy.

START POSTERS

P.001 | Investigating the role of Yap and Taz in the development of neural crest in zebrafish

Ruqaiya Al Jabri[1,2]; Robert N. Kelsh[1]

[1]*University of Bath, Bath, UK;* [2]*Sultan Qaboos University, Muscat, Oman*

Yes associated protein (Yap) and it's paralogue Transcription activating factor with PDZ domain (Taz) are the nuclear executors of the Hippo pathway. They have been extensively studied in recent years due to their involvement in many cellular process including proliferation, survival, tissue tension and stem cell maintenance. We are exploring the role of these two proteins in the development of neural crest cells which has been neglected to date, using the zebrafish as a model system. Double mutants for both genes ($yap^{-/-}$/$taz^{-/-}$), are severely retarded, showing incomplete arrest of development at 18hpf and surviving only up to 30hpf before starting to disintegrate. In contrast single mutants for either genes are morphologically normal, while embryos mutant for 3 alleles show a mild morphological phenotype. Double mutants show a dramatic decrease in melanocytes and melanoblast numbers compared to age matched WT siblings. We used *sox10* in situ hybridisation to show that neural crest cells are formed, but the expression pattern is abnormal, perhaps reflecting more general morphological disruption. Further investigations using markers for other neural crest derivatives will distinguish whether yap and taz play a specific role in just melanocytes or whether they have broader roles in neural crest development.

P.002 | Rest (RE1-silencing transcription factor) affects melanocyte development

Hitomi Aoki; Takahiro Kunisada

GIFU University, Gifu, Japan

Rest (RE1-silencing transcription factor), also known as Nrsf (neuron-restrictive silencer factor), is a well-known transcriptional repressor of neural genes in non-neural tissues and stem cells. Rest is expressed during embryonic development to prevent neural gene expression in non-neural cells, but it is dispensable for embryonic neurogenesis in vivo. The role of Rest/Nrsf in control of neural crest cells (NCCs) fate has little been examined. NCCs have the pluripotency to differentiate into neuronal and non-neuronal lineages including melanocytes. Analyses of *Rest* functions in vivo have been hampered by early embryonic lethality of *Rest* null mice. To evaluate the role of Rest in NCCs we developed a conditional *Rest* knockout (CKO) system and observed the established NCC-specific homozygous *Rest* CKO mice cause neonatal death. The heterozygous NCC-specific *Rest* CKO mice are viable and some of them showed the white spotting phenotype. A reduction in the number of melanoblasts is observed in NCC-specific *Rest* CKO embryonic skin. Little is known about the Rest function in melanocyte stem cells and their niche in hair follicles. To evaluate the role of Rest in hair pigmentation, we generated melanocyte lineage-specific *Rest* CKO mice and their niche, keratinocyte-specific *Rest* CKO mice, however, they did not show the white spotting phenotype. Therefore, the expression of REST during the early neural crest specification stage was necessary for the normal development of melanoblasts to cover all of the skin. Interestingly, it is recently reported that Rest induction suppresses apoptotic cell death and the aged-related stress or toxic effect. By using a model of irradiation induced hair graying, we are now investigating the Rest function in hair graying among various loss-of-function conditional depletion of *Rest* mice and we also developed a gain-of-function mice using a doxycycline inducible Rest overexpression system

P.003 | A negative feedback loop involving Kctd15 and Tfap2 paralogs regulates melanocyte differentiation in zebrafish

Mikaela M. Mallin; Hannah E. Seberg; Andrea R. Hallberg; Greg Bonde; Robert A. Cornell

University of Iowa, Iowa City, IA, USA

Pigmentation disorders and melanoma can result from dysregulation of the gene regulatory networks governing melanocyte development. Potassium channel tetramerization domain containing 15 (Kctd15) is a potent inhibitor of the transcriptional activity of Transcription factor activating-enhancer binding protein 2 (Tfap2) paralogs. In addition, expression of Kctd15 in early zebrafish embryos is strongly Tfap2 dependent. We hypothesized that Tfap2 and Kctd15 function as a negative feedback loop governing melanocyte differentiation. To test this model, we amplified the full-length open reading frame of zebrafish *kctd15a* and cloned it into an expression vector driven by the *mitfa* promoter; the vector also contains a second cassette in which the *mitfa* promoter drives expression of *mitfa* cDNA. We injected this construct, or a control version that contains the gene encoding mCherry, into $mitfa^{-/-}$ zebrafish embryos, which are normally devoid of melanocytes. In control-injected embryos, melanocytes were robustly restored. However, in embryos injected with *mitfa:kctd15a*,

melanocytes were smaller, paler, and abnormally shaped, suggesting that inhibition of Tfap2 paralogs via Kctd15a overexpression severely impairs differentiation of melanocytes. To determine a mechanism for the regulation of *kctd15a* expression by Tfap2 paralogs, we conducted ATAC-seq on *tfap2a/c* double-mutant zebrafish and identified a potential Tfap2-dependent enhancer adjacent to *kctd15a*. This enhancer is conserved from zebrafish to humans, and comparison to ChIP-seq profiles indicated that it is bound by both TFAP2A and MITF in human melanocytes. We cloned the candidate enhancer sequence into a GFP-reporter vector and observed reporter activity in zebrafish melanocytes at 36hpf. These findings suggest that Tfap2 is regulated in part by Kctd15a via a negative feedback loop, and identify Kctd15a as a potential modulator of Tfap2 activity in melanocytes and melanoma.

P.004 | Characterization of the pigment cell deficient zebrafish mutant, crasher

Samantha Neuffer[1]; Lauren Clancey[2]; Matt Lambert[3]; Alisha Beirl[2]; Cynthia Cooper[1]

[1]Washington State University Vancouver - Department of Molecular Biosciences, Vancouver, WA, USA; [2]Washington State University Vancouver - College of the Arts and Sciences, Vancouver, WA, USA; [3]Washington State University Vancouver - School of Biological Sciences, Vancouver, WA, USA

Pigment plays an essential role in protecting DNA from UV radiation. When pigment development goes awry, melanomas or albinism can reduce an organism's quality of life. We have identified a pigment cell deficient zebrafish (*Danio rerio*) mutant named *crasher* that shows a reduction in black pigment cells, melanophores, at day one and a reduction in silver pigment cells, iridophores, at day seven. The mutation maps to a region of the genome containing seven genes, including possible candidates, *gna11* and *gna15*. Both are G q class alpha subunits and pair with G-Protein Coupled Receptors (GPCRs). Mutations in gna11 are associated with excessive pigmentation in mice, and mutations in *gna15* are associated with skin cancer. Still, we know little about the function of these genes during pigment development in vertebrates. The other candidates are either not associated with pigment development, or have functions that do not explain the loss of both iridophores and melanophores. To better understand the function of the *crasher* mutation during pigment cell development, we will identify the mutated gene and examine the expression of genes important for pigment cell development at different developmental stages. Additionally, zebrafish pigment cell development is easily tracked through microscopy. By studying zebrafish with mutant pigment phenotypes, such as *crasher*, we can learn about novel roles for candidate genes in developmental processes that establish and maintain pigment in vertebrates.

P.005 | Investigating the interaction of MITF and TFAP2 paralogs at shared regulatory elements

Hannah E. Seberg[1]; Mikhail V. Kulak[2]; Eric Van Otterloo[1]; Greg Bonde[1]; Ronald J. Weigel[1]; Robert A. Cornell[1]

[1]Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA, USA; [2]Department of Surgery, University of Iowa, Iowa City, IA, USA

The melanocyte gene regulatory network is relevant to the pathogenesis of pigmentation disorders and switching between invasive and proliferative phenotypes in melanoma. We have recently shown that transcription factor TFAP2A binds 70% of active enhancers in melanocytes, including many bound by the melanocyte "master regulator" MITF. It is therefore surprising that Tfap2a-/- mice and zebrafish have only mild pigmentation phenotypes, and relatively few melanocyte genes show altered expression in mouse melanocytes depleted of *Tfap2a*. We have begun to address, 1) mechanisms by which TFAP2A and MITF interact, and 2) why the loss of *Tfap2a* has so little effect on gene expression. Towards the first question, we hypothesize that TFAP2A acts as a pioneer transcription factor for MITF at shared regulatory elements. We anticipate that in the absence of TFAP2A, MITF binding at these loci will be reduced. To test this, we deleted TFAP2A from A375 melanoma cells and are conducting ChIP-qPCR for MITF. Towards the second question, we hypothesize that TFAP2 paralogs compensate for depletion of TFAP2A. We predict that eliminating all TFAP2 paralogs in melanocytes will have a profound effect on gene expression, resembling loss of MITF. Supporting this view, we recently showed that mice with double conditional knockout of *Tfap2a/b* in the neural crest exhibit a far more severe reduction in melanocytes than either single mutant. To further examine the redundant activity of paralogs, we are generating zebrafish lines triple mutant for *tfap2a/c/e*. We introduced a 157 bp deletion into zebrafish *tfap2e* using zinc-finger nucleases. Homozygous $tfap2e^{\Delta 157}$ mutants do not display a notable pigmentation phenotype, and $tfap2a^{low}/tfap2e^{\Delta 157}$ double mutants largely retain the $tfap2a^{low}$ mutant phenotype, suggesting that *tfap2c* may compensate for loss of both paralogs. Finally, we are testing whether forced Mitfa expression induces ectopic melanocytes in these mutants. These experiments further illuminate the transcriptional control of melanocyte development.

P.006 | Structural analysis of the mouse choroid in a melanocyte-deficient mouse strain

Hirotoshi Shibuya[1]; Akiteru Maeno[2]; Kaoru Ichimura[1]; Masaru Tamura[3]; Shigeharu Wakana[3]; Toshihiko Shiroishi[2]; Koji Ohba[4]; Kazuhisa Takeda[5]; Shigeki Shibahara[5,6]; HIroaki Yamamoto[1]

[1]Nagahama Institute of Bio-Science and Technology, Nagahama, Shiga, Japan; [2]Mammalian Genetics Laboratory, National Institute of Genetics, Mishima, Shizuoka, Japan; [3]Technology and Development Team for Mouse Phenotype Analysis, Japan Mouse Clinic, RIKEN BRC, Tsukuba, Ibaraki, Japan; [4]Department of Endocrinology and Applied Medical Science, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; [5]Department of Molecular Biology and Applied Physiology, Tohoku University School of Medicine, Sendai, Miyagi, Japan; [6]Faculty of Sports Science, Sendai University, Shibata-Gun, Miyagi, Japan

Mammalian melanocytes are settled in various organs and tissues. Melanocytes contribute to UV protection and the determination of coat color in the skin. However, little is known about the function(s) of extracutaneous melanocytes except for cochlear melanocytes that are essential for hearing acuity. Thinking about the localization of melanocytes in microenvironments not exposed to the sun, it remains to be elucidated whether such neural crest-derived melanocytes play a specific role at the tissue/organ where they reside in the dim light. We have focused on melanocytes in the eye that localize in the melanin-rich layer of the eye called the "uvea", which consists of the choroid, iris and ciliary body. The choroid normally develops rich capillary networks surrounded by melanocytes. What are these extracutaneous melanocytes doing in such sun-protected habitats? As the first step, in order to elucidate whether choroidal melanocytes contribute to the structure of their habitats, we used a melanocyte-deficient mouse mutant strain, *Mitf*$^{mi\text{-}bw}$. This recessive allele comprises an insertion of a 7.2 kb novel L1 element into the intron sequence located between exons 3 and 4, that abolishes expression of the Mitf-M isoform that is indispensable for melanocyte development. Thus those mice lack mature melanocytes all over the body. By comparing structural differences in the choroids of wild-type and of melanocyte-deficient *Mitf*$^{mi\text{-}bw}$/Mitf$^{mi\text{-}bw}$ mutant mice, our observations suggest that choroidal melanocytes support the morphogenesis and/or maintenance of the normal structure of that tissue. We would like to discuss the functional divergence of melanocytes localized in tissues where they reside around blood vessels.

P.007 | Investigating the role of Pcdh10a in melanocyte migration in zebrafish

Jason S. Williams; Kristin Artinger

University of Colorado Denver AMC, Aurora, CO, USA

Neural crest derived melanocyte precursors migrate along discrete pathways to reach their final destination in the skin. A mechanism by which neural crest cells undergo directed migration is via contact inhibition of locomotion (CIL), where weaker adhesion between cells is required for cells to move collectively forward. How neural crest cells maintain a weaker adhesion is not well understood. Cell adhesion proteins such as Protocadherins, similar to classic cadherins in that they function in cell adhesion and cell guidance, are good candidates to mediate a weaker adhesion required for contact inhibition. Here we tested the hypothesis that *pcdh10a* functions in zebrafish neural crest derived melanocyte precursors migration by regulating actin distribution thereby promoting CIL. Through expression and loss of function analysis, we have determined that *protocadherin10a* (*pcdh10a)* is expressed in dct+ melanocytes during neural crest migration. Loss of *pcdh10a* function results in the development of fully melanized melanocytes within the ventral pathway adjacent to the notochord and fail to reach their final position in the skin. Live cell imaging analysis suggests two phenotypes in melanocyte precursor migration: 1) dorsally located cells aggregate and cluster together; and 2) cells that are able to migrate ventrally detach from the migrating stream. In addition, actin localization in *pcdh10a*-/- neural crest cells migrating in the ventral pathway is disrupted in that actin localization along the medial cell membrane closest to the neural tube is increased. These data in combination suggest that *pcdh10a* controls migration via CIL, and in the absence of pcdh10a, a stronger adhesion between neural crest cells is observed, resulting in clumping of cells during migration and differentiation of melanocytes in ectopic locations.

This work is funded by a pre-doctoral NRSA fellowship from NIDCR (1F31DE024953) to J.S. Williams

P.008 | Characterisation of the inflammatory response in acute and chronic changes with pigmentation in the skeletal muscle of Atlantic Salmon (*Salmo salar*)

Håvard Bjørgen[1]; Øystein Wessel[1]; Subramani Kumar[2]; Espen Rimstad[1]; Erling O. Koppang[1]

[1]Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway; [2]Centre for Biotechnology, Anna University, Chennai, TN, India

Melanised focal changes are common findings at slaughter in the muscle fillet of farmed Atlantic salmon. Initially, the condition appears as a focal, intra-muscular bleeding that over time develops into a chronic, melanised phenotype. Histologically, the acute manifestation is dominated by myocyte necrosis, invasion of inflammatory cells and haemorrhage. The chronic phenotype appears as a granulomatous inflammation with infiltration of melano-macrophages, i.e. melanin-producing leukocytes. Such cells are normally found in the lymphoid organs of fishes, but they are also known for their participation in granuloma formation.

The aetiology of the condition has remained obscure until the recent association with *Piscine reovirus* (PRV) infection. PRV is a commonly occurring viral agent in farmed salmon. The virus replicates in erythrocytes and is therefore abundantly present in the muscle when a bleeding is induced. However, the initial reason for the development

of the intra-muscular bleedings is unknown. A primary viral myopathy might be causative, though it is possible that the virus appears secondary to a yet elusive cause. Nonetheless, the persistence of viral antigen is likely to be the driving power in the pathogenesis.

In the present study, the inflammatory response in relation to a PRV infection was investigated in both acute and in chronic muscular changes. Immunohistochemistry (IHC) and RT-qPCR was applied, targeting different immune cells and –genes. All changes were PRV positive by both IHC and RT-qPCR. No other infectious agents were found. The changes were characterised by varying amounts of macrophage-like cells and T-cells. B-cells were not detected.

P.009 | Development of focal melanised lesions in muscle tissue of farmed Atlantic Salmon

Håvard Bjørgen[1]; Randi Haldorsen[2]; Odd Medhus[2]; Øyvind Oaland[2]; Øystein Wessel[1]; Espen Rimstad[1]; Erling O. Koppang[1]

[1]*Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway;* [2]*Marine Harvest ASA, Bergen, Norway*

In ectothermic vertebrates, melanin production may occur in subpopulations of leucocytes. These cells, called melano-macrophages in fish, are abundant in lymphoid organs and also at sites of inflammation. The function of melanin production in melano-macrophages is unknown. Pigmented muscle lesions are common in farmed Atlantic salmon with an average of 20% affected individuals at slaughter in Norway. The current hypothesis is that such changes initiate as haemorrhages (focal red changes) which develop into focal melanised changes. Histologically, focal melanised changes are characterized as sites with chronic inflammation with abundant presence of melano-macrophages. Recently, the development of chronic inflammatory lesions were associated with the presence of piscine orthoreovirus (PRV). In this study, we followed the development of focal red and melanised changes in a population of farmed salmon. For one year, seven samplings, each including 600 individuals, were investigated. The occurrence of muscle changes were registered and samples were harvested for histological examination and transcriptional and immunohistochemical analysis for PRV infection. Initially, the study population was PRV-free. However, at the forth sampling, virus was detected in the population. We here show that focal red and melanised changes do occur in the fish prior to detection of PRV infection. The histological appearance of the focal melanised changes revealed great diversity from presence of melano-macrophages dispersed between seemingly non-affected myocytes to severe granulomatous changes with virus infection. In addition, the occurrence of focal melanised changes increase over time. Different macroscopic histological manifestations of the changes are presented in addition to histological changes in other organs.

P.010 | Driver mutation-dependent immune signature suggest role of MDSCs in melanoma patients

Dana Davis; Martin D. McCarter; Richard P. Tobin

University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA

Recent evidence suggests that patients with melanomas expressing mutated forms of NRAS or exhibiting a high mutational burden have improved responses to immunotherapies compared to patients with tumors expressing mutated BRAF. It is hypothesized that in tumors with high mutational burden, a large pool of neoantigens offer more targets for inducing an immune response. While the NRAS mutated tumors in our analysis exhibited higher mutational burden than BRAF mutated tumors, the mechanisms that result in improved response rates as well as improved overall and progression free survival of patients with tumors possessing mutated NRAS have not been fully elucidated. Using The Cancer Genome Atlas (TCGA) database, we analyzed the expression of immunologically relevant genes in tumors with known driver mutations to determine if alterations in these mutations may affect the immune response. We found that patients with mutations in BRAF had higher expression of IL-8, TGFβ, TLR2, and TLR4 than patients with mutations in NRAS. The higher expression of these genes in BRAF mutated tumors was associated with increased expression of genes often found in immunosuppressive myeloid cells including myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). Secretion of IL-8 by melanoma recruits MDSCs to the tumor microenvironment where, with TAMs and regulatory T cells, these cells suppress T cell-mediated immunity. Additionally, IL-8 expression correlated with the expression of CD47 (a don't eat me signal) in BRAF mutated, but not in NRAS mutated, tumors. Melanoma molecular markers such as these may help guide targeted rational combinations of immunotherapies to improve the immune response in select melanoma patients.

P.011 | Targeting glutamatergic signaling and PD-1 checkpoint inhibition to treat melanoma in an experimental system

Ann Robinson[1]; Raj Shah[1]; Suzie Chen[1,2]

[1]*Ernest Mario School of Pharmacy, Department of Chemical Biology, Rutgers University, Piscataway, NJ, USA;* [2]*Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA*

Melanoma, the most dangerous type of skin cancer, becomes refractory to many treatments at advanced stages, creating a need for novel therapeutic strategies to combat the disease. Current work in our lab investigates the effect of combining immunotherapy and targeted therapy to treat melanoma in an experimental system.

Metabotropic glutamate receptor 1 (mGluR1, GRM1), when aberrantly expressed in melanocytes, is constitutively activated by enhanced levels of the ligand glutamate via autocrine loop, thereby stimulating cell proliferation and melanoma development. Riluzole

inhibits the release of glutamate, thus reduces extracellular glutamate levels, thereby disrupting glutamatergic signaling. Current immunotherapy treatments known as checkpoint inhibitors have shown some success in treating various solid tumors including melanoma. Anti-PD-1 antibody functions by interrupting the interaction between programmed cell death protein 1 (PD-1) on T cells, and its ligand PD-L1, present on many cell types. Normally, the PD-1 axis promotes peripheral tolerance, but when PD-L1 is expressed by tumors, it can restrict cytotoxic T cell activity within the tumor microenvironment. Studies by others have proposed an inverse relationship between glutamate levels and T cell functions. We hypothesize that in the presence of riluzole, the alteration of extracellular glutamate level modifies the tumor microenvironment and promotes T cell functions.

To test our hypothesis, we allografted GRM1 transformed mouse C57BL/6 melanocytes into flanks of syngeneic C57/BL6 mice. When tumors became palpable, we treated animals with riluzole or anti-PD-1 alone, or combination of both.

Preliminary data shows animals receiving monotherapy or combination therapy develop smaller tumors, and can be kept alive longer than animals receiving vehicle treatment. Based on this preliminary data, we conclude that inhibiting glutamate signaling and PD-1/PD-L1 interaction can enhance antitumor activity.

P.012 | TLR9 agonist therapy expands central memory CD8 + T cells with robust anti-melanoma activity

Aubrey Smith; Megan Wyatt; Michelle Nelson; Stefanie Bailey; Jacob Bowers; Chrystal Paulos

Medical University of South Carolina, Charleston, SC, USA

To determine the mechanisms by which innate immune activation via Toll Like Receptor 9 (TLR9) signaling improves antitumor T cell immunity, we used the pmel-1 melanoma mouse model. B16F10-bearing mice were preconditioned with 5 Gy TBI and given a combination ACT therapy consisting of transferred pmel-1 $CD8^+$ T cells primed in vitro with the TLR9 agonist CpG oligodeoxynucleotide (ODN). Here we report that simply adding CpG to a T cell culture dramatically augments their engraftment, function and antitumor activity when infused into tumor-bearing mice. In fact, this therapy was as effective and safer than exogenous administration of CpG. Moreover, we found that pmel-1 $CD8^+$ T cells primed with CpG possessed a central memory phenotype and were more functional, co-secreting many cytokines including IFN-g, IL-10 and Granzyme B. Additional investigation implied that CpG indirectly augmented the memory phenotype of pmel-1 CD8 T cells by directly activating dendritic cells. Therapeutic effectiveness of this therapy was associated with enhanced persistence of the infused T cells. Collectively, our results identify how to safely and more effectively use TLR agonists to enhance T cell-based immunotherapy. Our findings have clinical implications for the design of next generation immune-based therapies for cancer patients.

P.013 | Immunomodulatory effect of c-kit signaling on myeloid cells in melanoma bearing mice

Ren-In You

Tzu Chi University, Hualien, Taiwan

Several tyrosine kinase inhibitors (TKI) for c-kit directed targeted therapy have been considered effective for some types of tumors. Recent studies showed that inhibition of oncogenic c-kit signaling by TKI reduces Indoleamine-2,3-dioxygenase (IDO) expression in mouse gastrointestinal stromal tumor. Myeloid cells surrounding the tumor express IDO, which are capable of inhibiting effective T-cell priming in tumor-draining lymph nodes thereby suppressing the anti-tumor activity of the immune system. The functions of IDO is catalyzing tryptophan by cleaving the pyrrole ring to form kynurenine thereby leading to tryptophan deprivation, also maintaining homeostasis of immune response ia non-enzymatic cell signaling actions. In this report, we found that the C57BL/6 mice that received TKI-treated myeloid cells, displayed a delayed onset of B16 melanoma growth. The immunoregulatory enzyme IDO mediated tryptophan metabolism was interfered by TKI to inhibit B16 tumor cell growth. Furthermore, there were changes in the phosphorylation status of IDO and tryptophan metabolites downstream during TKI treatment in myeloid cells surrounding B16 tumor. These findings support c-kit as part of a pathway that suppresses tryptophan metabolism in myeloid cells. The observations lead to identification of key regulators for the plasticity of myeloid cells in targeted therapy to alter tryptophan metabolism and could be considered to optimize the efficiency during melanoma immunotherapy.

P.014 | Feasibility of neuromelanin formation in Drosophila melanogaster

Hanine Barek; Manickam Sugumaran

University of Massachusetts Boston, Boston, MA, USA

Melanin is a widely distributed phenolic biopolymer in nature. Different kinds of melanin have been characterized in animal systems. The brown to black eumelanin and the yellow to red pheomelanin that occur in the skin and hair serve the role of UV protection and coloration. While both are formed by oxidative polymerization reactions, dopa serves as the precursor for eumelanin and cysteinyldopa serves as the precursor for pheomelanin. A different type of melanin called neuromelanin occurs in certain regions of brain such as catecholaminergic neurons in *substantia nigra* and *locus coeruleus* and its absence is associated with neurodegeneration. This neuromelanin includes eumelanin and pheomelanin structures but with the exception of using dopamine rather than dopa as the precursor. The existence of neuromelanin seems to be limited to higher animals and is believed to be absent in the brain of lower species. In this study, we address the possible existence of neuromelanin in insects. Comparative analyses

show a number of similarities between neuromelanin and insect melanin. Excess dopamine triggers melanogenesis and occurs without melanosomal involvement in both of these systems. Neuromelanin and insect melanin seems to provide a protective role in detoxification of reactive quinone intermediates generated by the oxidation of catecholamines. Therefore, a study was undertaken to see if insects have the potentials to make neuromelanin. The brain tissue of *Drosophila melanogaster* was isolated and activities of enzymes associated with melanogenesis were determined. Preliminary studies reveal the presence of some crucial enzymes that are known to be involved in the melanogenesis of other tissues. Our results therefore indicate that *Drosophila melanogaster* has a potential enzymatic machinery to make neuromelanin.

P.015 | Bioorganic chemistry of eumelanin

Jason Belitsky

Oberlin College, Oberlin, OH, USA

A challenge to pigment cell research from a biochemical perspective is that many questions remain about the fundamental chemistry of pigments such as eumelanin, the brown-to-black form of melanin in humans. Similarly, in the materials community, there has been an explosive growth in the interest in melanin-related materials such as polydopamine, but there is still much to be learned about the structure and properties of these materials. At Oberlin, we are using the tools of bioorganic chemistry to investigate the formation and structure of eumelanin, as well as to develop melanin-inspired materials for environmental applications. Beyond our individual lab, approximately sixty Oberlin undergraduates per year are involved in this research through a course-based research experience, "Bioorganic Chemistry of Eumelanin," in our second-semester organic chemistry class ("Bioorganic Chemistry" CHEM 254). Initiated by the oxidative transformation of tyrosine/L-dopa to dihydroxyindoles, eumelanin is thought to form from the self-assembly of heterogeneous dihydroxyindole oligomers. We are investigating this process by two complimentary routes: synthesis of well defined dihydroxyindole oligomers and small molecule screening for modulators of biomimetic oxidative polymerizations. Screening by CHEM 254 students has uncovered inhibitors, aggregation promoters and delayers, and, most recently, small molecule catalysts for synthetic eumelanin polymerizations. Eumelanin is known to bind a wide variety of metal ions and organic compounds; in addition to using these interactions as probes to interrogate eumelanin formation and structure, these molecular recognition interactions can be leveraged for water purification and heavy metal sensing applications. In particular, we are developing synthetic eumelanin-based water purification agents and catechol-based colorimetric sensors for lead. The latter project has assumed particular importance given the recent incidences of lead contamination in Flint, Michigan and other US cities. This presentation will give an overview of the melanin-related projects in our lab and their extension to the course-based research experience format.

P.016 | Role of AMP-activated protein kinase signaling in melanocytes

Sung Eun Chang[1]; Seunghyun Bang[1]; Hanju Yoo[2]; Young Jin Lim[3]; Youngsup Song[2]

[1]Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [2]Department of Biomedical Sciences, University of Ulsan College of Medicine, Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea; [3]Department of Anesthesiology, Seoul National University hospital, Seoul, Korea

Adiponectin is an important adipokine which lowers obesity and diabetes by increasing insulin sensitivity via AMPK pathway. In our recent microarray data, *adiponectin* decreased in the melasma lesion. Thus we investigated the effect of adiponectin and AICAR, a cell permeable activator of AMPK in melanocytes. We showed that adiponectin and AICAR reduced melanin content, MITF, tyrosinase, TRP-1 and TRP-2 via AMPK/CREB regulated transcriptional coactivator (Crtc) inhibitory phosphorylation. This anti-melanogenic effect was correlated with decreased transcriptional activity of CREB and lentivirus-mediated knockdown of Crtc decreased MITF, tyrosinase, TRP-1 and TRP-2. These data emphasize the dermatologic beneficial effects of adiponectin and AMPK activators and suggest a clinical strategy for using its analogues in the treatment of melasma and post-inflammatory hyperpigmentation after acute skin injury.

P.017 | Facial hyperpigmented spots have thicker stratum corneum and drier skin

Steven Zhao[1]; Bradley Jarrold[1]; Timothy Laughlin[1]; Yoko Munakata[2]; Reiko Yamamura[2]; Megumi Watanabe[2]; Tomohiro Hakozaki[1]

[1]The Procter & Gamble Company, Mason, OH, USA; [2]P&G Innovation Godo Kaisha, Kobe, Japan

To better understand the underlying biology of 5 different facial hyperpigmented spot types: solar lentigo (SL: age spots), seborrheic keratosis (SK), melasma, freckles and post inflammatory hyperpigmentation (PIH), we collected 2 mm biopsies of facial spot as well as adjacent non-spot tissue from 77 Asian women, ages 20–70. Histological feature analysis was conducted to observe morphological alterations in different spot types compared to non-spot tissue. Laser Capture Microdissection was conducted to fractionate all biopsies into 3 compartments; suprabasal epidermis, basal epidermis and dermis. Gene expression changes in each compartment were assessed using Affymetrix U-219 microarrays. Additionally, the hydration level of SL spots and adjacent non-spot skin was assessed among 19 Japanese subjects (total 80 spots) using a moisture meter (ASA-MX100) with a 2 mm probe.

Compared to adjacent non-spot tissue, the stratum corneum (SC) thickness was significantly increased in 3 spot types: SL, SK and melasma. Transcriptomic analysis indicated that keratinocyte differentiation and skin keratinization themes are significantly up-regulated in

multiple spot tissues, which could partially explain the thicken SC observed in these 3 spot types.

Interestingly, increased SC thickness did not directly result in increased hydration. The skin hydration assessment demonstrated that SL spot tissue was significantly dehydrated compared with adjacent normal tissue ($p < .05$). Transcriptomic analyses indicated that the skin barrier function in most types of spot tissues could be significantly interrupted due to the reduced expression of tight-junction proteins (CLDN1 and/or CLDN4), which may partially contribute to the dehydration observed in SL spot tissues.

Our results demonstrate that some spot types including SL have thicker SC than adjacent non-spot tissue. Additionally, the hydration level of SL spots is lower. Both of these factors are considered to impact delivery of treatment materials, thus it is important to account for these attributes when seeking effective spot treatment technologies.

P.018 | Tyrosinase-catalyzed oxidation of 4-(4-hydroxyphenyl)-2-butanone (raspberry ketone): Implications for melanocyte toxicity

Shosuke Ito[1]; Maki Hinoshita[1]; Erina Suzuki[1]; Makoto Ojika[2]; Kazumasa Wakamatsu[1]

[1]Department of Chemistry, Fujita Health University School of Health Sciences, Toyoake, Japan; [2]Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

The exposure of human skin to 4-(4-hydroxyphenyl)-2-butanone (raspberry ketone, RK) is known to cause chemical/occupational leukoderma. RK has a structure closely related to 4-(4-hydroxyphenyl)-2-butanol (rhododendrol), a skin whitening agent that was found to cause leukoderma in the skin of consumers in 2013. Rhododendrol is a good substrate for tyrosinase and causes a tyrosinase-dependent cytotoxicity to melanocytes (ref 1–5), cells that are responsible for skin pigmentation. Therefore, it is expected that RK exerts its cytotoxicity to melanocytes through the tyrosinase-catalyzed oxidation to cytotoxic *o*-quinones. The results of this study demonstrate that the oxidation of RK by mushroom tyrosinase rapidly produces 4-(3-oxobutyl)-1,2-benzoquinone (RK-quinone), which is gradually converted to (*E*)-4-(3-oxo-1-butenyl)-1,2-benzoquinone (DBL-quinone). These quinones were identified as their corresponding catechols after reduction by ascorbic acid. RK-quinone and DBL-quinone quantitatively bind to the small thiol *N*-acetyl-L-cysteine to form thiol adducts, and can also bind to the thiol protein bovine serum albumin through its cysteinyl residue. DBL-quinone is more reactive than RK-quinone, as judged by their half-lives (6.2 min vs 10.5 min, respectively), and decays rapidly to form an oligomeric pigment (RK-oligomer). RK-oligomer can oxidize GSH to GSSG with a concomitant production of hydrogen peroxide, indicating its pro-oxidant activity, similar to RD-oligomer. These results suggest that RK is cytotoxic to melanocytes through the binding of RK-derived quinones to thiol proteins and the pro-oxidant activity of RK-oligomer (reported in ref 6).

References: 1. Ito et al., PCMR 27, 744, 2014. 2) Sasaki et al., PCMR 27, 754, 2014. 3) Ito et al., PCMR 27, 1149, 2014. 4) Ito et al., PCMR 28, 295, 2015. 5) Ito et al., PCMR 30, 63, 2017. 6) Ito et al., Chem Res Toxicol 30, 859, 2017.

P.019 | Quantum dynamics of branching reactions involved in melanogenesis

Hideaki Kasai[1]; Ryo Kishida[2]

[1]National Institute of Technology, Akashi College, Akashi, Japan; [2]Department of Applied Physics, Osaka University, Suita, Japan

Melanogenesis is initiated by tyrosinase-catalyzed oxidation of tyrosine, dopa, or similar phenols/catechols to form corresponding *o*-quinones. These *o*-quinones are in the branching point of melanogenesis [1]. Until cellular cysteine is consumed enough, the *o*-quinones are bound with cysteines, resulting in pheomelanogenesis. At a lower concentration of cysteine, the side-chain nucleophile in the *o*-quinone can undergo intramolecular cyclization, resulting in eumelanogenesis. The cyclization and cysteine binding proceed via various elementary processes including protonation, deprotonation, and intramolecular proton rearrangement. For instance, cyclization of *o*-quinone amines proceeds via a bond formation between amino group and benzene ring, which is followed by deprotonations from the amino nitrogen and the attacked carbon. Due to the small mass of proton, such proton transfer processes are essentially quantum phenomena that cannot be completely described by classical mechanics. In the present study, we investigated the above-mentioned proton transfer processes based on quantum dynamics theory. We calculated the potential energy curves for proton transfer processes using density functional theory based calculations. For the proton quantum tunneling on the obtained potential energy curve, we carried out the coupled-channel calculation which has been developed in author's groups [2].

We compared cyclizations of dopaquinone and dopamine quinone. For dopaquinone cyclization, we assumed that a carboxyl oxygen acts as the proton acceptor. For dopamine quinone cyclization, we placed water molecules as the proton acceptor. Our results showed that amino deprotonations of dopaquinone and dopamine quinone require activation energy of 0.22 eV and 0.45 eV, respectively. Quantum dynamics calculation showed that, in the case of dopaquinone, proton tunneling can proceed even at a half of activation energy, whereas dopamine quinone cycilzation showed a less remarkable tunneling effect.

Our findings emphasize the importance of quantum effects in melanogenesis.

[1] R. Kishida et al. J. Electron. Mater. (2017) https://doi.org/10.1007/s11664-017-5299-x.

[2] W. Brenig, H. Kasai, Surf. Sci. 213 (1989) 170.

P.020 | Melanin structure research; quantum chemical study and experimental results of 5, 6-dihydroxyindole oligomer as eumelanin model molecules

Kou Shiratsuki[1]; Keiji Monda[1]; Takahito Nakamura[1]; Hidekazu Okuda[2]; Takayuki Sota[2]; Kenzo Koike[1]

[1]*Kao Corporation, Tokyo, Japan;* [2]*Waseda University, Tokyo, Japan*

It has been well known that eumelanin is a photoprotective brown-to-black macromolecule produced by oxidative polymerization of 5,6-dihydroxyindole (DHI) and/or other precursors. However, its three-dimensional (3D) structure(s) as macromolecules and relationship between their absorption spectra and oxidation status of each unit are less understood. Previously we tried to analyze some structures of eumelanin by quantum chemical study and demonstrated some possible assemblies of DHI tetramers with 2,4'- or 2,7'-homo linkages at IPCC2011 (Bordeaux). In these studies, all quantum chemical calculations were carried out using Gaussian 03. The molecular structures have been optimized using Hartree–Fock theory with the 6-31G(d) basis set, and thermochemical effects have been calculated to estimate the molecular stability using the Gibbs free energies at 300K. The electronic energies for the corresponding molecular structures have been estimated at the B3LYP/6-31++G(d,p) level of theory.

As an advanced step, absorption spectra of melanin structures were estimated based on the quantum chemical study, and also actual spectra of melanin oligomers (DHI melanin) were measured with a spectrophotometer. It is thought that the oxidative status can be affected the spectrum of melanin as to our simulation results. The further structure analyses will be needed for confirmation of the simulation.

P.021 | A new in vitro pigmented human skin 3D model for studying glycation impacts and testing protective compounds

Robin Kurfurst[1]; Marion Albouy[2]; Olivier Jeanneton[1]; Aurelie Boher[2]; Amelie Thepot[2]; Sylvianne Schnebert[1]; Karl Pays[1]; Bruno Bavouzet[1]

[1]*LVMH RECHERCHE, Saint Jean de Braye, France;* [2]*LabSkin Creations, Lyon, France*

Glycation is a process responsible for skin aging through induction of deleterious formation of advanced glycation end-products (AGEs). Glycation is induced and promoted by many factors such as oxidative stress or UV exposure which drastically lead to AGEs accumulation. AGEs alter skin physiology by impairing deposition, organization and physicochemical properties of dermal extracellular matrix as well as affecting epidermal keratinocyte differentiation. In addition to these modifications, recent studies demonstrated that AGEs also promote melanogenesis through activation of AGEs receptors in melanocytes, strengthening role of glycation on skin pigmentation associated with photo-aging.

Studies about glycation employing skin tissue-engineering strategies rely on 3D constructs based on pre-glycated collagen before cell seeding. This approach allows only examining impacts of AGEs on extracellular matrix though glycation also have direct effects on cells.

We developed a new glycated full-thickness pigmented skin equivalent model by inducing continuous AGEs production during model formation. We used a unique scaffold made of collagen-glycosaminoglycan-chitosan polymer colonized by fibroblasts, providing a genuine dermis-type matrix well suited for epidermisation. We showed that our skin equivalent model integrating melanocytes, cultured for 42 days and systemically treated with a combination of glyoxal/methylglyoxal, displayed a high accumulation of N-(6)-carboxymethyl-lysine (CML) and a strong increase in melanin synthesis without changes in melanocyte number. An aging phenotype consisting of poor epidermal stratification and differentiation and dermal alterations was also observed.

AGEs-mediated alterations were prevented by aminoguanidine which validated the functionality of our model. An edelweiss extract was studied on this reconstructed skin and it exhibited protective activities against glycation e.g. by preventing CML formation and reducing melanin synthesis.

This approach is relevant to explore molecular mechanisms underlying impacts of long-term glycation on skin including melanogenesis and provides for the first time a unique model for studying new compounds for protecting skin against glycation-induced damage.

P.022 | Structural modification in oxidative coupling reaction of dopamine to cysteine-containing peptidic moieties

Kenta Nakao[1]; Yusuke Kumura[2]; Makoto Ojika[3]; Shosuke Ito[2]; Kazumasa Wakamatsu[1,2]

[1]*School of Health Research, Graduate School of Health Sciences, Fujita Health University, Toyoake, Japan;* [2]*Department of Chemistry, Fujita Health University School of Health Sciences, Toyoake, Japan;* [3]*Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan*

Background and Objectives: Although it is known that DA binds covalently to proteins through the sulphydryl group, the fate of protein-bound DA compounds during subsequent oxidation is little understood. We examined whether and how protein-bound DA structure is modified by the subsequent oxidation.

Methods: We used DPRA (Ac-R-F-A-A-C-A-A-COOH) as a model peptide, BSA as a representative protein, and β-lactoglobulin as a model protein, which contains an accessible and reactive Cys and can be converted into amyloid fibrillar form. We performed the oxidative coupling of DA with these cysteine-containing compounds and the products were analyzed periodically by spectral changes and HCl hydrolysis.

Results: The oxidative coupling reaction of DA with DPRA afforded DPRA-5-*S*-DA (40% after preparative HPLC) as a major product.

HCl hydrolysis of DA-BSA conjugates obtained by mushroom tyrosinase oxidation afforded 5-*S*- and 2-*S*-cysteinylDA in a quantitative yield. Absorption spectra showed a maximum at 480 nm for up to 60 min, indicating the production of a rather stable *o*-quinone structure. This was gradually replaced by a flat absorption suggesting a conversion to melanic moiety. On the contrary, DA-β-lactoglobulin conjugates afforded a much lower yield (17%) of cysteinylDA isomers even at the early phase, suggesting a much faster modification of the structure. The oxidative coupling of DA with BSA by Cu^{2+} proceeded gradually and steadily and reached to a peak at 5 hr in 77% yield followed by a slow decline to 61% at 24 hr. The oxidation with Fe^{2+} proceeded at a rate half that by Cu^{2+} and reached to 71% yield at 24 hr.

Conclusion: The results show that the cysteinyl residues in various proteins can bind covalently with DAquinone generated not only by tyrosinase but also by redox active ions. The subsequent fate of the conjugates depends on the structural feature of proteins.

P.023 | Melanin from 5,6-dihydroxyindole-2-carboxylic acid methyl ester with a potential for dermo-cosmetic applications

Alessandra Napolitano[1]; Raffaella Micillo[1]; Mariagrazia Iacomino[1]; Lucia Panzella[1]; Kenzo Koike[2]; Marco d'ischia[1]

[1]*University of Naples Federico II, Naples, Italy;* [2]*Kao Corporation, Tokyo, Japan*

The superior antioxidant properties of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) melanin with respect to 5,6-dihydroxyindole and DOPA melanins (Panzella et al. *Angew. Chem. Int. Ed.* 2013, 52, 12684–12687; Jiang et al. *Free Radic. Biol. Med.* 2010, 48, 1144–1151) would hint to its exploitation in a variety of applications including dermocosmetic formulations. Major limitations include the unfavorable solubility properties, particularly in hydroalcoholic or more hydrophobic media, and the relative ease to undergo degradation e.g. by photooxidation under UVA with loss of properties as recently reported (Ito et al. *Pigment Cell Melanoma Res.* 2016, 29, 340–351). This work was aimed at assessing whether melanin obtained by oxidation of the methyl ester of DHICA, *MeDHICA melanin*, could combine the marked antioxidant properties of DHICA melanin with more favorable solubility properties warranted by esterification of the carboxyl group. *MeDHICA melanin* was obtained in 65% yield by aerial oxidation of the monomer in phosphate buffer at pH 8.5 until its complete consumption (HPLC analysis). MALDI MS analysis provided evidence for a clean collection of intact oligomers as main constituents of this melanin. EPR features were similar to those of DHICA melanin confirming a good degree of homogeneity of the free radical components, but spin density values were almost one order of magnitude higher. In currently used antioxidant assays *MeDHICA melanin* performed well and in some cases better than DHICA melanin. In addition, this capacity was found to be unaltered after exposure to air in aqueous buffer over one week or following photoexposure to solar simulator over 3 hr. *MeDHICA melanin* proved fairly soluble in different water miscible organic solvents, with a complete solubilization in DMSO at 0.3 mg/ml conc. All together these properties would point to *MeDHICA melanin* as a stable bio-based antioxidant ingredient for dermocosmetic formulations and other health related applications.

P.024 | Keap1 knockdown in melanocytes induces cell proliferation and survival via HO-1-associated β-catenin signaling

Ji Young Kim[1]; Hemin Lee[1]; Eun Jung Lee[1]; Tae-Gyun Kim[1]; Hyoung-Pyo Kim[2]; Sang Ho Oh[1]

[1]*Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea;* [2]*Department of Environmental Medical Biology, Institute of Tropical Medicine, Yonsei University College of Medicine, Seoul, Korea*

Background: Nrf2-Keap1 signaling pathway protects cells against photo-oxidative stress. Yet in recent works, its role in melanogenesis together with cell protection functions against oxidative stress has been gaining interest. However, its effect on melanogenesis still has contradictory results from different studies.

Objective: he aims of our study were to investigate the effect of Keap1 silencing in melanocyte on melanogenesis and its associated mechanism.

Methods: Primary human epidermal melanocytes and melan-a cell line were used for this experiment. RNA sequencing was done to identify genes involved in melanocyte biology using Keap1 knockdown through siRNA techniques. And melanogenesis and the expression of melanogenesis-associated molecules were evaluated in Keap1 silenced melanocyte to examine the effects of Keap1 on melanogenesis, melanocyte growth, and related pathways.

Results: RNA-sequencing data revealed that Keap1 knockdown in primary human epidermal melanocytes (PHEMs) induced cell survival-related gene expression. Additionally, siRNA-mediated inhibition of Keap1 led to upregulation of MITF and melanogenesis-associated molecules along with Nrf2 activation in PHEMs. HO-1, a major gene that is upregulated in RNA-sequencing using Keap1-silenced PHEMs, protected melanocytes against H_2O_2-induced cell death and upregulated MITF and β-catenin expression. Further, increased expression of melanogenesis-associated molecules after Keap1 silencing was validated to occur through HO-1-associated β-catenin activation in a Keap1 and HO-1 double knockdown experiment.

Conclusion: This work suggests that Keap1 silencing in melanocytes induced melanogenesis and the expression of melanogenesis-associated molecules through HO-1-associated β-catenin activation. Keap1 downregulation in melanocytes is important for cell proliferation and survival.

P.025 | Ets Homologous Factor (EHF), a novel factor involved in melanogenesis

Shoko Sassa[1]; Yoshihiro Hamanaka[1]; Chihiro Kondo[1]; Tamio Suzuki[2]

[1]POLA Chemical Industries, Inc., Yokohama, Japan; [2]Yamagata University Faculty of Medicine, Yamagata, Japan

In solar lentigos (age spots, SL), melanogenesis is augmented and the melanin is profusely deposited in basal cells in epidermis. Variety of melanogenic factors, which are secreted from keratinocytes and stimulate melanin synthesis such as α-MSH in SL is known to be involved in melanogenesis. Even though they are released from keratinocytes in an orchestrated manner, the whole picture of the underlying mechanism is still remained unsolved. Besides, single nucleotide polymorphism (SNPs) is reported to be involved in skin tone recently. For elucidating how melanogenesis is controlled in an orchestrated manner, identifying melanogenesis master regulator was conducted by focusing on SNPs.

DNA has been extracted from the blood samples of 298 Japanese women healthy volunteers. By genomic analysis, distinctive SNP were identified on the locus of Ets homologous factor (EHF), which is one of the transcriptional factors, having a positive correlation with SL severity.

In human keratinocyte, the expression of tumor necrosis factor (TNF), Interleukin-6, endothelin, granulocyte-monocyte colony stimulating factor (GM-SCF) mRNA were found to be upregulated in EHF knock down samples. For investigating the paracrine effect on melanogenesis, the co-culture system of EHF knocked down keratinocyte and melanocyte were constructed, resulting that in the system with EHF knocked down keratinocyte, melanin production was induced dramatically.

A novel factor EHF has been identified and proved to be involved in melanogenesis. EHF knocked down keratinocyte produced several melanogenic factor and augmented melanin production in the keratinocyte-melanocyte network in vitro. Taken together, these results suggest that EHF may be a novel suppresser of melanogenic factors in keratinocytes, and its function might be deteriorated in SL.

P.026 | Melatonin and its metabolites enhance the DNA repair in human melanocytes exposed to UVB

Zorica Janjetovic[1,3]; Stuart G. Jarrett[2]; Elizabeth F. Lee[3]; Corey Duprey[3]; Russel J. Reiter[4]; Andrzej T. Slominski[1,5,6]

[1]University of Alabama at Birmingham, Birmingham, AL, USA; [2]Department of Toxicology and Cancer Biology, The Markey Cancer Center, College of Medicine, University of Kentucky,, Lexington, KY, USA; [3]Department of Pathology and Laboratory Medicine, University of Tennessee HSC, Memphis, TN, USA; [4]Department of Cellular & Structural Biology, UT Health Science Center, San Antonio, TX, USA; [5]Comprehensive Cancer Center, Cancer Chemoprevention Program, and Nutrition Obesity Research Center, University of Alabama at Birmingham, Birmingham, AL, USA; [6]VA Medical Center, Birmingham, AL, USA

Ultraviolet radiation (UVR) induces DNA damage in skin cells by producing cyclobutane pyrimidine dimers (CPD), pyrimidine photoproducts (6-4)PPs plus augmenting the production of reactive oxygen species (ROS) with deleterious effects on skin. It also induces tumor suppressor factor p53, as a part of response to DNA damage. Melatonin and its metabolites: 6-hydroxymelatonin (6-OHM), N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), *N*-acetylserotonin (NAS), and 5-methoxytryptamine (5-MT), reduce DNA damage caused by UVB irradiation in human melanocytes. We measured the DNA repair capacity of melatonin and its metabolites in melanocytes exposed to UVB. Treatment with melatonin or its metabolites caused significant reduction of CPD levels in cells exposed to UVB. DNA damage and repair were further assessed by comet assay. We exposed cells to UVB and treated them with the mentioned compounds. DNA damage and repair assessment showed that melatonin and its derivatives significantly reduced the tail moment of the comets ($p < .001$). Melatonin is known to induce phosphorylation of p53 at Ser-15, thus activating p53. We tested the ability of melatonin and its metabolites to induce p53 phosphorylation at Ser-15 as a response to UVB damage. All molecules tested significantly enhanced the expression of Ser-15 phosphorylated p53. Further, melatonin and its metabolites actions directly affect nucleotide excision repair (NER). Using an Oligonucleotide retrieval immunoprecipitation (ORiP) technique we proved that melatonin or its metabolites significantly enhanced the XPC and XPA interactions with the DNA substrate. Thus, by documenting the melatonin's capabilities to induce DNA repair mechanisms in melanocytes (shared also by its metabolites), we identify melatonin as the natural protector against UVR.

P.027 | Isolation of neural crest stem cells from hair follicle bulge and differentiation induction into melanocyte precursors by BMP-4 and α-MSH treatment

Tae-Hoon Kim[1]; Chang-Hoon Seo[2]; Young-Kwan Sung[2]; Ki-Ho Kim[1]

[1]Department of Dermatology, College of Medicine, Dong-A University, Busan, Korea; [2]Department of Immunology, School of Medicine, Kyungpook National University, Daegu, Korea

Background: The repigmentation in the combination treatment of follicular unit extraction (FUE) graft and NBUVB is much superior to that in interfollicular epidermis micropunch graft and NBUVB. So we guess a better therapeutic outcome of FUE graft may be due to prolonged life span and survival of hair follicle melanocytes. Various stem cells such as pluripotent epithelial stem cells, melanocyte stem cells (MSCs) and Neural crest stem cells (NCSCs) are present in the bulge of human hair follicle. NCSCs can migrate and differentiate into multipotent lineages via bipotent precursor of Schwann cell and melanocyte. BMP4 can induce MITF expression in NCSCs and postulated MSCs, and α-MSH subsequently promotes differentiation of MITF-expressing cells along the melanocyte lineage.

Objective: The aim the study is to ascertain whether the NCSCs could be isolated directly from human hair follicle bulge instead of embryonic stem cells, and induced to differentiate along the melanocyte

lineage by BMP-4 and α-MSH. And we try to utilize the NCSCs as the cell sources for better repigmenting therapy in vitiligo treatment in terms of cell survival and life span of melanocytes

Methods and Results: After we obtained a population of cells with NCSC marker, SOX10, during the emigrated cell culture from hair follicle bulge portions of adult human scalp; Firstly, a promoted proliferation of SOX10(+) cells by basic FGF were observed compared to control. Secondly, the emigrated hair bulge cells did not spontaneously differentiate into MITF-expressing cells, but into SOX2-expressing Schwann cell progenitors after prolonged cultivation. Thirdly, the differentiation into MITF-expressing cells was promoted after BMP-4 and α-MSH treatment.

Conclusion: The data showed that basic FGF promoted the proliferation and survivals of NCSCs with spontaneous differentiation into SOX2(+) Schwann cell progenitors, but not into MITF-expressing cells. And BMP-4 and α-MSH promoted a differentiation into MITF-expressing cells.

P.028 | Niche-derived KIT ligand is essential for maintenance of melanocyte stem cells

Yasuaki Mohri[1]; Naotaka Serizawa[1]; Hironobu Morinaga[1]; Sean Morrison[2]; Emi Nishimura[1]

[1]Tokyo Medical and Dental University, Tokyo, Japan; [2]University of Texas Southwestern Medical Center, Dallas, TX, USA

Melanocyte stem cells (McSCs), somatic stem cells of the melanocyte lineage that serve as a reservoir for melanocytes in the skin, have been identified in hair follicles and sweat glands. Hair follicle stem cells (HFSCs), located in the hair follicle bulge and in the hair germ (HG), provide a functional niche for follicular McSCs by secreting cytokines such as TGF-β and Endothelin, yet the exact molecular mechanisms by which the niche dominantly determines McSC fates in physiological settings are still unknown. Our previous studies showed that McSCs that are kept in a quiescent state do not require KIT signaling for their maintenance, while their activated progeny in hair bulbs requires KIT signaling for their survival and maturation for hair pigmentation. Here we report that KIT ligand (KITL) is expressed by HFSCs, the niche cells for McSCs that enable them to play dominant roles in stem cell maintenance and renewal thereby maintaining cyclic hair pigmentation. Analysis of HFSC-specific *Kitl*-deficient mice shows that McSCs become depleted from the niche during their activation for self-renewal after the induction of the *Kitl*-deficiency in HFSCs and display a prominent hair graying phenotype in subsequent hair cycles. These results demonstrate that niche-derived KITL is critical for the process of self-renewal of McSCs.

P.029 | Dysregulation in basic cellular processes during quiescence drives melanocyte stem cell loss

Joseph W. Palmer; Melissa L. Harris

University of Alabama at Birmingham, Birmingham, AL, USA

One aspect of aging is stem cell exhaustion— stem cells lose their regenerative potential and the body slowly declines as a result. Hair graying has long been associated with the aging process, characterized by a decrease of melanocyte stem cells (McSCs) residing in the hair follicle. While the presence of ectopic pigment is a commonly used indicator for dysregulation of this population with age, the underlying mechanism has not yet been fully described. Previous studies have shown that quiescent McSCs maintain an undifferentiated state. By utilizing an acute hair graying mouse model known to drive premature differentiation of McSCs, we confirmed that in quiescence this predisposition did not induce differentiation. Implying that during quiescence, McSCs may acquire genetic changes that predetermine their fate upon reactivation. To investigate whether aging quiescent McSCs show sufficient genetic change to influence stem cell loss, purified McSCs were collected from C57BL/6J mice at breeding age and 2-years. Whole genome gene expression analysis was performed, and the data refined using an in-house bioinformatics pipeline. Although 1006 genes were found to be differentially expressed, no changes in pigmentation or differentiation pathways were observed. Instead the data revealed changes in key pathways related basic cellular functions with a cluster of 67 ribosomal genes being down regulated with age. Taken together these results suggest that disruption in basic cellular processes, ultimately drives McSC loss with age. Our aim is identifying the key drivers behind early dysregulation of stem cell maintenance for use as a biomarker in detection of long-term stem cell viability. Using the gene list produced here, four other mouse strains from different backgrounds that vary in life expectancy will be compared to determine whether this signature pattern is seen across strains and how it plays a role in the overall longevity of these different backgrounds.

P.030 | Gene expression analysis of melanocyte stem cell sub-populations in bulge and secondary hair germ region of hair follicle

Bishal Tandukar[1]; Sandeep Joshi[1]; Theresa Hodges[2]; Anup Mahurkar[2]; Sean Daugherty[2]; Amol Shetty[2]; Thomas J. Hornyak[1,3,4]

[1]Department of Biochemistry and Molecular Biology, University of Maryland Baltimore, Baltimore, MD, USA; [2]Institute of Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA; [3]Department of Dermatology, University of Maryland School of Medicine, Baltimore, MD, USA; [4]Research and Development Service, VA Maryland Health Care System, Baltimore, MD, USA

Melanocyte stem cells (McSCs) are key components of the hair follicle (HF) stem cell system that are derived from neural crest during embryogenesis and are responsible for regeneration of differentiated melanocytes during successive HF cycles. We have described McSC subsets that can be distinguished by expression of CD34 by McSCs located within the bulge/lower permanent portion (LPP) and lack of its expression in McSCs in the secondary hair germ (SHG). Whether these two cell subpopulations arise independently or exist in a developmental hierarchy is not yet known.

Our *Dct*-H2BGFP bitransgenic mouse model permits accurate identification of the McSCs and melanocytes in the murine HF through GFP expression. To further explore the differences between CD34 + McSCs

from bulge/LPP and CD34- McSCs from the SHG, we compared the transcriptomes of the McSC subsets using RNA-seq. (Method) McSCs were isolated by fluorescence-activated cell sorting (FACS) from telogen HFs of P56 murine skin. Analysis of RNA-seq data showed higher expression of neural crest stem cell markers including *Ngfr*, *Gli1*, *BMP7*, *Snai1* and *Twist2* in CD34 + /bulge McSCs compared to CD34-/SHG McSCs. Similarly, CD34-/SHG McSCs showed higher expression of melanogenic markers and melanocyte specific transcription factors including *Tyrp1*, *Dct*, *Tyr*, *Mitf*, *Pax3*, *Slc45a2* and *Pmel17*. The results were confirmed using quantitative real-time PCR. Ingenuity Pathway Analysis (IPA) revealed higher expression of molecular components of pathways related to embryonic stem cell pluripotency in CD34 + /bulge McSCs while CD34-/SHG McSCs showed higher expression of genes related to melanocyte development and pigmentation signaling. These results suggest that CD34 + /bulge McSCs share characteristics of neural crest stem cells while CD34-/SHG McSCs represent a stem cell population that is more committed to melanocyte differentiation.

P.031 | Derivation of induced pluripotent stem-like cells from melanoma cell lines

Edgardo Castro-Perez[1,2]; Vijayasaradhi Setaluri[1,2]

[1]*Department of Dermatology, UW-Madison, Madison, WI, USA;* [2]*William S. Middleton Memorial Hospital, Madison WI, Madison, WI, USA*

Patient-derived induced pluripotent stem cells (iPSC) have become a valuable tool to model diseases in vitro and screening of drugs for treatment. More recently, cancer cell-derived iPSCs have been generated to study cancer cell plasticity in relation to the tissue of their origin and differential response to therapy. However, it is not clear whether melanoma cells can be reprogrammed to iPSC cells. Here, we report that both primary and metastatic melanoma cells can generate iPSC-like cells, albeit less efficiently than primary fibroblasts. Additionally, the efficiency of reprogramming of melanoma cells is significantly higher for primary than for metastatic melanoma cell lines. Moreover, melanoma-derived iPSC-like cells expressed the stem cell marker alkaline phosphatase and showed expression of reprogramming factors. Interestingly, we found that like in fibroblasts, transduction of metastatic melanoma cells with reprogramming factors induced senescence and cell death, whereas in primary melanoma cells there was no induction of senescence or apoptosis markers. We found that high senescence and cell death were inversely associated with success in reprogramming. Double staining of OCT4 or NANOG showed that senescent-positive cells displayed less expression of either OCT4 or NANOG. We also found that regardless of their senescence status, continued cell proliferation following transduction with reprogramming factors is required for successful reprogramming. Ongoing studies are aimed at understanding the relationship of this plasticity of melanoma cells to the cellular precursors of melanoma, tumor trans-differentiation and progression and melanoma drug-resistance.

P.032 | Domains controlling the subcellular localization and stability of MITF-M

Valerie Fock[1]; Sigurdur Gudmundsson[1]; Vivien Ionasz[1]; Jon August Stefansson[1]; Vivian Pogenberg[2]; Margret Ogmundsdottir[1]; Eirikur Steingrimsson[1]

[1]*University of Iceland, Reykjavik, Iceland;* [2]*EMBL Hamburg, c/o DESY, Hamburg, Germany*

Microphthalmia-associated transcription factor (MITF) is a member of the basic helix-loop-helix leucine zipper (bHLH-Zip) family and functions as a master regulator of the melanocytic lineage. Mutations in human *MITF* have been associated with melanoma, Waardenburg syndrome type 2A (WS2A) and Tietz syndrome. MITF-M is the predominant isoform expressed in melanocytes and melanoma cells and, unlike other MITF isoforms, is found constitutively nuclear. Surprisingly, very little is known about the domains in MITF-M that control its subcellular localization and stability. To address this question, we generated EGFP-MITF-M fusion proteins truncated in their N- or C-termini and studied their localization pattern and turnover rate in 501mel and HEK293T cells. Our data indicate that MITF-M mutants lacking the basic region or leucine zipper domain showed a prominent cytoplasmic localization. Protein sequence analysis identified several clusters of basic amino acid residues in the DNA binding and HLH-Zip domains of MITF-M as potential nuclear localization signals. Consistent with that, amino acid substitution of residues 203/205/206, 214-217 or 255/256/259/263/265 strongly interfered with the nuclear localization of the protein. Importantly, a number of these residues, including R203, K206 and R217, are found mutated in patients with WS2A and Tietz syndrome. Interestingly, structural characterization of MITF confirmed that these three nuclear localization signals are exposed for interactions in the absence of DNA. By performing cycloheximide assays, we found that dimerization-deficient MITF-M mutants had a significantly reduced half-life when compared to the wild type protein. These findings suggest that MITF dimers are more stable than monomers. Taken together, we have shown that, in addition to its established role in DNA binding and dimer formation, the bHLH-ZIP domain of MITF modulates the transcription factor's subcellular localization and stability.

P.033 | Keratinocyte-paracrine factors in melanocyte homeostasis

Manale El Kharbili; Nema Sobhani; Neil Box; Tamara Terzian

University of Colorado, Department of Dermatology, Aurora, CO, USA

Cutaneous melanoma is the deadliest form of skin cancer with an incidence that continues to increase in the United States (477% increase from 1950 to 2000, SEER). Ultraviolet radiation (UVR) plays a major role in melanomagenesis by inducing tumor-initiating mutations and promoting proliferation of transformed melanocytes. BRAF (~50%) is the most frequently mutated gene in melanoma and BRAFV600E represents the most commonly occurring isoform (>90%). This

oncogenic mutation leads to constitutive activation of the mitogen-activated protein kinase (MAPK) signaling pathway and higher oncogenic potential through a variety of mechanisms including increased invasiveness, metastasis and immune escape capacity, and reduced apoptosis. Interestingly, BRAFV600E is associated with intense sun damage-induced superficial spreading melanomas. UVR may stimulate the expansion of initiated melanocytes through the upregulation of melanocyte growth factors secreted by surrounding keratinocytes. Here, we investigate the role of keratinocytes in regulating normal and BRAFV600E mutant melanocyte proliferation in response to high doses of UVR using human culture systems. Our data demonstrate the contribution of paracrine factors produced by keratinocytes to melanocyte cell cycle progression and proliferation. We aim to identify key keratinocyte-derived signals factors mediating this cross-talk that participate in melanocyte homeostasis and that may impact melanoma initiation and progression.

P.034 | Tyrosine phosphatase, TC-PTP and SH-PTP2 mediate dephosphorylation of STAT3 in TPA-induced growth inhibition of melanoma

Tetsushi Iwasaki[1]; Miwa Yamauchi[1]; Takeshi Fukumoto[2]; Liang Zhu[1]; Ayano Itai[1]; Masanobu Sakaguchi[3]; Taiki Nagano[1]; Shinji Kamada[1]; Masahiro Oka[3]

[1]Kobe University, Kobe, Japan; [2]The Wister Institute, Philadelphia, PA, USA; [3]Tohoku Medical and Pharmaceutical University, Sendai, Japan

The signal transducer and activator of transcription 3 (STAT3) is a transcription factor involved in the expression of various oncogenic proteins. STAT3 is normally activated by tyrosine 705 phosphorylation in the molecule when adequate ligands bind cell surface receptors. However, constitutive activation of STAT3, namely constitutive tyrosine 705 phosphorylation, has been observed in melanoma cells. It has been elucidated that the constitutive activation of STAT3 plays a crucial role in the growth and metastasis in these cells. Thus, STAT3 has been considered to be a strong candidate for melanoma therapy. We have previously found that the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibits both the proliferation and the DNA synthesis in melanoma cells concomitant with inactivation of STAT3 through decreasing the phosphorylation level at Tyr705 of STAT3. In this study, we demonstrated the molecular mechanisms by which TPA inhibits melanoma growth through dephosphorylating Tyr705 phosphorylation by pharmaceutical and biochemical analysis using various melanoma cell lines. The growth inhibition of melanoma cells was disabled by introduction of a small interference RNA (siRNA) for STAT3 or several protein tyrosine phosphatases (PTPs) including TC-PTP and SH-PTP2. TPA-induced STAT3 dephosphorylation was also blocked by siRNAs for the PTPs. These data suggest that the TPA-induced growth inhibition of melanoma cells is due to the dephosphorylation of STAT3 and that multiple PTPs contribute to the dephosphorylation of STAT3.

P.035 | Endogenously producing lumisterol derivatives have anti-proliferative effects on human melanoma and epidermal melanocytes

Tae-Kang Kim[1]; Allen S. Oak[1]; Zongtao Lin[2]; Wei Li[2]; Robert C. Tuckey[3]; Andrzej T. Slominski[1]

[1]University of Alabama at Birmingham, Birmingham, AL, USA; [2]University of Tennessee Health Science Center, Memphis, TN, USA; [3]University of Western Australia, Perth, WA, Australia

Lumisterol (L3) is a photoisomer formed from previtamin D3 by prolonged exposure to UVB and was assumed to have no biological activity, unlike vitamin D3 which is activated by hydroxylation to form the key regulatory hormone, 1,25-dihydroxyvitamin D3. Recently we discovered that purified CYP11A1 can act on L3 producing 20(OH)L3, 22(OH)L3, 24(OH)L3 and 20,22$(OH)_2$L3. In order to test whether CYP11A1 could similarly metabolize L3 in vivo we analyzed extracts of epidermis, serum and the pig adrenal gland using LC/qTOF-MS. Results showed that 20(OH)L3, 22(OH)L3, 24(OH)L3 and 20,22$(OH)_2$L3 were all present in human epidermis and serum. The production of 20(OH)L3 was increased by addition of exogenous L3 to incubation mixtures of adrenal fragments and HaCaT cells. The CYP11A1-derived L3 derivatives displayed anti-proliferative effects on SKMEL-188 melanoma cells as determined using the MTS assay. IC_{50} values were 1.0 x 10^{-11} M for 20(OH)L3, 22(OH)L3 and 24(OH)L3 and 6.7 x 10^{-11} M for 20,22$(OH)_2$L3. These L3 derivatives also inhibited melanoma colony formation for colony sizes ranging from 0.1 mm to 0.2 mm, at concentrations below 10^{-9} M. 20(OH)L3, 22(OH)L3 and 20,22$(OH)_2$L3 inhibited the growth of normal human melanocytes over the 72 hr treatment period at concentrations below 10^{-10} M for 20(OH)L3 and 22(OH)L3, and 10^{-8} M for 20,22$(OH)_2$L3. Using SKMEL-188 melanoma cells transduced with a VDR-GFP fusion construct the effects of the hydroxylumisterols on the translocation of the VDR to the nucleus were determined. 20(OH)L3, 22(OH)L3, 24(OH)L3 and 20,22$(OH)_2$L3 stimulated this VDR translocation in a dose-dependent manner, statistically different from the control at a concentration of 10^{-7} M. In summary, we have discovered that the CYP11A1-derived hydroxylumisterols are made in vivo and enter the bloodstream, and that they show anti-proliferative effects on malignant and normal human melanocytes, possibly through VDR, with the potential to be used for melanoma or skin-disorder therapy.

P.036 | LncRNA SPRIGHTLY acts as an intra-nuclear organizing hub for pre-mRNA Necessary for melanoma development

Bongyong Lee[1]; Anupama Sahoo[1]; John Marchica[1]; Erwin Holzhauser[2]; Xiaoli Chen[2]; Jian-Liang Li[1]; Tatsuya Seki[3,1]; Subramaniam Govindarajan[1]; Fatu B. Markey[4]; Mona Batish[4]; Sonali J. Lokhande[5]; Shaojie Zhang[2]; Animesh Ray[5,6]; Ranjan J. Perera[1]

[1]Sanford Burnham Prebys Medical Discovery Institute, Orlando, FL, USA; [2]University of Central Florida, Orlando, FL, USA; [3]Medical and Biological Laboratories, Nagoya, Japan; [4]Rutgers University, Newark, NJ, USA; [5]Keck Graduate Institute, Claremont, CA, USA; [6]California Institute of Technology, Pasadena, CA, USA

Molecular mechanisms by which long noncoding RNA (lncRNA) molecules may influence cancerous condition are poorly understood. The aberrant expression of SPRIGHTLY lncRNA, encoded within the drosophila gene homolog *Sprouty-4* intron, is correlated with a variety of cancers including human melanomas. We demonstrate by SHAPE-Seq and dChIRP that SPRIGHTLY RNA secondary structure has a core pseudo-knotted domain. This lncRNA interacts with the intronic regions of six pre-mRNAs: *SOX5*, *SMYD3*, *SND1*, *MEOX2*, *DCTN6*, and *RASAL2*, all of which have cancer related functions. Hemizygous knockout of *SPRIGHTLY* by CRISPR/Cas9 in melanoma cells significantly decreases SPRIGHTLY lncRNA levels, simultaneously decreases the levels of its interacting pre-mRNA molecules, decreases anchorage-independent growth rate of cells, and the rate of in vivo tumor growth in mouse xenografts. These results provide the first demonstration of a lncRNA's 3-dimensional coordinating role in facilitating cancer-related gene expression in human melanomas.

P.037 | Autoinflammation from the imbalance of IL-1 over IL-1Ra contributes to tumor progression in metastatic melanoma

Yang Li; Miyako Okamoto; Yuchun Luo; Charles A. Dinarello; Mayumi Fujita

University of Colorado, Aurora, CO, USA

Sustained inflammation in tumor microenvironment promotes tumor growth and disease progression. We have demonstrated an autoinflammatory property of metastatic melanoma, characterized by constitutive activation of IL-1R signaling and NLRP inflammasome. In this study, we investigated the involvement of agonistic and antagonistic cytokines of IL-1R signaling in 6 primary and 4 metastatic human melanoma cell lines. Agonistic cytokines (IL-1α and IL-1β) were spontaneously produced and secreted in advanced-stage melanoma cells, and their levels were further enhanced by IL-1R stimulation. However, these cytokines were rarely produced or secreted in primary melanoma cells. In contrast, antagonistic cytokine (IL-1Ra) was produced and secreted in primary melanoma cells but not in metastatic melanoma cells, even after IL-1R stimulation, demonstrating the imbalance of agonistic over antagonistic cytokines in metastatic melanoma. Secreted IL-1 from metastatic melanoma was biologically active as an autocrine factor to induce IL-1 itself and other inflammatory cytokines (IL-6, IL-8 and MCP-1) in vitro and in vivo. Tumor-derived IL-1 was also active to induce paracrine effects on stromal cells, including macrophages, lymphocytes, keratinocytes and endothelial cells. In a xenograft mouse model, secreted IL-1 from metastatic melanoma recruited CD11b/Gr1 myeloid-derived suppressor cells in the tumor microenvironment and contributed to tumor growth, both of which were inhibited by injecting IL-1Ra subcutaneously. In summary, our findings demonstrate that autoinflammation derived from the imbalance of IL-1 over IL-1Ra contributes to melanoma progression in metastatic melanoma.

P.038 | cdkn2ab overexpression induces a nevus-like state in fish pigment cells

Janine Regneri[1]; Brigitta Wilde[1]; Barbara Klotz[1]; Martina Regensburger[1]; Michael Hausmann[1]; Manfred Schartl[1,2,3]

[1]Physiological Chemistry, University of Wuerzburg, Wuerzburg, Germany; [2]Comprehensive Cancer Center, University Clinic Wuerzburg, Wuerzburg, Germany; [3]Texas Institute for Advanced Study and Department of Biology, Texas A&M University, College Station, TX, USA

In humans, the *CDKN2A* locus encodes two tumor-suppressor proteins, INK4a and ARF. Loss of these proteins due to inactivating mutations or epigenetic silencing is a frequent event in malignant melanoma. Furthermore, both proteins are important mediators of cellular senescence in human nevus cells. Fish, like all other non-mammalian vertebrates, have the ancestral gene of mammalian *CDKN2A* and *CDKN2B*, which is termed *cdkn2ab*. *Cdkn2ab* does only encode Ink4a, while Arf-like transcripts are absent. To analyze whether the fish *cdkn2ab* gene has a similar function in pigment cell tumor formation as its human counterpart, we studied its role in the teleost fish *Xiphophorus*. These fish develop hereditary melanomas that originate from precursor lesions consisting of so called macromelanophores, which are giant melanophores that show morphological features of senescent pigment cells comparable to human nevus cells. We found high *cdkn2ab* mRNA and protein levels in melanomas of high and intermediate malignancy, while benign precursor lesions showed slightly lower expression levels. Ectopic expression of *cdkn2ab* in *Xiphophorus* melanoma and fibroblast cell lines led to a growth arrest and the development of a senescent phenotype, characterized by an enlarged and flattened cell shape and large nuclei with prominent nucleoli. In the melanoma cell line, we furthermore noted a strong increase in the proportion of bi- and multinucleated cells upon *cdkn2ab* overexpression. Multinucleated cells are also a hallmark of human nevi. In line with this, we were able to demonstrate that fish macromelanophores are frequently multinucleated. As the *CDKN2A* encoded proteins are components of the main pathways mediating senescence in human nevus cells, our findings point to a similar tumor-suppressive function of fish *cdkn2ab* and human *CDKN2A* in pigment cells.

P.039 | Cystine depletion induces pro-metastatic features in melanoma

Madlen Staus[1]; Susanne Kneitz[1]; Carsten Ade[2]; Werner Schmitz[2]; Almut Schulze[2]; Mathias Rosenfeldt[3]; Svenja Meierjohann[1]

[1]Physiological Chemistry, University of Wuerzburg, Wuerzburg, Germany; [2]Molecular Biology & Biochemistry, University Wuerzburg, Wuerzburg, Germany; [3]Institute of Pathology, University Wuerzburg, Wuerzburg, Germany

Maintenance of a well-adjusted redox balance is essential for cells to preserve the functional integrity of cellular components and to prevent oxidative damage from proteins, lipids and nucleic acids. Consequently, antioxidant mechanisms are required to maintain redox balance and ensure cellular survival. With regard to low molecular cellular antioxidants, the amino acid cysteine plays a dominant role, as it is an important antioxidant itself and a key player in glutathione synthesis. One of the main sources of cysteine is the cystine/glutamate antiporter xCT.

Here, we show that inhibition of the transporter by cystine depletion has only a minor effect on melanoma proliferation, but leads to profound alterations of the melanoma transcriptome, as assessed by RNA-Seq.

We observed that cystine depletion leads to a strong compensation of parallel antioxidant pathways, including the thioredoxin and cysteine biosynthesis pathways. Interestingly, the largest regulated gene set affected the KEGG pathway "mesenchymal-epithelial transition", including several MMPs and growth factors. In line with these results, we found that cystine depletion enhances cellular migration and invasion of melanoma cells.

P.040 | p16INK4A controls mitochondrial biogenesis in melanoma cells and melanocytes through a CDK4/Rb-independent pathway

Ethika Tyagi[1]; Bin Liu[1]; Jared Rutter[2]; Douglas Grossman[1,3]

[1]Huntsman Cancer Institute, Salt Lake City, UT, USA; [2]Department of Biochemistry, University of Utah Health Sciences Center, Salt Lake City, UT, USA; [3]Department of Dermatology, University of Utah Health Sciences Center, Salt Lake City, UT, USA

The tumor suppressor $p16^{INK4A}$ (p16) inhibits cell cycle progression through the CDK4/Rb pathway, and is silenced or deleted in melanoma. We have previously shown that p16 regulates cellular oxidative stress, independent of its cell cycle control. With the aim to identify the impact of p16 loss on mitochondrial functions, we designed studies for p16 manipulation in p16-null primary fibroblasts (PMF) and lentivirus mediate over expression of p16 in primary melanocytes as well as melanoma cells. We observed an increase in mitochondrial mass analyzed by MitoTracker staining and expression of mitochondrial respiratory subunit proteins in p16-null PMFs as compared to wild-type (WT) PMFs. These findings in p16-null PMFs were associated with increased expression of mitochondrial biogenesis transcription factors PRC and TFAM and increased mitochondrial superoxide, analyzed using MitoSox staining. Loss of p16 also demonstrated reduced mitochondrial respiration capacity consistent with electron microscopy findings showing that p16 null PMFs mitochondria have abnormal morphology. One biological consequence of elevated ROS in p16-deficient PMFs was enhanced migration, which was reduced by the ROS scavenger N-acetylcysteine. Interestingly, p16-deficient PMFs display increased mitochondrial membrane potential measured by JC-1 staining, which was also required for their enhanced migration. We used the lentiviral approach for over expression studies of p16 in p16-deficient PMFs, primary human melanocytes and A375 melanoma cells. The mitochondrial and migration phenotype was restored by forced expression of p16. Similarly, over-expression of p16 led to decreased expression of some mitochondrial respiratory proteins, enhanced respiration, and decreased migration. Inhibition of Rb phosphorylation in melanocytes and melanoma cells, either by addition of chemical CDK4 inhibitors or RNAi-mediated knockdown of CDK4, did not mimic the effects of p16 loss. These results suggest that p16 plays a key role in the regulation of mitochondrial biogenesis and function, and this alternate tumor-suppressor function is independent of the canonical CDK4/Rb pathway.

P.041 | The importance of a negative control in the histologic assessment of surgical margins in lentigo maligna

Nathan J. Luby; Glen M. Bowen; Shadai F. Arce

University of Utah, Salt Lake City, UT, USA

Background: Lentigo maligna (LM) is a subtype of melanoma in situ in chronically sun-damaged skin. The distinction between the histologic surgical margins of a LM and the surrounding junctional melanocytic hyperplasia (JMH) inherent to chronically sun-exposed skin is ambiguous given overlapping histologic features.[1] The only statistically distinct histologic feature between LM and JMH is the melanocyte count (MC).[1,2] In a U.K. study, the risk LM recurring after surgery was predicted by a regression model of MC: 0-20 cells/400x magnification was categorized as low risk, 21-30 cells as intermediate risk, and >31 cells as high risk.[2] We hypothesized that MC from the U.K. are not representative of patients in the Intermountain West at higher altitude and lower latitude.

Methods: Freshly frozen negative control samples from 45 patients undergoing staged excisions for LM were immunostained with antisera against SOX10, a nuclear protein expressed in melanocytes with MC quantified as described above.

Results: The mean MC was 20.3 (median = 20.5). The range was 9.0 – 36.7. Using the predictive model for LM recurrence from the U.K. cohort,[2] 22/45 (49%) of our negative controls were low-risk, 19/45 (42%) were intermediate-risk, and 4/45 (9%) were in the high-risk category. Using the U.K. predictive model, 51% of our negative control samples would be considered as intermediate- or high-risk for local LM recurrence.

Conclusion: Applying the U.K. standard to predict local recurrence of LM in patients from the Intermountain West is of limited value, since Caucasians in this region have a higher melanocyte density in

chronically sun-exposed sites compared to U.K. patients. We advocate establishment of a baseline MC in patients with LM from a negative control biopsy and judging surgical margins for LM as a deviation from the baseline MC.

References:: 1. Dermatol Surg. 2011;37:657–663.
2. J Plast Reconstr Aesthet Surg. 2014;67(10):1322–32.

P.042 | Melanoma thickness can predict level of skin invasion

Dejan V. Nikolic[1,2]; Violeta V. Stanimirovic[3]; Aleksandra T. Nikolic[1,4]; Marko D. Nikolic[1]

[1]Faculty of Medicine, Belgrade, Belgrade, Serbia; [2]University Medical Center Bezanijska Kosa, Belgrade, Serbia; [3]Medicines and Medical Devices Agency, Belgrade, Serbia; [4]Institute for Cardiovascular Diseases Dedinje, Belgrade, Serbia

Introduction: Melanoma is the most deadly skin cancer, accounting for only about 1% of all cases, but the vast majority of skin cancer death. Incidence is rising every year and has doubled since 1973 and has increased 15 times in the last 40 years. This is a more rapid increase than for any other cancer.

Patients and Methods: The sample studied consisted of 497 primary cutaneous melanoma lesions, identified by anatomopathological reports. The variables evaluated were: level of invasion (Clark) and tumor thickness (Breslow). The rate of skin invasion was calculated depending of tumor thickness.

Results: The total number of 497 melanomas was divided in three categories, thin-up to 1 mm, medium-from 1–2 mm, thick-over 2 mm. In the group of 159 thin melanomas, the most presented level was II 75.5%, followed by level III 19.5%. Medium group of 105 melanomas was divided by invasion, 49.5% level III, followed by level II 39.5%. Group of 233 thick melanoma over 2 mm showed the most presented level of tumor by Clark IV 46.4% and 38.6% level III.

Discussion: There has been a significant rise in overall 5-year survival in patients with melanoma. This may be due to earlier diagnosis, when tumors are still at a thinner depth, as well as improved treatment and surgical techniques. The mortality rate for melanoma has increased at a much slower rate and has remained stable over the past 10 years.

P.043 | The Baseline neutrophil-lymphocyte, platelet-lymphocyte and lymphocyte-monocyte ratios as biomarkers of survival in stage I-III cutaneous melanoma

Alyss V. Robinson[1]; Claire Keeble[1]; Howard Peach[1]; Maria Marples[1,2]; Donald Dewar[1]; Ryckie G. Wade[1,3]

[1]Faculty of Medicine and Health, University of Leeds, Leeds, UK; [2]Leeds Cancer Centre, St James' University Hospital, Leeds, UK; [3]Department of Plastic and Reconstructive Surgery, Leeds General Infirmary, Leeds, UK

Background: The host inflammatory environment represented by the peripheral blood neutrophil-lymphocyte (NLR), platelet-lymphocyte (PLR) and lymphocyte-monocyte ratio (LMR) changes in response to malignancy. These biomarkers are strongly associated with adverse outcomes in metastatic melanoma and a number of other cancers, but the evidence is limited in relation to early stage melanoma. This study sought to investigate the association between these biomarkers and survival in stage I-III cutaneous melanoma.

Methods: This retrospective cohort study describes a consecutive series of patients who underwent wide excision and sentinel lymph node biopsy (SLNB) for cutaneous melanoma over 10 years. Cox regression and Kaplan Meier survival analyses were used to determine the association between NLR, PLR and LMR and overall and melanoma-specific survival (OS and MSS).

Findings: Five hundred and sixty-nine patients were included. The median follow up was 2.86 years (IQR, 1.67, 4.72; minimum 8 months, maximum 10.7 years). During surveillance, 67 (11.8%) participants died of which 57 (85%) were attributable to melanoma. Tumours with regressional change were associated with a higher NLR (p = .005). Improved overall survival was associated with a baseline NLR <2.5 (HR 3.49 [95% CI 1.36, 8.93], p = .009) and PLR <100 (HR 2.78 [1.23, 6.32], p = .014), whereas LMR was not significantly associated. A combined high NLR/PLR ratio was also strongly associated with overall survival (p = .001). However, peripheral blood biomarkers were not significantly associated with melanoma-specific survival.

Interpretation: A baseline NLR <2.5 and PLR <100 was associated with worse overall survival, which is in contradiction to previous studies in more advanced disease. Further studies are required to validate NLR and PLR as risk stratification tools in early disease.

P.044 | Baseline neutrophil-lymphocyte ratio adds prognostic value to sentinel lymph node biopsy in cutaneous melanoma

Alyss V. Robinson; Claire Keeble; Howard Peach; Maria Marples; Donald Dewar; Ryckie G. Wade

Faculty of Medicine and Health, Leeds, UK

Background: The neutrophil-lymphocyte ratio (NLR) changes in response to cancer and provides prognostic information in a number of malignancies. In melanoma, a high NLR is associated with nodal metastasis and survival in high-risk and advanced-stage disease. This study sought to investigate the association between baseline NLR, nodal status and survival in early cutaneous melanoma.

Methods: This retrospective cohort study describes a consecutive series of patients who underwent wide excision and sentinel lymph node biopsy (SLNB) for cutaneous melanoma over 10 years. Logistic regression was used to determine the association between NLR and sentinel lymph node status. Thereafter, we stratified sentinel lymph node (SLN) status according to baseline NLR. Cox regression and Kaplan Meier survival analyses were used to determine the association between the combined NLR/SLN status and survival.

Findings: Overall 569 patients were included, of which 147 (25.8%) had metastasis of melanoma to the SLN. During surveillance, 67 (11.8%) patients died of which 57 (85%) were attributable to melanoma. An adjusted high NLR (>1.9) was able to predict the presence of associated with SLN metastasis (adjusted OR 1.82 [95% CI 1.05–3.17], p = .034), primary tumour regression (p = .005), and the absence of extracapsular spread (p = .013). After stratification, patients with a positive SLNB but and poor immune response (NLR <2.5) experienced a significantly worse overall survival than all other groups (adjusted HR 6.89 [95% CI 2.99–15.9], p < .001). There was no difference in survival outcome between groups with an NLR >2.5 and a positive SLNB, and those with an NLR <2.5 and negative SLNB (p = .291).

Interpretation: A high NLR in the presence of nodal disease may indicate a successful host immune response. Stratifying patients by SLN status and NLR provides additional prognostic information, which may be used to direct adjuvant therapy.

P.045 | Clinical and dermoscopic monitoring of pigmented and amelanotic spitz naevi in children

Elodie Bou Assi; Bertille Bonniaud; Marie-Hélène Aubriot-Lorton; Blandine Bel; Géraldine Jeudy; Pierre Vabres

CHU Dijon Bourgogne, Dijon, France

Background: Classical management of Spitz naevi (SN) relies on excision to exclude melanoma. However, dermoscopic monitoring has shown spontaneous involution of Spitz naevi in children. Hence, it appears as an alternative to surgical excision.

Objectives: To report the clinical and dermoscopic outcome of Spitz naevi after long-term dermoscopic follow-up.

Methods: Clinical and dermoscopic features of 3 hypochromic and 11 pigmented SN were reviewed. Median initial age of patients was 4.9 years. Six nevi were excised on first visit or after short-term monitoring and microscopically confirmed. Eight underwent long-term dermoscopic monitoring (14 months to 7 years).

Results: Most naevi were located on the extremities (7/14) or on the face (4/14). Pigmented SN were predominant in children with skin type III-IV. No hypochromic SN were present in skin types IV-V. At baseline, pigmented SN showed a starburst (8/14) or reticular pattern (3/14). Non-pigmented SN exhibited a vascular (2/14) or homogeneous pattern (1/14). Specific SN structures (crystalline structures, peripheral streaks, superficial black network or negative pigment network) were observed in 12 cases. At the end of follow-up, five nevi were in complete or near complete involution after 31 to 87 months. One nevus evolved from starburst to reticular after 27 months. Two reticular nevi remained stable after 14 and 26 months.

Conclusions: Spontaneous involution is common both in pigmented and non-pigmented SN, as previously reported, but it requires several years. Hence, we advise long-term dermoscopic monitoring of pigmented SN. Amelanotic SN without rapidly progressive features may also be monitored.

P.046 | Development of peptide therapeutics targeting the CtBP transcriptional complex as a potential approach for treating melanoma

Caiguo Zhang; Melanie Blevins; Hong Li; Rui Zhao; Mingxia Huang

University of Colorado School of Medicine, Aurora, CO, USA

Carboxyl-terminal binding protein (CtBP) is a transcriptional corepressor that regulates the expression of multiple epithelial-specific and pro-apoptotic genes. Overexpression of CtBP in human cancers promotes epithelial-mesenchymal transition, stem cell-like features, and cell survival. Since CtBP is overexpressed in many human melanoma samples, we explore the possibility of targeting CtBP as a therapeutic approach for treatment of melanoma.

To regulate the expression of its target genes, CtBP binds to a conserved peptide sequence, PXDLS, found within its transcription factor partners and is thus recruited to its target promoters. Disrupting the interaction between CtBP and its transcription factor partners may be a direct means of altering CtBP-mediated transcriptional repression and a potential mechanism for future cancer therapies. To that end, we generated peptides containing the PXDLS motif fused to cell-penetrating peptides (CPP). These peptides disrupt the ability of CtBP to interact with a protein partner, E1A, with IC_{50} values of 11–12 μM in an AlphaScreen assay. These peptides, termed CPP-E1A, were also capable of entering both lung carcinoma and melanoma cells, disrupting its interaction with transcription factor partners, and inhibiting CtBP-mediated transcriptional repression. A drawback of the CPP-E1A is their short half-life (~4 hr) inside the cell. To overcome the instability and test the long-term effect, we constitutively express CPP-E1A from a plasmid in several melanoma cell lines. CPP-E1A expressing cells exhibit slower proliferation, decreased migration, and reduced sphere-forming ability. Future effort will be focused on optimization of the CPP-E1A peptides to increase their stability and efficacy.

P.047 | Patient and tumor characteristics for all pediatric melanoma patients within the Colorado cancer registry 1988–2015

Blake M. Snyder[1]; Jacqueline Guidry[1]; Jessica Mounessa[2]; Robert Dellavalle[1]; Cory Dunnick[1]

[1]University of Colorado School of Medicine, Denver, CO, USA; [2]Stony Brook Medicine, Stony Brook, NY, USA

Background and Importance: Melanoma incidence is increasing overall in the general population. However, trends in the pediatric

population have been less well defined, particularly in patients less than 10 years of age.

Objective: To analyze demographic and clinical tumor trends in melanoma in the Colorado pediatric population.

Methods: De-identified case information for pediatric melanoma in the state of Colorado 1988–2015 was obtained from the Colorado Cancer Registry. Demographic information (age, gender, and county), clinical tumor information (Breslow's depth, Clark's level, mitotic rate, ulceration status, and regression), year of diagnosis and TNM stage were recorded. Data were analyzed using descriptive statistics.

Results: A total of 144 cases of pediatric melanoma cases were reported to the Colorado Cancer Registry between 1988 and 2015. A bimodal distribution across the pediatric cohort existed, with 20.8% of patients diagnosed at <1 year of age and 68.8% of patients diagnosed at >14 years of age. Geographic variability in the incidence of pediatric melanoma was noted.

Conclusions and Relevance: Our study is consistent with historical data on the age of diagnosis and incidence of pediatric melanoma. We did not find a statistically significant rise in incidence in pediatric melanoma in Colorado over the past three decades. We further identified recent patient and tumor characteristics of pediatric melanoma in Colorado that may help to guide screening efforts for early detection of melanoma in children.

P.048 | The global burden of melanoma: Results from the global burden of disease study 2015

Chante Karimkhani[1]; Mohsen Naghavi[2]; Christina Fitzmaurice[2,3]; Robert P. Dellavalle[1,4,5]

[1]Department of Dermatology, University of Colorado, Aurora, CO, USA; [2]Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, USA; [3]Department of Medicine, Division of Hematology, University of Washington, Seattle, WA, USA; [4]Dermatology of Epidemiology, Colorado School of Public Health, Aurora, CO, USA; [5]Dermatology Service, US Department of Veterans Affairs, Eastern Colorado Health System, Denver, CO, USA

Background: Despite recent improvements in prevention, diagnosis, and treatment, vast differences in melanoma burden still exist between populations. Comparative data can highlight these differences and lead to focused efforts to reduce the burden of melanoma.

Objectives: To assess global, regional, and national melanoma incidence, mortality, and disability-adjusted life year (DALY) estimates from the Global Burden of Disease 2015 study.

Methods: Vital registration system and cancer registry data were used for melanoma mortality modeling. Incidence and prevalence were estimated using separately modeled mortality-to-incidence (MI) ratios. Total prevalence was divided into four disease phases and multiplied with disability weights to generate years lived with disability (YLDs). Deaths in each age group were multiplied with the reference life expectancy to generate years of life lost (YLLs). YLDs and YLLs were added to estimate DALYs.

Results: The five world regions with the greatest melanoma incidence, DALY, and mortality rates were Australasia, North America, Eastern Europe, Western Europe, and Central Europe. With the exception of regions in sub-Saharan Africa, DALY and mortality rates were greater in males than females. DALY rate by age was highest in those aged 75–79 years, 70–74 years, and 80 + years.

Conclusions: The greatest burden from melanoma falls on Australasian, North American, European, elderly, and male populations, consistent with previous investigations. These substantial disparities in melanoma burden worldwide highlight the need for aggressive prevention efforts. GBD results can help shape melanoma research and public policy.

P.049 | Presence of online support groups within melanoma organizations

Nazanin Kuseh Kuseh Kalani Yazd[1]; Ravi Patel[2]; Robert P. Dellavalle[1,3]

[1] University of Colorado School of Medicine, Denver, CO, USA; [2]Medical College of Georgia, Augusta, GA, USA; [3]Denver Veterans Affairs Medical Center [VAMC] Department of Dermatology, Denver, CO, USA

Studies show that internet usage amongst cancer patients is common. These individuals use online forums to read about patient experiences, share personal stories, and participate in support groups.

Our study investigated the assistance available to melanoma patients in the form of online support groups. To determine this, a Google search of "melanoma organization" was performed and the top ten organizations were identified. Each organization's website was searched to determine what support options were available and whether an online support system was present. Out of the ten, only three organizations, The Melanoma Research Foundation (MRP), Cancer Survivors Network (CSN), and AIM at Melanoma (AIM) offered their own online support forum. Five organizations provided links to external support. Of note, MRP and CSN both had in-site forums while AIM used Facebook technology. MRF led the group with 107 posts/month followed by CSN at 11 posts/month and AIM at 1 post/month.

We categorized the first 60 posts from the three aforementioned support forums into five categories. These included discussions on support, treatment, diagnosis, side effects, and cost. MRF contained 19 topics about support, 18 about treatment, 13 about diagnostic advice, 8 about side effects, and 2 about cost issues. AIM contained 27 topics about support, 23 about treatment, and 10 about side effects. Lastly, CSN contained 20 topics about support, 18 about treatment, 15 about diagnostic advice, and 7 about side effects. Support/coping advice ranked number one in all three forums in post frequency with number two being treatment advice.

Due to the increasing usage of the internet amongst cancer patients and evidence for positive emotional reassurance from online support groups, more organizations should investigate the institution of these support mediums for a real-time solution to the complex social, emotional, and educational needs of their patient populations.

P.050 | Melanoma campaign CAN/NOT improve revealing of THIN/THICK malignant melanomas

Dejan V. Nikolic[2,1]; Aleksandra T. Nikolic[2,3]; Violeta V. Stanimirovic[4]; Marko D. Nikolic[2]

[1]University Medical Center Bezanijska Kosa, Belgrade, Serbia; [2]Faculty of Medicine, Belgrade, Serbia; [3]Institute for Cardiovascular Diseases Dedinje, Belgrade, Serbia; [4]Medicines and Medical Devices Agency, Belgrade, Serbia

Introduction: Media campaigns are an important intervention for influencing behaviour modifications to encourage the population to reduce sun exposure as main risk for malignant melanoma. Concept that having a tanned skin is not healthy since it implies the skin being damaged by solar ultraviolet radiation are effectively changing people's behaviors and their motivations through public campaigns.

Patients and Methods: First Melanoma Center in Balkan was established in 1999, two months after six Belgium dermatologist initiated media campaign called Euromelanoma campaign. During the period of 18 years, 1999–2017, more than 50.000 patients came for medical examination of moles. Total number of almost 1.000.000 images were taken on *MoleMaxII*.

Results: The total number of melanoma patients for the last 18 years, was 1040 from which 40%(416) patients were found with thin melanomas, melanomas that are thinner then 1 mm, according to Breslow. From the total number of 1040 melanomas, 9%(93) of them were melanoma in situ. Comparing results made 10 years after establishing Melanoma center where 37% from the total number of patients were thin melanomas, and 6% of melanomas were in situ. *We* can conclude that there is increase in trend of revealing thin melanomas in First Balkan Melanoma Center due to intensive media campaign.

Discussion: Annual screening campaigns coupled with intense media promotion have become common place in many countries and Serbia for last 10 years. Despite improvements in the early recognition of melanoma and the use of novel diagnostic techniques that enhance our diagnostic capabilities, disease-related mortality remains a significant public health issue. Revealing of thick melanomas can be decreased by self-selected screening campaigns, health care professional surveillance, and specialized pigmented lesions clinics underscoring in early detection programs, especially in high-risk groups. Revealing the number of thin/melanoma in situ in higher number can lead to definitely cure these patients from melanoma without death consequences.

P.052 | Phenotypic characterization and the UV response of melanocyte cultures expressing a mutant p16 allele and/or a MC1R loss of function variant

Barbara Hernando[1]; Steven Guard[2]; Kevin Choi[2]; Viki Swope[2]; Ayesha Anwar[2]; Renny Starner[1]; Pamela Cassidy[3]; Ana Luisa Kadekaro[2]; Dorothy Bennett[4]; Sancy Leachman[3]; Zalfa Abdel-Malek[2]

[1]Jaume I University of Castellon, Castellon, Spain; [2]University of Cincinnati, Cincinnati, OH, USA; [3]Oregon Health and Sciences University, Portland, OR, USA; [4]St. George's University of London, London, UK

Heterozygous germline mutations in *p16* result in dramatic hereditary predisposition to melanoma, and co-inheritance of a loss of function (LOF) variant of the melanocortin 1 receptor (*MC1R)* gene increases their risk for melanoma further. We established primary melanocyte cultures from donors that were heterozygous for V126D, 23ins24, or 5'UTR-34G>T *p16* mutations and expressed either wild type or a heterozygous LOF *MC1R* variant. We compared these primary cultures' behavior and response to ultraviolet radiation (UV) in vitro. Melanocytes expressing wild type *p16* and *MC1R*, heterozygous for a LOF *MC1R* allele, or expressing two mutant *p16* alleles were included as controls. All melanocyte cultures expressed the appropriate molecular weight p16 protein, with the exception of 23ins24 mutation carriers, which expressed an additional higher molecular weight protein. Expression of a *p16* mutant allele did not affect MC1R activity. *P16* and *MC1R* genotypes did not affect melanocyte proliferation rate or their ability to undergo replicative senescence. The exception were melanocytes heterozygous for 23ins24 mutation, which proliferated slowly from early passage, and the two rare cultures expressing two mutant *p16* alleles that showed minimal β-galactosidase staining at late passage. Carriage of a *p16* mutation, regardless of *MC1R* genotype, did not affect the response of melanocytes to UV, as evidenced by hypo-phosphorylation of Rb protein, cell cycle arrest, phosphorylation of JNK and p38, accumulation and transactivation of p53, hydrogen peroxide generation, and repair of DNA photoproducts. Deep RNA sequencing revealed differential gene expression in V126D *p16* melanocytes, as compared to melanocytes co-expressing D294H *MC1R*, or wild type for *p16* and *MC1R* under control conditions or post UV irradiation. Using primary cultures of keratinocytes and fibroblasts that we established from the above donors, we plan to investigate how these *p16* germline mutations might affect these cells and their interaction with melanocytes.

P.053 | Whole-exome sequencing in relatively slow-growing metastatic melanoma from ungual melanoma

Naoki Oiso[1]; Kazuko Sakai[2]; Tomohiko Narita[1]; Shigeto Yanagihara[1]; Kazuto Nishio[2]; Akira Kawada[1]

[1]*Department of Dermatology, Kindai University Faculty of Medicine, Osaka-Sayama, Japan;* [2]*Department of Genome Biology, Kindai University Faculty of Medicine, Osaka-Sayama, Japan*

Approximately, half of melanomas occur on the acral sites in Japan. Acral lentiginous melanoma (ALM) does not differ in its prognosis from non-ALM, but ALM has a different genetic background from non-ALM, i.e., infrequent *BRAF* mutations and frequent *KIT* mutations. Recently, we experienced a case of lymph node metastatic melanoma from ungual melanoma with an interval of 5 years after excision. The patient provided written informed consent to participate in the study according to a protocol approved by the Genetic Committee of Kindai University Faculty of Medicine. The protocol was conducted according to the Declaration of Helsinki Principles. We then performed whole-exome sequencing of DNAs from the tumor and whole blood with a mean depth of coverage of > 100X. The recurrently altered pathways in melanomas include (1) proliferation and survival, (2) cell cycle progression, (3) apoptosis and senescence, (4) chromatin remodeling and transcriptional control, (5) survival and melanocyte development, 86) telomere maintenance, 87) chromatid segregation and genetic stability and (8) epigenetic regulation. Furthermore, our case showed mutations including (9) antigen presentation and (10) resistance to anti-tumor drugs. The high frequent somatic mutations in *KIT* and HLA-B might be associated with proliferation and escape from anti-tumor immunity. The low frequent somatic mutations like *MBD6* and *PTPN18* may be associated with resistance to anti-tumor drugs if applied. Whole-exome sequencing suggests that complexity of the somatic mutations underlies progression even in our case of the relatively slow-growing metastatic melanoma.

P.054 | Tumoral melanosis associated with metastatic melanoma treated with PD-1 inhibitors

Andrew S. Fischer[1]; Neda R. Black[2]; Brandon McNally[2]; Joshua Wisell[2]; Loren Golitz[2]

[1]*Department of Dermatology, University of Colorado, Aurora, CO, USA;* [2]*Department of Pathology, University of Colorado, Aurora, CO, USA*

Tumoral, or nodular, melanosis is characterized by a nodular, dermal collection of heavily pigmented melanophages without melanocytes. It is thought to represent an uncommon form of tumor regression in which massive pigment deposition remains in melanophages, perhaps after a strong immunologic response. Although largely speculative due to the few number of cases reported in the literature, tumoral melanosis in association with a primary melanoma may portend metastatic disease and an overall poor prognosis. With the advent of immunotherapy for advanced melanoma, few cases of tumoral melanosis have emerged in patients on immune checkpoint inhibitors. The significance of this has yet to be determined. We present the case of an 80-year-old male with stage III unresectable melanoma of the scalp who developed a good response to pembrolizumab after failing ipilimumab. He then presented with several new, darkly pigmented lesions on his scalp concerning for recurrent melanoma versus epidermotropic metastases. Biopsies of these lesions were performed and revealed a broad and dense dermal population of heavily pigmented melanophages with associated dermal fibrosis and attenuation of the epidermal rete ridge pattern. Immunohistochemical studies for S100, Melan-A, tyrosinase, and Sox-10 did not label these lesional cells, thus confirming the absence of a melanocytic proliferation. The patient was diagnosed with tumoral melanosis likely secondary to treatment with a PD-1 inhibitor. In this case, tumoral melanosis may have resulted from an extensive immunologic response, as would be invoked during a favorable response to immunologic therapy.

P.055 | PD-1 inhibition and vitiligo-like depigmentation in the treatment of non-melanoma solid cancers

Victor Huang[1,2]; Anna K. Dewan[1]; Nicole LeBoeuf[1,2]

[1]*Brigham and Womens Hospital, Boston, MA, USA;* [2]*Dana Farber Cancer Institute, Boston, MA, USA*

The relationship between vitiligo and melanoma has long been hypothesized based on cases of melanoma-associated depigmentation, spontaneous regression of melanoma, and halo nevi. The reciprocal relationship between risk and protective alleles in GWAS studies has provided genetic evidence supporting this hypothesis. Most recently, it has been observed that vitiligo-like depigmentation developing in the setting of melanoma vaccine and immunotherapy trials is associated with a more favorable prognosis. Vitiligo-like depigmentation occurs in 8% and 20% of patients on treatment with PD-1 and ipilimumab, respectively, and has therefore been interpreted as the clinical manifestation of effector T-cells specifically targeting melanocytic antigens. This model, however, does not sufficiently explain the emergence of vitiligo-like depigmentation in a non-melanoma context.

Herein, we describe the development of new or worsening vitiligo-like depigmentation in patients with NSCLC, metastatic RCC, and urothelial carcinoma and discuss the implications of vitiligo-like depigmentation in a non-melanoma context on our understanding of autoimmune depigmentation. The observation of depigmentation in a non-melanoma context suggests that the relationship between the pathophysiology of vitiligo and the immunologic state induced by anti-PD-1 therapy is more multifaceted than previously appreciated.

P.056 | Possible pathomechanisms of anti-PD-1 antibody-induced-leukoderma in advanced malignant melanoma patients

Yorihisa Kotobuki[1]; Atsushi Tanemura[1]; Takayoshi Morita[2]; Atsushi Tanaka[2]; Fei Yang[1]; Mari Wataya-Kaneda[1]; Ichiro Katayama[1]

[1]Department of Dermatology, Graduate School of Medicine, Osaka University, Osaka, Japan; [2]Experimental Immunology, Immunology Frontier Research Center, Osaka University, Osaka, Japan

Malignant melanoma is one of the most aggressive malignant neoplasms and there had been few reliable therapeutic options for advanced melanoma for a long time. Recent trials by using BRAF V600E inhibitors, MEK inhibitors and the immune checkpoint inhibitors actually give the breakthrough for the treatment of unresectable advanced melanoma.

Anti-PD-1 antibody, nivolumab, is the most relevant immune checkpoint inhibitor and was approved for advanced melanoma and in 2014 followed by non-small-cell lung cancer (NSCLC) in Japan. We administered nivolumab to 19 advanced melanoma patients and noticed vitiliginous macules named "anti-PD-1 antibody-induced-leukoderma (PD-1 leukoderma)" on 8 patients so far. Surprisingly, 6 out of 8 with PD-1 leukoderma had showed significant clinical response of "complete remission" or "partial response". Since PD-1 leukoderma has not been occurred in NSCLC patients in the treatment with anti-PD-1 antibody, PD-1 leukoderma in melanoma might be involved in melanoma and/or melanocyte specific immune response. In 2016, Freeman et al. reported that there are statistically significant OS differences in patients with anti-PD-1 antibody induced "vitiligo" compared with non-vitiligo. These results indicate that the occurrence of PD-1 leukoderma might be an estimated effective predictor of anti-PD-1 antibody.

To reveal a detail immune cellular response to melanocyte in the skin, PD-1 leukoderma was biopsied and analyzed immunohistochemically about residual melanocytes, melanocyte stem cells and immune-competent cells. Moreover, cellular immune response to melanocyte in circulation was examined by FACS analysis.

In this study, we will discuss the pathomechanisms of anti-PD-1 antibody-induced leukoderma.

P.057 | Knockout of BIM expression in melanoma cells enhances PDL-1 expression, a predictive biomarker for immune therapy

Nabanita Mukherjee[1]; Karoline Lambert[1]; Mayumi Fujita[1]; Yiqun G. Shellman[1]; David A. Norris[1,2]

[1]University of Colorado, Denver/Anschutz Medical Campus, Aurora, CO, USA; [2]Department of Veterans Affairs Medical Center, Denver, CO, USA

The interaction between PD-L1 on tumor cells with PD-1 on T-cells leads to immune evasion by tumor cells. Blocking this interaction by PD-1 or PD-L1 inhibitors has been successful in melanoma, but relapse still occur after initial tumor regression. To optimize immunotherapy responses, we must understand the molecular characteristics that influence these responses.

BIM, a pro-apoptotic BCL-2 family protein, is a downstream signaling molecule of the PD-1 pathway in T cells. High levels of BIM in T cells are associated with clinical benefit in patients with metastatic melanoma treated with PD-1 inhibitors. However, it is not known whether the expression of BIM in tumor cells affects immunotherapy response.

We used shRNA or CRISPR/Cas9 genome-editing to knock-down (KD)/knock out (KO) BIM expression in multiple melanoma cell lines, and examined whether BIM in melanomas influences several positive predictive biomarkers for immunotherapy targeting PD-1 or PD-L1. WB and FACS analyses indicated that KD/KO of BIM increased the basal level of PD-L1 in some melanoma lines. Further, PD-L1 is dramatically induced in response to Interferon-γ treatment (24 and 96 hrs) in all KD/KO lines tested ($p < .05$). These results suggest that down-regulation of BIM in melanoma cells may sensitize them to immunotherapy. We also looked at two additional tumor biomarkers that independently predict positive responses: (a) Interferon-γ induced growth inhibition of tumor (by means of cytokine-induced killing) and (b) HLA-DR expression (facilitating recognition by T-cells). KD/KO of BIM did not alter Interferon-γ induced growth inhibition. However, BIM KO/KD increased the basal level of HLA-DR in a subset of melanoma cell lines. Currently, we are testing the role of BIM in patient samples, which have either responded or relapsed from immunotherapy treatment. Taken together, these data suggest that BIM expression in melanoma tumor cells significantly modulate factors that control response to immunotherapy.

P.058 | Upregulation of notch signaling induces apoptosis in MAPKi-resistant melanoma cells

Dareen M. Mikheil[1]; Carlos I. Rodriguez[2]; Vijayasaradhi Setaluri[1,2]

[1]William S. Middleton Veterans Hospital, Madison, WI, USA; [2]University of Wisconsin-Madison, Madison, WI, USA

Notch signaling has been implicated in different aspects of melanoma biology including tumor initiation, progression and metastasis. Preclinical and clinical studies targeting Notch signaling for treatment of melanoma have produced conflicting results. However, the role of Notch signaling in resistance of melanoma cells to Mitogen Activated Protein Kinase (MAPK)-targeted therapy has not been investigated. In this study, we found that although NOTCH proteins are upregulated in metastatic melanoma cell lines, inhibition of Notch signaling did not affect cell survival. Furthermore, treatment with Notch inhibitors did not alter the sensitivity of BRAF(V600E)-melanoma cells to BRAF(V600E) and MEK inhibitors, vemurafenib and selumetinib, respectively. However, we noted that both intrinsic and acquired resistance to MAPK inhibition (MAPKi) is associated with down-regulation of NOTCH1 and its active form the Notch Intracellular Domain (NICD). Overexpression of NICD induced apoptotic cell death in MAPKi-resistant cells but not in the NOTCH-high, MAPKi-sensitive melanoma cells. These data suggest that MAPKi-resistance

is mechanistically linked to downregulation of Notch and that reactivation of Notch signaling is sufficient to overcome the resistance to killing independent of MAPK inhibition. Understanding the mechanisms of action of Notch in drug resistance will have an impact on the design of better therapeutic strategies for melanoma.

P.059 | Alpha-1 antitrypsin suppresses melanoma progression by inducing melanocyte differentiation antigen expression and dampening immune checkpoint responses

Jenny Mae Samson[1]; Yang Li[1]; Yuchun Luo[1]; Zili Zhai[1]; Erika Guevara[1]; Ryan Eom[1]; David Pearson[1]; Charles A. Dinarello[1]; Mayumi Fujita[1,2]

[1]*University of Colorado Anschutz Medical Campus, Aurora, CO, USA;* [2]*Denver VA Medical Center, Denver, CO, USA*

Recent advances in treating cancers with immune checkpoint inhibitors have fueled the investigation of new therapeutic modalities. Alpha-1 Antitrypsin (AAT) is a serine protease inhibitor with immunomodulatory effects shown to dampen proinflammatory responses. Treatment of B16F10 melanoma by intraperitoneal injection of recombinant human AAT suppressed tumor growth. Due to the decline in AAT effect from the development of neutralizing antibodies, we used C57BL/6 transgenic mice (TG) expressing hAAT under a surfactant protein C promoter to investigate the AAT effects on the microenvironment. B16F10 growth was suppressed in TG mice compared to wildtype mice (WT), with histological analyses revealing reduced mitosis, increased apoptosis, enhanced melanin pigmentation and increased CD3 + T cell infiltration in TG tumors. However, AAT induced no direct effects on cell growth or apoptosis in B16F10 melanoma cells in vitro. These findings suggest that AAT suppresses melanoma growth by affecting tumor cells and immune cells in the tumor microenvironment. Mechanistic studies demonstrated that AAT reduced expression of PD-1 in T cells and PD-L1 in B16F10 melanoma cells. We observed increased expression of melanocyte differentiation antigens in AAT-treated melanoma cells, subjecting them to cytotoxic T cell killing. Indeed, AAT led to the enhanced lysis of B16F10 cells by activated T cells in vitro. Thus, our data demonstrate that AAT suppresses melanoma progression by promoting tumor antigen expression and dampening immune checkpoint responses.

P.060 | Early intravasation of tyrosinase positive cells in a mouse model of melanoma metastasis

Raul Torres; Xiaoshuang Li; Lidia Kos

Florida International University, Miami, FL, USA

Melanoma is the deadliest type of skin cancer that leads skin cells to mutate and form malignant tumors. Due to its aggressiveness, melanoma has a high propensity to metastasize and invade other essential organs to seed distant metastases. The main goal of this study is to define when metastatic melanoma cells leave the primary tumors and reach the circulating system to potentially initiate metastases. To achieve this, we have created a spontaneous melanoma metastatic mouse model (Dct-Grm1/K5-Edn3) where metastasis to the lungs is 80% penetrant. We crossed the Dct-Grm1/K5-Edn3 mice to CreERT2/ROSAmT/mG mice to indelibly label Tyrosinase expressing cells within the primary tumors with green fluorescence protein (GFP) by topical application of 4-Hydroxytamoxifen (4-HT). In vivo lineage tracing of GFP-labeled cells showed that primary tumor derived Tyrosinase expressing cells, or their progeny, can seed successful metastases in the lung demonstrating their tumor initiating capacity. To establish the timing of metastatic cell intravasation, we labeled Tyrosinase expressing cells in the mice at three different stages during tumor progression: pre-nevus stage, nevus stage and mature tumor stage. GFP-labeled cells were found entering blood vessels within the tumor and in close proximity to nevi. Interestingly, GFP-labeled cells were also found in close association with blood vessels prior to the appearance of detectable nevi at sites where tumors generally develop. These results indicate that tyrosinase positive cells have the ability to disseminate very early and continue to do so during the process of melanomagenesis. Further characterization of the early aggressive cells in melanoma will allow for the development of new prognostic tests and novel therapeutic strategies to eliminate metastasis.

P.061 | The role of neuronal nitric oxide synthase ((nNOS) in interferon-gamma-induced melanoma progression

Shirley Fong; Sergei Shevtsov; Sun Yang

Chapman University School of Pharmacy, Irvine, CA, USA

Interferon-gamma (IFN-γ) produced by immune cells plays a critical role in tumor immune surveillance; however, a Phase III clinical trial surprisingly showed pro-tumorigenesis activity of IFN-γ in patients with early stage melanoma. To date, the underlying molecular mechanisms of IFN-γ-stimulated melanoma progression have not been well defined.

As shown in our previous studies, neuronal nitric oxide synthase (nNOS) overexpressed in melanoma stimulates disease progression associated with induction of nitric oxide (NO). Knockdown of nNOS significantly reduced tumor growth and lung metastasis in vivo. For the first time, our study shows that IFN-γ markedly induced the expression level of nNOS in melanoma cells, while such induction was absent with IFN-alpha treatment, a FDA-approved adjuvant for melanoma chemotherapy. Consistently, intracellular NO levels are also elevated in cells treated with IFN-γ and diminished with IFN-α exposure. STAT3-mediated signaling pathway was markedly activated by IFN-γ treatment in melanoma A375 cells. In addition, Reverse Phase

Protein Array (RPPA) analysis demonstrated that IFN-γ treatment induced the expression of genes associated with proliferation, invasion, and immunosuppression, such as BRAF, HIF1α, fibronectin, and PD-L1; However, genes associated with DNA repair and apoptosis, such as p21 and ATM, were found decreased after IFN-γ exposure. The inhibitory function of PD-L1 expression in melanoma is known to facilitate tumor cells escape from immune surveillance; Our study revealed that in melanoma cells treated with IFN-γ, PD-L1 expression was increased by 30% of control, while it was decreased when cells were exposed to IFN-α (87% of control). Inhibiting nNOS-mediated NO pathway using siRNA and specific nNOS inhibitors was shown to effectively diminish these changes induced by IFN-γ in human melanoma cells, suggesting nNOS-NO signaling plays an important role in IFN-γ-stimulated disease progression. Our study will enhance the fundamental understanding of melanoma pathogenesis, leading to the development of novel pharmacologic inhibitors for melanoma therapy.

P.062 | CDK1 enhances tumor initiating potential of cancer cells through Sox2 phosphorylation

Dinoop Ravindran Menon[1]; Yuchun Luo[1]; John J. Arcaroli[2]; Sucai Liu[1]; Stacey M. Bagby[2]; Wells A. Messermith[2]; Mayumi Fujita[1,3]

[1]*Department Of Dermatology, University Of Colorado Denver, Aurora, CO, USA;* [2]*Department Of Medicine, University Of Colorado Denver, Aurora, CO, USA;* [3]*Denver VA Medical Center, Denver, CO, USA*

Cancer stemness is often associated with high tumor initiating potential and disease relapse. In this study, we have identified a distinct population of HLA-(A/B/C) high (HLA^{hi}) cells present in patient-derived xenograft tumors of melanoma, colon and pancreatic cancer that are enriched in tumor-initiating potential. While probing for differentially expressed stemness genes in HLA ^{hi} and HLA ^{lo} cells using NanoString® nCounter analysis, CDK1 was found to be consistently upregulated in HLA ^{hi} population. Inhibition of CDK1 suppressed tumor growth in vivo. CDK1 inhibition also reduced clonogenicity in vitro and tumor-initiating capacity in vivo. A stem cell gene antibody array performed on CDK1 immunoprecipitated samples from human melanoma cells identified a pluripotent stem cell marker SOX2 as a binding partner of CDK1. Luciferase and CHIP analysis confirmed the importance of CDK1 – SOX2 interaction for the transcriptional activity of SOX2. Inhibiting CDK1 abrogated SOX2 S249-251 phosphorylation, resulting in the inhibition of nuclear translocation of SOX2 and downregulation of SOX2 target genes. Together, the study implies a novel role of CDK1 in cancer stemness by regulating SOX2 function. Thus the CDK1-SOX2 interaction could serve as a potential target for therapeutic intervention in cancer.

P.063 | Loss of Brn2 promotes in vivo melanomagenesis

Michael B. Hamm; Madeleine Le Coz; Pierre Sohier; Lionel Larue

Institut Curie, Orsay, France

Every 32 min a person died of melanoma in the EU last year, making melanoma to the deadliest form of commonly encountered skin cancer due to its rapid progression. Melanomagenesis is a multistep process including melanoma initiation and progression. Over the last ten years a series of proteins have been shown to be functionally important to induce melanomagenesis in vivo. These proteins are lost such as Nf1, p16, and Pten or possess point mutations such as Braf^{V600E}, Nras^{Q61K}, β-catenin$^{ST/AA}$ and Mitf^{E318K}. Brn2 is a POU-family transcription factor that has been linked to melanoma for its potential role during the melanoma phenotype-switch. However, its role in vivo during melanomagenesis remains unclear. In order to evaluate the role of Brn2 in this process, Brn2 was specifically deleted in melanocyte using two mouse melanoma models : (i) Braf^{V600E} and (ii) Braf^{V600E} Pten loss. This approach allowed to show that Brn2 loss induces melanoma initiation and progression in vivo. Functionally, Brn2 acts synergistically with Pten to bypass of senescence, making Brn2 a potent tumor suppressor in melanoma.

P.064 | In vitro and in vivo anti-melanoma effect of *T. hirsuta* extract and its bioactive component daphnane diterpenoid gnidilatidin

Myra O. Villareal; Yuki Sato; Hiroko Isoda

University of Tsukuba, Tsukuba City, Japan

Melanoma is the most aggressive type of cancer with a very poor prognosis. At present, surgery to remove cancerous tumors, followed by cytotoxic chemotherapy, is the main treatment for melanoma. However, currently available anti-cancer drugs produce unwanted side effects such as nausea and fatigue, to name a few, giving rise to the growing need to discover new candidate therapeutics against cancer, such as the use of medicinal plants known to contain natural products of therapeutic relevance. In this study, we determined the effect of melanogenesis inhibitor *Thymelaea hirsuta* (TH) extract and one of its bioactive components, daphnane diterpenoid gnidilatidin, on melanoma. Results of both in vivo and in vitro assays have shown that both TH and gnidilatidin can inhibit melanoma metastasis by inhibiting the expression of metalloproteases (MMP2 and MMP9) and CD44. Elucidation of mechanism underlying their effects was done by determining the effect of gnidilatidin on the global gene expression in B16 cells, the results of which supported the findings on the inhibitory effect of TH on metastasis of melanoma cells. This is

the first report on the potential therapeutic effects against melanoma of TH and gnidilatidin.

P.065 | Utilization of reactive oxygen species to prolong the efficacy of BRAF inhibitors in melanoma

Long Yuan[1]; Rosalin Mishra[1]; Safnas Abdulsalam[2]; MacKenzie Nolte[1]; Edward J. Merino[2]; Joan T. Garrett[1]

[1]*College of Pharmacy, Cincinnati, OH, USA;* [2]*Department of Chemistry, University of Cincinnati, Cincinnati, OH, USA*

BRAF mutations occur in about 50% of melanoma patients. FDA approved BRAF and MEK inhibitors have improved the prognosis of patients with BRAF mutations.

However, all responders develop resistance within one year of treatment. Recent observations demonstrate that BRAF inhibitors induce reactive oxygen species (ROS) in melanoma cells. A100, identified from a library screen, self-cyclizes into a stable bicyclic ring after ROS quenching and causes DNA double strand breaks. We propose to examine if ROS targeted therapy in BRAF mutant melanomas will inhibit tumor growth and evade resistance to BRAF inhibitors. BRAF-mutant A375, SK-MEL-24 and WM-115 dabrafenib resistant melanoma cells were generated using increasing doses of dabrafenib. Flow cytometry experiments showed that ROS levels as assessed by both the H2DCFDA and MitoSOX assays are increased in A375, SK-MEL-24 and WM-115 melanoma cells after acquisition of resistance to dabrafenib compared to parental cells. Crystal violet and 3-D matrigel assays were done to analyze the effect of both the drugs on cell proliferation of dabrafenib resistant and parental melanoma cell lines. Cell lines show statistically significant greater inhibition of growth in combination treatment groups compared to single agent treatment groups. We identified that ERK phosphorylation is reduced in melanoma cell lines subjected to dabrafenib treatment alone or the combination of both dabrafenib and A100. DNA damage signaling is altered in combination treatment groups as assessed by p-ATM and γ-H2AX levels. These data suggest that the combination of a ROS quenching agents with BRAF inhibitor could be a potential strategy to treat melanoma patients having BRAF mutations.

P.066 | Investigation of BLOC-2 regulated melanosome cargo delivery

Shanna L. Bowman[1,2]; Yueyao Zhu[2]; Erika Holzbaur[2]; Michael Marks[1,2]

[1]*Children's Hospital of Philadelphia, Philadelphia, PA, USA;* [2]*University of Pennsylvania, Philadelphia, PA, USA*

Hermansky-Pudlak Syndrome (HPS) is a group of genetic disorders that cause oculocutaneous albinism, excessive bleeding, and also immunodeficiency, pulmonary fibrosis, and granulomatous colitis in some patients. HPS symptoms result from malformation of lysosome-related organelles (LROs). For example, oculocutaneous albinism is caused by defects in melanin synthesis in melanosomes, LROs that synthesize skin and ocular melanin pigment. Excessive bleeding in HPS patients results from defects in formation of platelet dense granules, LROs that are necessary for secretion of blood clotting factors. Many of the affected genes in HPS encode subunits that function in five protein complexes that regulate membrane trafficking processes in the cell: Biogenesis of Lysosome-related Organelles Complex (BLOC)-1, 2, and 3, Adapter Complex-3 (AP-3), the HOPS, and the Rab GTPase, RAB38. Proper formation of LROs relies on the membrane trafficking functions of these complexes to deliver protein cargo, such as melanin synthesizing enzymes, from membrane tubules that are derived from early endosomes. Previous work in our laboratory has shown that BLOC-2-depleted melanocytes are hypopigmented, and BLOC-2 is required for these endosomal tubules to contact melanosomes for efficient cargo delivery. Here, we investigated the mechanism of BLOC-2's role in endosomal tubule targeting to melanosomes in melanocytes from a mouse HPS model. Using high resolution, live cell fluorescence microscopy, we show that melanosome-directed endosomal tubule carriers have shorter lifetimes and length, do not move processively toward melanosomes, and travel at a higher velocity in BLOC-2-depleted melanocytes. We are currently investigating the role of the microtubule cytoskeleton and molecular motors in BLOC-2-mediated endosomal tubule contacts with melanosomes. Further, understanding the mechanisms of melanosome biogenesis serves as an important model to increase our understanding of the improper formation of other tissue specific LROs in HPS.

P.067 | Cell mechanics and signaling in skin: The role of caveolae

Lia Domingues[1]; Floriane Gilles-Marsens[1]; Ilse Hurbain[1,2]; Weiwei Shen[3]; Cédric Blouin[3]; Christophe Lamaze[3]; Christelle Gueré[4]; Nathalie André[4]; Katell Vié[4]; Graça Raposo[1,2]; Cédric Delevoye[1,2]

[1]*Institut Curie, PSL Research University, CNRS, UMR144, Structure and Membrane Compartments, Paris, France;* [2]*Institut Curie, PSL Research University, CNRS, UMR144, Cell and Tissue Imaging Facility PICT-IBiSA, Paris, France;* [3]*Institut Curie, PSL Research University, INSERM, U1143, CNRS, UMR 3666, Membrane Dynamics and Mechanics of Intracellular Signaling Laboratory, Paris, France;* [4]*Laboratoire Clarins, 31 chaussée Jules César, Pontoise, France*

Skin pigmentation and photoprotection rely on two epidermal cell types, keratinocytes and melanocytes. Keratinocytes secrete hormones, growth factors and exosomes that modulate melanocyte functions, like melanin pigment synthesis, storage in newly formed organelles (melanosomes) and further transfer to neighboring keratinocytes. We showed that one such specific regulation depends on the miR203 (non-coding RNA), which is highly expressed in exosomes secreted from UV-stimulated keratinocytes. MiR203 raised the melanin content and expression level of key melanosomal enzymes (Tyrosinase) in primary melanocytes and melanoma cells. Mir203 can target, and subsequently down-regulate many components, including caveolin-1 (Cav1). Cav1 is the scaffold protein of caveolae,

invaginated plasma membrane structures contributing to lipid homeostasis, signaling transduction, mechanosensing and endocytosis. Interestingly, we showed that UV-stimulated melanocytes increased the expression of Cav1 and related caveolae components. Therefore caveolae represent an ideal molecular platform candidate to modulate keratinocyte-melanocyte communication in skin.

Depleting Cav1 expression (siRNA or miR203) or impairing the formation and function of caveolae in melanocytes increased the melanin content, number and pigmentation status of melanosomes and tyrosinase expression. Also, the transfer of the pigment to keratinocyte was impaired, indicating together that Cav1 and caveolae control the pigmentation and activity of melanocytes. Given that melanin transfer occurs through poorly defined mechanisms, we then examined the melanocyte-keratinocyte interface at the ultrastructural level by analyzing caveolae in different samples — from human skin biopsies to synthetic epidermis and in vitro co-culture. We identified specific caveolae enrichment and polarization at melanocyte-keratinocyte contact sites, indicative of a specific role for caveolae during melanocytes-keratinocyte communication.

Altogether, our data suggest that caveolae play a dual function in pigmentation: a negative role on the melanin and melanosomes production, and a positive control of melanin transfer between epidermal cells. Both processes would be temporally regulated in skin upon UV exposure to tune the pigmentation.

P.068 | Reversal of hypopigmentation in Hermansky-Pudlak Syndrome models by MC1R signalling

Philip S. Goff[1]; Shyamal Patel[1]; Nyamekye Obeng-Adjei[2]; Michael S. Marks[2]; Elena V. Sviderskaya[1]

[1]Cell Biology and Genetics Research Centre, Molecular and Clinical Sciences Research Institute, St. George's, University of London, London, UK; [2]Department of Pathology & Laboratory Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA, USA

Pigmented melanosomes mature from highly acidic, non-pigmented precursors by the delivery of melanogenic enzymes and transporters primarily from early endosomes. The onset of pigmentation correlates with pH neutralisation, at least in part through delivery of transporters such as OCA2. Melanocortin-1 receptor (MC1R) signalling, via a cAMP-dependent pathway, regulates melanogenic genes and transporters (including OCA2) and consequent eumelanin synthesis.

Hermansky-Pudlak syndrome (HPS) comprises 10 known autosomal recessive disorders characterised by hypopigmentation, bleeding tendency and other variable symptoms due to defects in the biogenesis of lysosome-related organelles (LROs) such as melanosomes. HPS7-9 result from loss of biogenesis of lysosome-related organelle complex 1 (BLOC-1), which is required for the delivery of many melanogenic enzymes and transporters, including OCA2, to melanosomes. BLOC-1-deficient melanocytes are severely hypopigmented because their melanosomes lack these components despite the presence of tyrosinase.

We aim to determine whether MC1R signalling and pH modulation can enhance pigmentation in BLOC-1-deficient melanocytes by altering melanosomal protein trafficking and/or compensating for trafficking defects by cargo overexpression. We show that treatment of BLOC-1-deficient melanocytes with cAMP agonists induces a significant two-fold increase in melanin content. By immunofluorescence and bright field microscopy, the melanin within cAMP agonist-treated BLOC-1-deficient melanocytes predominantly overlapped with a marker of lysosomes but not of melanosomes. This suggests that cAMP signalling leads to mistargeting of a cohort of melanosomal proteins toward lysosomes. To determine whether the enhanced pigmentation reflected the neutralisation of pH by enhanced OCA2 expression, we overexpressed OCA2 or an inactive OCA2 mutant in BLOC-1-deficient melanocytes. Our data show that overexpression of active OCA2, but not the inactive V443I OCA2 mutant, significantly enhanced pigmentation in BLOC-1-deficient melanocytes.

Our data suggest that approaches to neutralise the pH of endolysosomal organelles might restore pigmentation to BLOC-1-deficient melanocytes. These results could lead to novel treatments for hypopigmentation in HPS patients.

P.069 | Dermal fibroblasts internalize phosphatidylserine-exposed melanosome-rich globules and apoptotic melanocytes

Moemi Yoshida[1]; Satoshi Yoshimoto[1]; Yoshiaki Ohagi[1]; Hiroki Yanagi[1]; Masayuki Yagi[2]; Takahito Nishikata[3]; Toshiyuki Ozawa[4]; Daisuke Tsuruta[4]; Masamitsu Ichihashi[5]; Hideya Ando[1]

[1]Okayama University of Science, Okayama, Japan; [2]Rosette Co., Tokyo, Japan; [3]Frontiers of Innovative Research in Science and Technology [FIRST], Konan University, Kobe, Japan; [4]Department of Dermatology, Osaka City University Graduate School of Medicine, Osaka, Japan; [5]SAISEI MIRAI Clinic, Kobe, Japan

It is well known that fibroblasts possess a phagocytotic ability. Here we show that dermal fibroblasts preferentially incorporate melanosome-rich globules released from melanocyte dendrites as well as apoptotic melanocytes via phosphatidylserine-mediated endocytosis. Cultured normal human melanocytes produced melanosome-rich globules along various sites of their dendrites and time-lapse imaging showed that dermal fibroblasts scraped those globules and incorporated them. Annexin V staining revealed that phosphatidylserine, which is normally located on the inner layer of plasma membrane and is known as an 'eat me' signal that triggers the initiation of internalization, was exposed on the surface of melanosome-rich globules. The ability of dermal fibroblasts to uptake melanosome-rich globules was comparable to macrophages. Further, the co-culture of fibroblasts and melanocytes in fibroblast culture medium revealed that dermal fibroblasts actively internalized phosphatidylserine-exposed apoptotic melanocytes. These results suggest that dermal fibroblasts contribute to the clearance of unwanted obstacles in the dermis, such as melanosome-rich globules and apoptotic melanocytes, that occasionally drop down from the epidermis.

P.070 | Negative charges as well as carboxy groups are triggers for the phagocytosis of dermal fibroblasts

Satoshi Yoshimoto[1]; Moemi Yoshida[1]; Yoshiaki Ohagi[1]; Hiroki Yanagi[1]; Masayuki Yagi[2]; Yuji Yamashita[3]; Tetsuji Hirao[3]; Haruka Koda[4]; Takahito Nishikata[4]; Masamitsu Ichihashi[5]; Hideya Ando[1]

[1]Department of Applied Chemistry and Biotechnology, Okayama University of Science, Okayama, Japan; [2]Rosette Co., Tokyo, Japan; [3]Cosmetic Science Laboratory, Chiba Institute of Science, Chiba, Japan; [4]Frontiers of Innovative Research in Science and Technology FIRST, Konan University, Kobe, Japan; [5]SAISEI MIRAI Clinic, Kobe, Japan

Although dermal fibroblasts are known to incorporate phosphatidylserine-exposed melanosome-rich globules that are released from the dendrites of epidermal melanocytes, the phagocytotic mechanisms of fibroblasts are poorly understood. Here we show that the electric charge and the functional group of the membrane phospholipid side chain is involved in the mechanism of internalization of melanosome-rich globules by fibroblasts. Cultured normal human dermal fibroblasts incorporated few uncoated polystyrene particles with a diameter of 5 μm, while they preferentially internalized polystyrene particles that had been coated with phosphatidylserine. Since phosphatidylserine has a carboxy group (negative charge) and an amino group (positive charge), carboxylate-modified particles or amine-modified particles were added to the culture medium of fibroblasts. The results showed that only carboxylate-modified particles were internalized by fibroblasts. However, the internalization of sulfo-modified particles, which also have a similar negative charge to carboxylate-modified particles in their zeta potential values, was significantly decreased. These results suggest that not only a negative charge but also a carboxy group are essential for the mechanism of internalization of phosphatidylserine-exposed melanosome-rich globules by dermal fibroblasts.

P.071 | Deodorized beta-mercaptoethylamine hydrochloride as a depigmenting agent for the treatment of melasma: Results of in vitro, in vivo and human studies

Behrooz Kasraee

Cornavin Dermatology Center, Geneva, Switzerland

Beta-mercaptoethylamine hydrochloride (MEA) is known for its potent depigmenting effect since 1960's when Chavin tested it through injecting MEA into the black goldfish skin. A few years later, different in vivo studies showed the higher depigmenting efficacy of this molecule compared to hydroquinone. However, MEA has never been utilizable in human mainly due to the very offensive odour it produced in topical products. Recently, we have developed a new technology to deodorize MEA. MEA thus became utilizable for the first time in a topical product. Deodorized MEA showed a significant melanogenesis inhibiting effect in different in vitro and in vivo models. A double-blind, placebo-controlled randomized human study in 50 patients with epidermal melasma showed the significant efficacy of deodorized MEA for the treatment of this hyperpigmentary disorder. MEA is biologically produced in mammalian cells and serves as an intracellular antioxidant. This molecule has been orally used in human, mainly for the treatment of cystinosis, and has a long history of safety for human use. The anti-mutagenic and anti-carcinogenic effects of MEA are previously shown in numerous studies. Deodorized MEA might serve as a new safe and effective skin depigmenting agent for the treatment of hyperpigmentary disorders such as melasma.

P.072 | Mutual cross talk between metabolism and epigenetics

Fabian V. Filipp

Systems Biology and Cancer Metabolism, Program for Quantitative Systems Biology, University of California Merced, Merced, CA, USA

Histone methylation is an epigenetic modification of chromatin undergoing dynamic changes and balancing tissue-specific demands of proliferation and differentiation. In melanocytes, aberrant histone methylation can facilitate oncogenic and tumor suppression programs by modulating gene expression. Histone remodelers such as lysine methyltransferases and lysine demethylases are seemingly opposite or contrary forces but may actually be part of an interconnected network complementing each other. We identify several layers of molecular communication in melanoma where epigenetic master regulators engage in crosstalk between tumor metabolism and histone remodeling. Epigenetic master regulators have the ability to cooperate with members of the transcriptional machinery, DNA methyltransferases, as well as other histone modifiers. High-throughput sequencing and omics data in combination with cancer systems biology analysis has the power to prioritize regulatory events epigenome-wide.

P.073 | Photobiomodulation – a possible role for age-related auto-fluorescence

James M. Gallas

Photoprotective Technologies, San Antonio, TX, USA

Melanin, and several related naturally-occurring pigments in the retina and ocular lens, display a wavelength-selective filtration of UV and high energy visible light that has long been *associated with photoprotection*. These pigments also display a level of fluorescence that can create glare within the ocular cavity and therefore compromise vision. However, this auto-fluorescence, whose intensity and emission maxima red-shifts and increases with age, may be *associated with repair* in the aged ocular system.

Previous photobiomodulation studies - relating to repair of the retina - used 670 nm LED light sources and found an increase in mitochondrial membrane potentials in aged murine RPE cells that was

associated with increased ATP production. We present here evidence that may support photo-biomodulation as a possible role for age-related auto-fluorescence.

Data in this presentation underscore an increasingly red-shifted auto fluorescence emission toward 670 nm for melanin and ocular pigment samples progressively-bleached using techniques which we have assumed to be equivalent to aging. A range of fluorescence excitation and emission maxima are presented that correspond to those ranges reported in red light and near infrared light-based photo-biomodulation studies – supporting our view of a wavelength-selective-based repair of retina cells by auto-fluorescent molecules.

A brief review of photo-biomodulation - empirical studies on cells and tissue exposed exclusively to LED light sources - is presented along with calculations that compare previously-reported LED intensities and doses external to the ocular media, with auto-fluorescence intensities and doses within the ocular media.

P.074 | Genome instability and aberrant cell cycle progression in mahogunin ring finger-1 null mouse melanocytes

Idoya Martínez-Vicente[1]; Cecilia Herraiz[1]; Marta Abrisqueta[1]; María Castejón-Griñán[1]; Julia Sirés[1]; Elena V. Sviderskaya[2]; Dorothy C. Bennett[2]; Conchi Olivares[1]; Jose C. García-Borrón[1]; Celia Jiménez-Cervantes[1]

[1]Department of Biochemistry, Molecular Biology and Immunology, School of Medicine, University of Murcia and Instituto Murciano de Investigación Biosanitaria IMIB-LAIB, El Palmar, Murcia, Spain; [2]Molecular and Clinical Sciences Research Institute, St George's, University of London, Cranmer Terrace, London SW17 0RE, London, UK

Mahogunin Ring Finger-1 (MGRN1) E3-ubiquitin ligase interacts with the melanocortin 1 receptor (MC1R) and competes with Gsα to decrease activation of cAMP signalling downstream of receptor. The mouse *Mahoganoid* mutation abolishes *Mgrn1* expression and yields a complex phenotype with fur darkening, congenital heart defects and neurodegeneration. Mgrn1 deficiency also causes a mitochondrial dysfunction that precedes neurodegeneration. Since mitochondrial dysfunction often leads to increased production of reactive oxygen species and oxidative stress, we analyzed oxidative DNA damage in control (melan-a6) and *mahoganoid* (melan-md1) cells. Immunochemical staining revealed higher levels of 8-oxodG in melan-md1 cells. Alkaline comet assays showed extensive DNA fragmentation in melan-md1 cells, indicative of DNA double strand breaks. FACS analysis of propidium iodide-stained cells indicated alteration of DNA contents. Assessment of karyotypes in chromosomal spreads showed that ~80% of *Mgrn1*-null cells were aneuploid, with chromosome numbers ranging from 8 to 100 and frequent polyploidy (~ 4n). In contrast, less than 1% of mitotic spreads from wild-type melan-a6 cells showed numerical abnormalities, suggesting compromised fidelity of chromosome segregation in Mgrn1-null cells. Melan-md1 cells proliferated at a slower rate than melan-a6 cells in 2D cultures and in 3D spheroids. Thus, their frequent polyploidy likely reflected a delay in G2 and/or an accumulation of tetraploid cells following failed division. Consistently, gene set enrichment analysis identified reduced expression in melan-md1 melanocytes of *AurkB*, *Incenp*, *Birk5*, *Cenpa* and other genes encoding centromeric proteins or proteins of the spindle assembly cell cycle checkpoint and proliferation markers such as *Mki67*. Work is underway to determine whether these cell cycle aberrations actually result from loss of Mgrn1 expression in melan-md1 melanocytes.

P.075 | Mahogunin ring finger-1 controls tyrosinase activity and melanin synthesis by regulation of melanosomal pH

Julia Sirés[1]; Conchi Olivares[1]; Ana Lambertos[1]; Cecilia Herraiz[1]; Marta Abrisqueta[1]; María Castejón-Griñán[1]; Idoya Martínez-Vicente[1]; Elena V. Sviderskaya[2]; Dorothy C. Bennett[2]; Rafael Peñafiel[1]; Celia Jiménez-Cervantes[1]; Jose C. García-Borrón[1]

[1]Department of Biochemistry, Molecular Biology and Immunology, School of Medicine, University of Murcia and Instituto Murciano de Investigación Biosanitaria IMIB-LAIB, El Palmar, Murcia, Spain; [2]Molecular and Clinical Sciences Research Institute, St George's, University of London, Cranmer Terrace, London SW17 0RE, London, UK

Mgrn1 is an E3-ubiquitin ligase mutated in dark-furred *mahoganoid* mice. We investigated the mechanisms of hyperpigmentation in *Mgrn1*-null melan-md1 melanocytes. Tyrosinase (Tyr) activity measured in situ was 10-fold higher in melan-md1 cells compared with melan-a6 control cells, but Tyr protein levels were comparable. Treatment with the lysosomotropic neutralizing agent NH_4Cl increased Tyr activity in situ, melanin content and organelle pH in melan-a6 cells, with minor effects on melan-md1 cells. Upon co-transfection with Tyr in HEK293 cells, MGRN1 decreased Tyr specific activity and pigment synthesis, and caused significant acidification of organelles as determined by DAMP staining. Therefore, Mgrn1 modified the melanosomal pH, causing post-transcriptional Tyr activation. Gene set enrichment analysis also suggested a differential regulation of organelle pH in melan-md1 cells. Polyamines (putrescine, spermidine and spermine) are ubiquitous polycationic basic compounds essential for cell proliferation, differentiation and survival that may accumulate in melanized melanosomes through electrostatic interaction with anionic melanins. Melan-md1 cells showed aberrant expression of genes encoding proteins of the polyamine biosynthesis pathway, suggesting a possible relationship with the higher melanosomal pH. However, difluoromethylornithine (DFMO), a specific inhibitor of the rate-limiting enzyme ornithine decarboxylase, lowered polyamine contents in melan-a6 cells and blocked cell proliferation but stimulated 2-3-fold both Tyr activity in situ and melanin content, thus ruling out a major role of altered polyamine levels in upregulating intramelanosomal pH. We next confirmed aberrant expression of genes encoding several subunits of the proton-transporting lysosomal vATPase and a very strong upregulation of *Mcoln3*, encoding the ion channel Mucolipin-3. Mutations in *Mcoln3* are known to yield pigmentation phenotypes and to modulate organelle pH. Manipulation of *Mcoln3* expression or

pharmacological modulation of Mcoln3 activity suggested a contribution of Mcoln3 to the regulation of Tyr activity, but the effects were modest, pointing to additional mechanisms of melanosomal pH neutralization in melan-md1 cells.

P.076 | Multiple immunoevasive mechanisms exhibited by ALDHhi human melanoma cells

Erika N. Guevara[1]; Dinoop R. Menon[1]; Yuchun Luo[1]; Mayumi Fujita[1,2]

[1]*Depatment of Derm, University of Colorado SOM, Aurora, CO, USA;* [2]*Denver VA Medical Center, Denver, CO, USA*

Cancers are comprised of heterogeneous subpopulations with various tumor initiating capacities. We have demonstrated that high aldehyde dehydrogenase (ALDH) activity identifies a highly tumorigenic population with stemness in human melanoma. ALDH is important for melanoma cell survival, proliferation, and chemoresistance. In this study, we investigated immunoevasive properties of $ALDH^{hi}$ population in human melanoma. In contrast to $ALDH^{lo}$ melanoma cells that displayed increased tumorigenesis in NSG mice compared with NOD/SCID mice, $ALDH^{hi}$ cells were equally tumorigenic in NOD/SCID and NSG mice, suggesting that $ALDH^{hi}$ melanoma cells were resistant to cytotoxicity by NK cells. In line with these data, $ALDH^{hi}$ melanoma cells expressed higher MHC class I compared to $ALDH^{lo}$ cells. $ALDH^{hi}$ melanoma cells also expressed lower levels of melanocyte differentiation antigens compared to $ALDH^{lo}$ melanoma cells, suggesting that $ALDH^{hi}$ melanoma cells were resistant to cytotoxicity by cytotoxic T cells (CTLs). However, $ALDH^{hi}$ melanoma cells upregulated other antigens such as cancer-testis antigens that could be recognized by CTLs. Therefore, we decided to seek more global protective mechanisms against cytotoxicity. NK cells and CTLs possess self-protective mechanisms to avoid self-destruction after secreting their cytotoxic granules. One mechanism is to express cathepsin B, a lysosomal cysteine protease that cleaves and inactivates perforin. We found that $ALDH^{hi}$ melanoma cells upregulated *CTSB* in 3D culture. Immunohistochemistry of human melanoma tissues and patient-derived xenografted melanoma tissues demonstrated colocalization of cathepsin B and ALDH expression in human melanoma cells. Together, we show that $ALDH^{hi}$ melanoma cells exhibit multiple immunoevasive properties, which may contribute to the ability of $ALDH^{hi}$ population to avoid cytotoxic immune attack in tumor microenvironment.

P.077 | A novel role of notch signaling in melanocyte pigmentation

Li Pan[1]; Bishal Tandukar[1]; Sandeep Joshi[1]; Emmanual Kalapurakal[1]; Thomas J. Hornyak[1,2,3]

[1]*Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, USA;* [2]*Research & Development Service, VA Maryland Health Care System, Baltimore, MD, USA;* [3]*Department of Dermatology, University of Maryland School of Medicine, Baltimore, MD, USA*

Notch signaling is an important cell-cell interaction mechanism, which involves in multiple processes during development. In mammals, both the canonical and non-canonical pathways have been reported. In melanocyte lineage, Notch signaling plays essential roles in embryonic melanoblasts and melanocyte stem cell maintenance via its target gene *Hes1*, as well as proper location and timed differentiation of melanoblasts in postnatal hair follicles (HFs). In addition, it is also involved in melanoma progression. However, the functional roles and molecular mechanisms of Notch signaling in melanocytes are still not fully understood.

We performed immunostaining of *Dct*-H2BGFP mice skin to detect the expression of active Notch signaling (NICD1) in HF melanocytes. The results showed NICD1 co-localized with GFP-expressing melanocytes in the HF bulb at anagen III, suggesting that Notch signaling is active in differentiated anagen HF melanocytes. To investigate functional roles of Notch signaling, we used DAPT, a γ-secretase inhibitor, to inhibit Notch signaling in melan-a cells. After 4 days of treatment, the pigmentation of melan-a cells was significantly decreased under 1 μM, 5 μM, 10 μM and 20 μM DAPT treatment, but cell growth was not affected. Quantitative RT-PCR results showed that the decrease in pigmentation observed was associated with lower expression not only of *Hes1*, but also of melanogenic enzyme genes *Dct*, *Tyrp1*, *Tyr* and transcription factors *Mitf*, *Sox10* and *Pax3*. To further confirm these results, a dominant-negative mutant of the Notch transcriptional co-activator Mastermind-like 1 (migDNMAML1), as well as a GFP control (migR1) and Deltex1 (MigDtx1), a distinct regulator of Notch signaling acting independently of the canonical RBPJ-κ pathway, were overexpressed in melan-a cells. Melanin content analysis showed that overexpression of Dtx1 but not DNMAML1 inhibited melan-a cell pigmentation compared to control.

Taken together, these data indicated that Notch signaling is required for melanogenesis and this novel role may involve the Notch non-canonical pathway.

P.078 | Astaxanthin abrogates stem cell factor-stimulated melanogenesis in human melanocytes via interruption of MSK1 phosphorylation in the p38/MSK1/CREB/MITFAxis

Shuko Terazawa[1]; Takao Niwano[2]; Hiroaki Nakajima[3]; Genji Imokawa[1,2]

[1]*Research Institute for Biological Functions, Chubu University, Kasugai, Japan;* [2]*Center for Bioscience Research & Education, Utsunomiya University, Utsunomiya, Japan;* [3]*School of Bioscience and Biotechnology, Tokyo University of Technology, Hachiouji, Japan*

We previously reported that astaxanthin (AX) abrogates the stem cell factor (SCF)-stimulated pigmentation of human epidermal equivalents (HEEs). In this study, we characterized the molecular mechanisms involved in the AX-induced anti-melanogenic effects in normal human melanocytes (NHMs). Pretreatment with AX suppressed the SCF-stimulated protein expression of tyrosinase (TYR)

and endothelin B receptor at 96 hr and of MITF at 1.5 hr post-stimulation. Although the major SCF-activated signaling cascade in NHMs was not interrupted by AX, the increased phosphorylation of CREB was significantly abrogated, indicating the presence of an alternative inhibitory cascade leading to CREB inactivation. We now report for the first time that SCF can stimulate the phosphorylation of p38, which generally occurs at the front line of the UVB-dependent stress-activated signaling pathway. Further, the p38 activation was abrogated by pretreatment with a neutralizing antibody to c-KIT, indicating its SCF-dependent activation. While it is known that activated p38 can stimulate the phosphorylation of CREB via the activation of MSK1 or MAPK-APK2, MSK1 but not MAPK-APK2 was distinctly activated by SCF treatment. Interestingly, whereas AX did not abrogate the SCF-induced activation of p38, it affected the increased phosphorylation of its downstream target, MSK1. The lineage connection of p38/MSK1 activation with CREB activation and its associated MITF expression was substantiated by our observations that while silencing of MSK1 abolished the activation of CREB at 15 min post-stimulation with SCF, inhibitors of p38 and of MSK1 abrogated the SCF-induced increase in MITF at 1.5 hr post-stimulation. These findings suggest that the suppressive effect of AX on the SCF-induced stimulation of melanogenesis in NHMs is mediated via interruption of MSK1 phosphorylation in the p38/MSK1/CREB/MITF axis, providing new evidence for the reactive oxygen species depletion-independent interruption by antioxidants of SCF-triggered signaling.

P.080 | Histopathologic analysis and inter-observer correlation of ashy dermatosis

Marcel Bekkenk; Marieke Berghuis; Myrna Bakker

Department of Pathology AMC Hospital Amsterdam, Academic Medical Center, Amsterdam, Netherlands

Background: Ashy Dermatosis (AD) also known as Erythema Dyschromicum Perstans (EDP) is a rare pigment disorder clinically characterized by 'ash –gray' discoloration of the skin. It is matter of debate whether Lichen Planus Pigmentosus (LPP) is a different disease or part of the AD/EDP spectrum. It is an acquired slowly progressive, persistent skin disease of unknown etiology. There is no well proven effective treatment. It is cosmetically very disturbing for patients. The aim of this study is to investigate if we can identify a clear histopathological pattern by an inter-observer correlation analysis.

Methods: All patients diagnosed in our institute as AD/EDP/LPP and available histopathology samples of the initial diagnostic lesion were reviewed by 4 dermatopathologists and independently scored for histopathological featured often found in EDP/LPP. Inter-observer correlation of diagnosis and the specific features was calculated.

Results: Histological samples of 54 patients were evaluated. The Fleiss' kappa for diagnosis was 0.18 which can be interpreted as a 'slight' correlation. In many biopsies other diagnoses seemed more likely than AD/EDP/LPP. The percentage of pairwise similarities for assessing characteristics was between 61 and 96% with kappa values between -0.01 and 0.41 (bad to reasonable agreements) Only four cases were diagnosed as EDP/LPP by all four investigators. Opinions differed between the experts what the most important histological feature in EDP/LPP is.

Conclusions: There was no unilateral agreement of histopathological pattern for AD/EDP/LPP according to the investigators. They scored histological characteristics differently. It seems to be a 'diagnosis on exclusion'. Especially features suggestive of lupus erythematosus lead to excluding the diagnosis of AD/EDP/LPP. It appears to be no clear clinico-pathological entity, but when clinical features are suggestive for AD/EDP/LPP and pathology can rule out other causes of secondary hyperpigmentation this results in the diagnosis of AD/EDP/LPP.

P.081 | Modeling melanocyte chemoresistance in zebrafish larvae

Kersten A. Peterson; Sam Neuffer; Miranda Bean; Leslie New; Allison B. Coffin; Cynthia D. Cooper

Washington State University Vancouver, Vancouver, WA, USA

Melanoma treatment is inefficient due to its inherent resistance to chemotherapy. Using model organism zebrafish, our long-term goal is to improve the treatment of melanocyte-related disorders, such as melanoma, by elucidating the cell biological mechanisms that promote chemoresistance in vivo. Due to the ease of adding chemotherapeutic drugs to fish water, conserved roles for pigment cell proteins to mammals and structurally similar skin architecture, zebrafish larvae offer a biologically relevant model for understanding chemoresistance in humans. Here, we test in vivo the hypothesis that melanosomes contribute to chemotherapy drug resistance via drug sequestration. We also compare three melanosome biogenesis proteins - microphthalmia-associated transcription factor (Mitfa), vacuolar protein sorting 11 (Vps11) and oculocutaneous albinism 2 (Oca2) to determine their respective contributions to chemoresistance. Zebrafish larvae harboring loss of function mutations in the *mitfa*, *vps11* or *oca2* genes are more sensitive to chemotherapeutic drug cisplatin damage as compared to wildtype larvae. In contrast, these mutations do not alter cisplatin sensitivity in iridophores (silver pigment cells) or lateral line sensory cells, suggesting that this increase in drug sensitivity is melanocyte specific. This is the first in vivo study to show an increase in chemotherapeutic drug sensitivity when melanosome-related mutations are present. The proteins tested represent novel drug targets for increasing the efficiency of melanoma chemotherapy treatment.

P.082 | Waardenburg syndrome type IIE in a Japanese patient caused by a novel non-frameshift duplication mutation in the SOX10 gene

Ami Hemmi[1]; Ken Okamura[1]; Shuji Izumi[3]; Ryushi Tazawa[2]; Jun Tohyama[2,5]; Yutaka Shimomura[4]; Yutaka Hozumi[1]; Tamio Suzuki[1]

[1]Department of Dermatology, Yamagata University Faculty of Medicine, Yamagata, Japan; [2]Bioscience Medical Research Center, Niigata University Medical and Dental Hospital, Niigata, Japan; [3]Department of Otolaryngology, Head and Neck Surgery, Niigata University Medical and Dental Hospital, Niigata, Japan; [4]Department of Dermatology, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan; [5]Department of Child Neurology, Nishi-Niigata Chuo National Hospital, Niigata, Japan

SOX10 plays an essential role for the development of cells in the neural crest lineage, including melanocytes and enteric ganglia neurons. Mutations in the *SOX10* have been found in patients with Waardenburg syndrome (WS), which is characterized by sensorineural hearing loss and abnormal pigmentation of the eye, hair, and skin. Non-truncating mutations in the *SOX10*, mainly detected in the central high mobility group (HMG) DNA-binding domain are associated with various phenotypes, ranging from WS2 (WS2E) or WS4 (WS4C; with Hirschsprung Disease: HD) to severe complex syndromes involving peripheral and central nervous system manifestation. Although the phenotypic heterogeneity remains unknown, previous studies have proposed some genetic modifiers, such as rs2435357 polymorphism in *RET* which have been investigated as a strong candidate contributing to the development of HD, might affect their phenotype. We previously reported a case with WS2 (without HD) harboring heterozygous p.N131D mutation in the *SOX10* who have no risk allele in rs2435357. Here, we additionally report a case of an 8-month-old Japanese girl presenting bilateral heterochromia irides and sensorineural hearing impairment, clinically diagnosed with WS2. Mutational screening for *MITF* and *SOX10* showed a novel heterozygous insertion mutation, c.381_386dupGCACCT, p.L129_H130insHL in the HMG domain of the *SOX10*. Genotyping for rs2435357 revealed that she also did not have the risk allele for the HD. Our sequential reports accentuated the importance of the rs2435357 as a genetic modifier for the patients with non-truncating mutation in the HMG domain of the *SOX10*.

P.083 | Genetic analysis of nevi in cardiofaciocutaneous syndrome

Tokimasa Hida; Sayuri Sato; Masae Okura; Hisashi Uhara

Department of Dermatology, Sapporo Medical University School of Medicine, Sapporo, Japan

Cardiofaciocutaneous syndrome (CFCS) is a genodermatosis characterized by cardiac, craniofacial, cutaneous and neurological abnormalities. Skin manifestations include multiple melanocytic nevi, palmoplantar keratoderma, keratosis pilaris and hair abnormalities. CFCS is caused by germ-line variations in genes related to a RAS/mitogen-activated protein kinase (MAPK) pathway. The genes known to be associated with CFCS are *BRAF*, *MAP2K1*, *MAP2K2* and *KRAS*. Variations in *BRAF* gene are non-V600E. Some of the gene products have an increased kinase activity comparable to V600E while others have reduced activities. It is unknown how the dysfunction in the RAS/MAPK pathway leads to the development of multiple nevi and whether the nevi in CFCS have potential risk of developing melanoma. In this study, we present a case of CFCS and the result of whole exome sequence analyses of a nevus tissue. A CFCS patient with a germ-line *BRAF* L485F variation had over 120 melanocytic nevi (Sato S et al. Eur J Dermatol 2017, in press). Fourteen nevi ranged from 6 to 9 mm in size and others were less than 6 mm. Dermoscopy of the nevi showed typical features of common melanocytic nevi. One compound nevus near the nose was excised. For a whole exome sequencing, genomic DNA was extracted from the patient's blood and the nevus tissue collected by laser microdissection. Sequencing libraries were generated using Agilent SureSelect Human All Exon kit. The clustering of the index-coded samples was performed, followed by sequencing on an Illumina sequencing platform. From the analysis of the nevus exome, SNPs, indels and copy number alterations were detected. In this presentation, we will discuss the nevus-causing somatic mutations in CFCS.

P.084 | Dyschromatosis symmetrica hereditaria and aicardi-goutières syndrome 6 caused by ADAR1 mutations; effects of skin color on phenotypes

Michihiro Kono; Mutsumi Suganuma; Yasushi Tomita; Masashi Akiyama

Nagoya University Graduate School of Medicine Department of Dermatology, Nagoya, Japan

We clarified that dyschromatosis symmetrica hereditaria (DSH), a hereditary pigmentation disorder, is caused by a heterozygous mutation of adenosine deaminase acting on RNA 1 gene (*ADAR1*) in 2003. In 2012, it was reported that *ADAR1* mutations cause Aicardi-Goutières syndrome 6 (AGS6), a genetic inflammatory disorder that affects the nervous system. Ten reported AGS patients had bi-allelic mutations or the identical heterozygous mutation p.Gly1007Arg in *ADAR1*. However, all these reported patients showed no dyschromatosis. We report the first case of DSH with neurological symptoms and brain calcification resulted from compound heterozygous *ADAR1* mutations, c.3444-1G>A and c.1600C>T (both novel), and present comprehensive functional studies of *ADAR1* mutations. The average A-I editing efficiency of three transfectants of half-wild/half DSH-causative mutants was approximately half (48.1 ± 13.4%) of that of only wild-type plasmid transfectants. The average A-I editing efficiency of transfectants of the 3 simple AGS-causative mutants was extremely low,

20.9 ± 2.7% of that of only wild-type plasmid transfectants, possibly leading to the AGS phenotype.

Transfectants of half-wild/half DSH with the neurological symptoms-causative exceptional mutant p.Gly1007Arg showed an A-I editing efficiency of 30.2% of that of only wild-type plasmid transfectants, between the above-mentioned values of transfectants with DSH-causative mutants and AGS-causative mutants. These results seemed to be consistent with the phenotypic severity of DSH and AGS.

We speculate that variability in the severity of skin manifestations is associated with background skin color. We suggest heterozygous *ADAR1* mutations cause DSH in East Asian patients and no apparent disease in non-East Asian individuals. In contrast, homozygous or compound heterozygous *ADAR1* mutations cause the combination of AGS and DSH in East Asian patients, but cause only AGS in non-East Asian patients.

P.085 | Progressive guttate and confluent leukoderma of the limbs: Report of 3 cases

Indira P. Kahawita[2]; Prasad Kumarasinghe[1]; Punya H. Abeygunawardena[3]; Nayana R. Ratnayake[4]

[1]*Fiona Stanley Hospital and University of Western Australia, Nedlands, WA, Australia;* [2]*Department of Dermatology, Base Hospital, Homagama, Sri Lanka;* [3]*Department of Dermatology, Base Hospital, Karawanella, Sri Lanka;* [4]*Department of Pathology, Base Hospital, Karawanella, Sri Lanka*

Progressive guttate and confluent leukoderma of the limbs(PGCLL) is a disorder of pigmentation seen in the dark skinned persons, first described by Kumarasinghe et al. in Singapore in 2007. We report 3 Sri Lankans with this entity.

Two males; 25 yrs and 28 yrs and a 19 year female presented with progressive, discrete and confluent hypopigmented macules on the shins with over 12 months' duration. Koebner phenomenon was seen in 1 case. None had involvement of other areas of the body, any history of repeated trauma to the sites, long term drug intake, a family history of vitiligo or other pigmentary disorders. Two had been treated with topical steroids and tacrolimus with no improvement.

There was no absence of epidermal melanocytes in all 3 skin biopsies. Case 2 showed reduced epidermal pigmentation compared to normal skin. In the dermis there was mild perivascular lymphocytic infiltration in case 1 and 3 while case 2 showed band like infiltration of lymphocytes. In case 3 there was mild fibrosis in the upper papillary dermis. None of the patients showed any response to local application of moderately potent corticosteroids.

The differential diagnoses of 'Progressive guttate and confluent leukoderma of the limbs(PGCLL)' and the characteristics of this condition will be discussed. It is important to diagnose this condition as a wrong diagnosis of vitiligo will cause anxiety and distress in the patients of skin of color. The progression of this condition is very slow. No treatment is known yet. Wider recognition of this condition will encourage more research into this condition.

P.086 | Cutaneous hyperpigmentation induced by daclatasvir, sofosbuvir, and ribavirin combination therapy

Chanat Kumtornrut; Narisa Brownell

Chulalongkorn University, Bangkok, Thailand

A 61-year-old Thai patient, known case of Hepatitis C (HCV)-related cirrhosis, underwent liver transplantation, presented with 3-month history of generalized skin darkening. He had been treated with daclatasvir (DCV), sofosbuvir (SFV) and ribavirin (RBV) combination therapy for three months before he developed the skin change. Physical examination showed photodistributed skin hyperpigmentation accompanying with nail lunular and oral mucosal hyperpigmentation. Two months after the discontinuation of these drugs, his cutaneous pigmentation started to fade. Other investigations showed no evidences of systemic diseases contributed to cutaneous hyperpigmentation.

DCV/SFV/RBV combination therapy has been used as a new treatment for hepatitis C virus. The reported adverse skin reactions from DCV/SFV/RBV are still limited, yet including erythema multiforme and Stevens-Johnson syndrome. At present, there is no reported cases of skin hyperpigmentation after using these medications.

We describe the first case of drug-induced cutaneous hyperpigmentation by DCV/SFV/RBV combination therapy. More cases with these treatment should be observed and the mechanism of hyperpigmentation should be studied to support our hypothesis.

P.087 | Melasma Extent Score (MES): Development, validation and reliability testing of a new outcome measure in melasma

Janny E. Lommerts; Philippe Smeets; Albert Wolkerstorfer; Marcel Bekkenk

Netherlands Institute for Pigment Disorders, Academic Medical Centre, Amsterdam, Netherlands

Background: Melasma is a common pigment disorder for which no core set of outcome domains is available. Commonly used measurement tools that are available, such as the Melasma Area and Severity Index (mMASI), mostly measure the severity of melasma by calculating the sum score of outcome domains. However, measurement tools that separately measure the outcome domains are lacking. We developed, validated and tested the reliability of a new tool which separately measures the extent of melasma; the Melasma Extent Score (MES).
Material and Methods: In the development phase, we based the different degrees of extent of the MES on clinical photographs of 110 patients with melasma. We validated and tested the reliability of the MES on 52 patients with melasma. Validation was performed by assessing the correlation of the MES with the gold standard (mMASI) and objective extent measurements. Reliability of the MES was assessed by measuring the intra-class correlation (ICC) of 3 different assessors (inter-observer reliability) and of the same assessor on 2 different time

points (intra-observer reliability). The user-friendliness and rapidity of both the MES and mMASI were measured on a VAS-scale (0-10).

Results: The correlations of the MES with the area measurement of the mMASI (r = 0.94) and objective surface measurements (r = 0.91) were very strong. Furthermore, both the inter-observer reliability (ICC=0.93, 95% CI 0.89-0.96) and intra-observer reliability (ICC=0.96, 95%CI: 0.93-0.98) of the MES were excellent. The user-friendliness and subjective assessment of the rapidity of the MES and mMASI were similar.

Conclusion: The Melasma Extent Score is a valid and reliable tool to measure the extent of melasma. Further research is needed on the responsiveness of the MES.

P.088 | Inter- and intra-observer agreement in dermatologists visual diagnoses of cutaneous hyperpigmented facial lesions and the application for objective assessment

Ankur Purwar[1]; Tomohiro Hakozaki[2]; Adams Wai Kin Kong[3]; Peicong Yu[3]; Huang Yi[3]; Hong Liang Tey[4]

[1]The Procter & Gamble Company, Singapore, Singapore; [2]The Procter & Gamble Company, Mason, OH, USA; [3]Nanyang Technological University, Singapore, Singapore; [4]National Skin Centre, Singapore, Singapore

In conventional research setting, diagnosis of hyperpigmented spot lesions relies on dermatologist's visual assessment, often with the aids of objective tools such as magnifier or dermatoscope. Thanks to the advances in clinical imaging systems and analysis tools, many methods are published on quantitative analysis of hyperpigmented lesions. However, none of these methods are discriminative on the types of pigmented lesions. For more effective personalized treatment, classification of hyperpigmented lesions is important for treatment strategy development and their efficacy evaluation because different type of lesions have different characteristics thus different solutions.

We explored if high resolution facial images, as an unaided eye, can be used in the differential diagnosis of facial hyperpigmented lesions, including solar lentigo, melasma, seborrheic keratosis, melanocytic nevi.

Here, we conducted a study to understand the inter- and intra-observer agreement in dermatologist diagnosis of these lesions using left/right sided high resolution cross-polarized facial images. In the 3 dermatologists examination, three-sided agreement was high at 97.15% however inter- and intra-observer agreement were moderate, with mean Kappa value of 0.436 ($p < .001$) and 0.507 ($p < .001$) respectively. Compared with the observed agreement (97.15%), the kappa value modulates the observed agreement by subtracting the expected agreement, which amounts to the degree of agreement due to chance, and is a more robust measure on the agreement. In this presentation, we will share our findings on the distribution and repeatability of dermatologist's diagnosis for different cutaneous hyperpigmented types. This consensus diagnosis might not be practical in most clinical settings however these findings can be relevant for tele-diagnostics applications.

P.089 | Rapid improvement of Riehl's melanosis by 1550 nm Erbium-glass fractional laser treatment

Jung-Won Shin; Ji-Young Choi; Jung-Im Na

Department of Dermatology, Seoul National University Bundang Hospital, Seongnam-si, Korea

Riehl's melanosis is a pigmentary disorder presented as a reticulated gray-brown to black hyperpigmentation on the face and neck. Histopathologically, it is characterized by pigmentary incontinence with infiltration of numerous dermal melanophages and lymphohistiocytes. Treatment of Riehl's melanosis is challenging. Although light based therapies including IPL and low fluence Q-switch ND:YAG laser have been reported to result in produce improvements, nearly 10 laser sessions and 6 months of treatment period were required. In this case-series study, we document five cases of Riehl's melanosis which showed rapid improvement in three sessions of 1550 nm erbium-glass fractional laser.

Five female patients who were clinically and histopathologically diagnosed as Riehl's melanosis were included in this study. Medical records including present illness, results of patch test, treatment history and treatment outcome were thoroughly reviewed. Patients' ages ranged from 49 to 60 years (mean age 55). Four patients developed skin lesions after using henna-containing hair dyes and demonstrated positive allergic reaction to hair dyes in the patch test. All the patients were instructed to avoid further exposure to causative agents. After using minocycline, topical tacrolimus and oral tranexamic acid to subside erythema for one month, the patients underwent erbium-glass fractional laser. The laser procedures were performed 1-5 sessions (average 2.6) at a month interval. The average modified Melasma Area and Severity Index (MASI) score decreased from 10.62 ± 5.98 to 4.32 ± 3.61 after the laser treatments. In physician's assessment, three patients got almost cleared (>75%), two patients showed marked improvement (51-75%), and one patient showed moderate improvement (25-50%). Notably, we could observe a clear clinical improvement even after a single session in most cases. In conclusion, 1550 nm erbium-glass fractional laser is a promising treatment modality for Riehl's melanosis that leads to rapid improvement via reducing dermal melanophages.

P.090 | A novel variant in the regulatory region of the SLC45A2 gene is associated with oculocutaneous albinism Type4 (OCA4) in Japanese

Tamio Suzuki; Ken Okamura; Masahiro Hayashi; Yutaka Hozumi

Department of Dermatology Faculty of Medicine, Yamagata University, Yamagata, Japan

Background: We reported that OCA4 (OMIM 606574) is one of the most common non-syndromic OCAs among Japanese patients, the

causative gene of which is *SLC45A2* (Inagaki K, et al. Am J Hum Genet 74: 466–471, 2004).

Objective: During our gene analyses for OCAs, we found that 12 patients had only one heterozygous pathological mutation in *SLC45A2* [we call them "suspected OCA4 (sOCA4) patients"]. Interestingly, most of the sOCA4 patients revealed a mild phenotype for eye manifestation, which means no nystagmus or mild visual impairment. In order to identify another responsible variant, we investigated the regulatory region in the *SLC45A2*.

Methods: We sequenced about ~1.2 kb upstream of the coding region of *SLC45A2* in which H3K4Me1, H3K27Ac, and H3K4Me3 marks revealed high peaks in the two representative sOCA4 patients. The newly identified variant was tested for the rest 10 sOCA4 patients and 110 normally pigmented Japanese. Furthermore, we compared the transcriptional activity of the wild allele and the variant allele using luciferase reporter assay.

Results: A novel 4 bp deletion variant at upstream of the *SLC45A2* (c.-492_489delAATG; GenBank Accession number: NM_016180) was found in eight of the twelve sOCA4 patients (66.7%). They had the heterozygous deletion variant. Four of the 110 normal controls also had the variant heterozygously (3.6%). Luciferase reporter assay showed that the variant allele had some transcriptional activity, however, the activity was significantly decreased compared with that of the wild allele.

Conclusion: The c.-492_489delAATG variant in the *SLC45A2* would be a responsible variant in combination with another pathological mutation having null activity for the mild OCA4 in Japanese.

P.091 | Producing new mouse gene-edited CRISPR lines for animal modelling a variety of types of Albinism

Almudena Fernandez[1,2]; Santiago Josa[1,2]; Andrea Montero[1]; Celia De Lara[1]; Yaiza Lopez[1]; Marta Cantero[1]; Irene Robles[1]; Davide Seruggia[1]; Lluis Montoliu[1,2]

[1]*CNB-CSIC, Madrid, Spain;* [2]*CIBERER-ISCIII, Madrid, Spain*

Albinism is a human rare genetic condition associated with impaired vision and, often, with hypopigmentation in the eyes and skin (oculocutaneous albinism, OCA) or only affecting the eyes (ocular albinism, OA). Currently we know up to 7 OCA types and 1 OA type. Approximately, 1:17,000 newborns can be potentially affected by any type of albinism. There are non-syndromic and syndromic types of albinism (e.g. Hermansky-Pudlak; Chediak-Higashi, 11 types altogether), the latter representing more severe forms of albinism, where other organs and cellular types, besides melanocytes and retinal pigment epithelium cells, can also be affected. To date we know of at least 20 loci whose mutations can be associated with the corresponding type of albinism (Montoliu et al. PCMR 2014; Montoliu & Kelsh PCMR 2014; Montoliu & Marks PCMR 2017). Traditionally, investigations on animal models for albinism have progressed using spontaneous mutants at any of these genes (e.g. extreme-dilution mottled, Lavado et al. JBC 2005; model for OCA1B) or through the generation of transgenic (e.g. Gimenez et al. J Comp Neurol 2005; model for OCA1B) or classical KO mouse lines (e.g. Incerti et al. Hum Mol Genet. 2000; model for OA1). Gene editing technologies, namely the novel CRISPR-Cas tools (Seruggia & Montoliu, Transgenic Res 2014; Mojica & Montoliu Trends in Microb. 2016), have permitted the functional analysis of the regulatory elements associated with these loci (Seruggia et al. Nucleic Acids Res. 2015) and the generation of more representative animal models, useful for the specific study of the phenotypic alterations caused by precise genetic mutations diagnosed among human albino patients, the so-called avatar mice. In this work we will share the current status of our efforts towards the generation and analyses of a multiple new mouse gene-edited CRISPR lines for animal modelling a large variety of types and sub-types of albinism.

P.092 | Rescue of the Albino phenotype in mice using gonad method

Hiromi Miura[1]; Naomi Arifin[1]; Masahiro Sato[2]; Masato Ohtsuka[1]

[1]*Tokai University, Isehara, Japan;* [2]*Kagoshima University, Kagoshima, Japan*

Albinism is a congenital disorder characterized by the absence of pigment in the skin, hair and eyes. It is often due to mutation of tyrosinase gene, encoding an enzyme that involved in the production of melanin. Therefore, it can be assumed that the pigmentation may rescue if this mutation is repaired.

We recently developed a novel system for generation of genome editing mice, called Genome-editing via Oviductal Nucleic Acids Delivery (GONAD) method. Since this strategy allows electroporation to deliver CRISPR-related components to the zygotes within the intact mouse oviduct in situ. In our previous report, we successfully generated gene-disrupted embryos/pups when delivered CRISPR components. Aim of this study is to test if the method can be used for making point mutation changes in genome. For this purpose, we designed experiments to rescue G308C mutation in the *tyrosinase* (*Tyr*) locus of albino mice that have a simple, causing a cysteine to serine mutation at amino acid 103. This mutation is responsible for defective tyrosinase activity, and the phenotype of this genetic change can be assessed by eye pigmentation or coat color. We designed a gRNA for a region spanning the point mutation and constructed ssODN that corresponds to the wild-type sequence of *Tyr*. The GONAD was performed in five pregnant females. A total of 32 offspring from these females were harvested at different stages of gestation, or at post-natal stage. Fifteen (47%) of these samples exhibited the expected phenotype of eye pigmentation and/or coat color. Moreover, sequence analysis demonstrated that at least one allele had the corrected sequence at the target site in all of the offspring that had been identified as pigmented ones. Taken together, these results suggest that GONAD can be used for high efficiency gene correction of tyrosinase mutation, and applied for gene therapy of albinism.

P.093 | Fibroblast-derived clusterin inhibits melanogenesis

Misun Kim[1]; Jiun Lee[1]; Tae Jun Park[2]; Hee Young Kang[1]

[1]Department of Dermatology, Ajou University School of Medicine, Suwon, Korea; [2]Department of Biochemistry and Molecular Biology, Ajou University School of Medicine, Suwon, Korea

Clusterin (CLU), also known as apolipoprotein J, appears to be extremely sensitive biosensor of oxidative injury in human tissues. CLU expression is highly induced during many types of oxidative stress including UV exposure. We analyzed the role of CLU in the regulation of skin pigmentation in line with the epidermal/dermal cross-talk between fibroblasts and melanocytes. CLU expressions were investigated with cultured skin cells, UV irradiated fibroblasts, and acutely UV irradiated in vivo skin. CLU mRNA and protein were highly expressed in fibroblasts, scarcely expressed in keratinocytes, and not in melanocytes. CLU expression was increased in the UV irradiated fibroblasts and the acutely UV irradiated in vivo skin. To investigate the effect of fibroblast-derived clusterin on melanogenesis, fibroblast was infected with CLU lenti-virus or shRNA. Normal human melanocytes were treated with conditioned medium derived from the fibroblasts and the melanogenesis was analyzed. The pigmentation was also assessed using the *ex vivo* skin and the artificial skin. The melanin contents and tyrosinase activity were significantly reduced in the melanocytes treated with conditioned medium from CLU overexpressed fibroblasts. The mRNA and protein expression levels of melanogenesis-associated proteins, microphthalmia-associated transcription factor (MITF) and tyrosinase were significantly downregulated expressions *via* TGFβ/Smad signaling activation. These results suggest that UV irradiation induces CLU in the skin and the fibroblasts derived-CLU inhibits melanogenesis *via* the cross-talk between fibroblasts and melanocytes.

P.094 | UV irradiated human dermal endothelial cells induce pigmentation: The role of vasculature in the development of UV-induced hyperpigmentary disorders

Misun Kim[1]; Tae Jun Park[2]; Takako Shibata[3]; Kiyoshi Sato[3]; Hee Young Kang[1]

[1]Department of Dermatology, Ajou University School of Medicine, Suwon, Korea; [2]Department of Biochemistry and Molecular Biology, Ajou University School of Medicine, Suwon, Korea; [3]Shiseido Global Innovation Center, Yokohama, Japan

We previously demonstrated that a hyperpigmented melasma lesion is characterized by increased vasculature, which may play a role in the development of hyperpigmentation. We report here in vitro evidence of the vascular modulation of skin pigmentation. In the presence of endothelial cells, the pigmentation of melanocytes and of *ex vivo* cultured human skin was reduced. Contrary to non-stimulated cells, UV-irradiated endothelial cells showed stimulatory action on skin pigmentation. The mRNA and protein expression levels of melanogenesis-associated proteins, microphthalmia-associated transcription factor (MITF) and tyrosinase were increased in the conditioned medium from UV irradiated endothelial cells compared to non–irradiated endothelial cells. Consistently, *ex vivo* skin pigmentation was significantly induced. Gene expression profiling was undertaken to identify melanogenic factors from UV-irradiated endothelial cells. Differential expressions of melanogenic and anti-melanogenic factors were shown in between the UV irradiated endothelial cells and non-irradiated cells by RNA sequencing analysis. Our study suggests that endothelial cells have a negative effect on pigmentation. However, their effect differs after they are irradiated with UV, as they then positively influence the induction of melanogenesis. This may play a role in the development of hyperpigmentary disorders.

P.095 | Mature adipocytes activate the phagocytotic activity of dermal fibroblasts

Yoshiaki Ohagi[1]; Satoshi Yoshimoto[1]; Moemi Yoshida[1]; Hiroki Yanagi[1]; Masayuki Yagi[2]; Masamitsu Ichihashi[3]; Hideya Ando[1]

[1]Department of Applied Chemistry and Biotechnology, Okayama University of Science, Okayama, Japan; [2]Rosette Co, Tokyo, Japan; [3]SAISEI MIRAI Clinic, Kobe, Japan

It is well known that melanin synthesis in epidermal melanocytes is regulated by paracrine factors derived from epidermal keratinocytes and from dermal fibroblasts. In addition, it has also been reported that adipocytes in subcutaneous adipose tissue are involved in the protection against fibroblast-related photoaging of the skin. Further, we have found that the exposure of phosphatidylserine on the outer membrane of melanosome-rich globules released from melanocyte dendrites is necessary for the internalization of those globules into fibroblasts. In this study, we evaluated the effects of paracrine factors derived from adipocytes on the phagocytotic activity of fibroblasts. We used COOH-beads as a substitute for melanosome-rich globules, since phosphatidylserine exposed on the surface of melanosome-rich globules contains a COOH group and we recently found that the COOH group is one of the triggers for the phosphatidylserine-mediated internalization of melanosome-rich globules by fibroblasts. When adipocyte-conditioned medium was added to the culture medium of fibroblasts, the internalization of COOH-beads by fibroblasts was significantly increased, compared to the addition of the original adipocyte medium as a control. Interestingly, the uptake of COOH-beads by fibroblasts was more prominent when the conditioned medium of differentiated mature adipocytes containing lipid droplets was added. These results suggest that the phagocytotic activity of dermal fibroblasts can be affected by adipocyte-secreted factors that might be involved in the underlying mechanisms of dermal pigmentation.

P.096 | Skin transcriptomic studies including ethnicity, UV radiations and pollution show specific epigenetic miRNA signatures involved in skin pigmentation

Morgan Dos Santos[1]; Dandan Jiang[1]; Valere Busuttil[2]; Hui Li[1]; Shanshan Jiang[1]; Catherine Serre[2]; Jean-Marie Botto[2]; Nouha Domloge[2]; Zhengzheng Lu[1]

[1]JALA GROUP, Shanghai, China; [2]Ashland, Sophia Antipolis, France

Among many skin features, pigmentation status is modulated between ethnies around the World. Common extrinsic skin stresses such as UV radiations require responses to protect the skin against DNA damage resulting in melanogenesis. Pollution, another environmental stress, can synergize with UV in skin pigmentation. Skin melanin pigments (eumelanin and pheomelanin) have been described to differ from Asian to Caucasian. To evaluate skin pigmentation, we considered samples from 4 different groups in the same age range (30–40 yo): Chinese individuals (UV-exposed skin or UV-protected skin, Shanghai, China), Chinese individuals (UV-exposed skin residing in polluted area, Jinan, China), Caucasian individuals (UV-protected skin, Lyon, France). Up to 8 donors were considered in each cohort and after total RNA extraction, miRNA microarray (Agilent) was used to study differential expression of microRNAs between cohorts.

Using databases and bioinformatics tools we were able to identify several miRNAs specifically modulated between the different cohorts (ethnicity, UV and pollution) involved in early and late phases of skin pigmentation. By looking at validated target genes for each of these miRNAs, we were able to identify genes involved in melanogenesis such as melanogenic enzyme TYRP1 and RAB family members influencing melanosomal transfer. Interestingly, we noticed that some of these target genes have also been described to play roles in autophagy and they may help in melanin recycling process.

To conclude, we identified specific miRNAs signatures, some who could potentially explain ethnic differences (Chinese vs. Caucasian) and some involved in UV and/or pollution responses in Chinese skin. Differential expression validation of selected microRNAs and target mRNAs is in progress. Finally, the comprehension of mechanisms and physiological pathways leading to epigenetic modifications associated with pigmentation in Chinese populations may help the development of new specific targeted skin lightening molecules.

P.097 | Genetic variants in IRF4, TYR, ASP and BNC2 contribute to ephelides occurrence in the absence of loss-of-function MC1R Variants

Barbara Hernando; Julio A. Deserio-Cuesta; Raquel Soria-Navarro; Inca Vilar-Sastre; Conrado Martinez-Cadenas

Jaume I University, Castellon de la Plana, Spain

Ephelides are small pale-brown spots commonly observed in fair-skinned and/or red-haired individuals. The presence of ephelides is largely genetically determined, with *MC1R* being the main freckle gene in European populations. However, freckled individuals without *MC1R* mutations have been also described, suggesting that other genetic variants might contribute to the appearance of ephelides.

We performed a genetic association study for presence of ephelides in 458 individuals of Spanish origin. Six SNPs located in 5 pigmentation-related genes (*IRF4*, *TYR*, *ASP*, *BNC2* and *SLC45A2*) were genotyped. Also, the entire *MC1R* coding region was sequenced, classifying individuals as RHC (red hair colour) or non-RHC carriers. A standardized questionnaire was used to collect pigmentation and sunlight sensitivity traits, solar exposure habits and sex.

Female sex and poor sun tolerance (skin phototypes I/II) were risk factors for having a freckling phenotype. Association between genetic variants and presence of ephelides were determined by multivariate regression analysis including in the model all genetic variants, sex and skin phototype. Carrying at least one *MC1R* RHC variant, *IRF4* allele rs12203592*T and *ASP* allele rs4911442*G showed an association with freckling phenotype at Bonferroni-corrected threshold ($p < 5.50 \times 10^{-3}$). In addition, we observed that genetic variants in *IRF4*, *ASP*, *TYR* and *BNC2* contributed to ephelides formation in the absence of *MC1R* RHC variants, while their effect (except for *IRF4*) was less significant in RHC carriers.

In conclusion, our results show an association between ephelides and genetic variants in *IRF4*, *MC1R*, *ASP*, *BNC2* and *TYR* in a Mediterranean population, confirming previous studies performed in North European populations. Other than *IRF4*, the genetic effect of these variants was moderate in individuals carrying *MC1R* RHC variants, while it was notably strong in those not harbouring a RHC variant.

P.098 | Mechanisms of the structural colors in bird feathers and involvement of pigment

Toyoko Akiyama

Department Biology, Keio University, Yokohama, Japan

The feather colors of birds are depend upon three systems; 1) melanin and pheomelanin produced by melanocytes, 2) carotenoids and pteridines from foods, 3) structural colors by nanoscale-structure of feathers. These systems are involved independently or cooperatively in expression of the feather colors. But the mechanism of structural color and the role of pigments in it are not completely determined. The main mechanisms of the structural color in birds are known as thin layer interference, multiple layer interference, amorphous network structure, and photonic crystals, etc. Shining purple and green colors in neck of domestic pigeon are revealed by thin layer interference of inside fine structure in the feather barbules. Melanin is contained in particles inside the barbules. Non-iridescent blue color in the back of kingfisher is derived by amorphous colloidal particles (amorphous network structure) in the barbs. A part of the feathers of a poll parrot, macaw, exhibit blue and red in upper side and back side, respectively. By the observation with SEM, blue of the upper side of feather barbs is thought to be derived by fine amorphous network structure without melanin. On the other hands, red color in the underside of

barbs is seemed to be resulted by the accumulation of dietary pteridine. Iridescent multiple colors in peacock are depend upon nanoscale multiple columnar structures (photonic crystals) inside the feather barbules and melanin is contained in the structure. But the feathers of white peacock with defect of pigmentation reveal almost no structural colors. Generally, structural colors have been thought to reveal without specific pigments and also to be enforced by the existence of pigments. In this report, we suggest that melanin is indispensable to show the structural colors in the case of peacock.

P.099 | Anti-melanogenic effect of phenolic compound extracted from Juglans Mandshurica fruit

Ji Young Kim; Yeon Jung Lee; Sein Lee; Sang Ho Oh

Department of Dermatology, Yonsei University College of Meidicine, Seoul, Korea

Juglans mandshurica Maxim. (Juglandaceae), known as the Manchurian walnut, is a traditional folk medicine used for treatment for dermatosis, cancer, gastritis, as well as diarrhea in Korea. Water extracts of *J. mandshurica* has been proved to have an anti-obesity effect by examining the inhibitory activity against pancreatic lipase. Recently, several compounds of neolignan and phenolic compounds were isolated from extract of *J. mandshurica* fruits and some phenolic compounds exhibited the potent lipolytic activities in in vitro experiment. A variety of chemical agents that decrease pigmentation have been identified and most of these agents are phenolic compounds affecting tyrosinase. Therefore, in this study, we investigated the effect of phenolic compounds extracted from *J. mandshurica* fruits on melanogenesis for its possibility of development of hypopigmenting agents.

Three kinds of compounds from *J. mandshurica* fruits were examined for anti-melanogenic effect. Of three, 2-[4-(3-Hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (HPMPP) has reproducibly the most powerful inhibitory effect on melanogenesis in B16 melanoma cells and primary human epidermal melanocytes. It significantly decreased the protein expression of tyrosinase and MITF and also reduced tyrosinase enzyme activity. I addition, melanin content as the end product of melanogenesis was also significantly decreased after HPMPP treatment. These results indicate that HPMPP has anti-melanogenic effect and thus it may be useful as a therapeutic agent for treating hyperpigmentary disorders and as a component of whitening cosmetics.

P.100 | A new approach for treating melasma: Hydroporation with anti-aging cocktail enhancing microenvironment of skin

Jung-Won Shin; Je-Byeong Chae; Kyoung-Chan Park

Department of Dermatology, Seoul National University Bundang Hospital, Seongnam-si, Korea

Melasma is a common hypermelanotic skin condition usually presenting with brown-colored macules and patches on sun-exposed areas. Although the main histopathologic finding of melasma is epidermal hyperpigmentation, there are also characteristic dermal changes in lesional melasma skin: solar elastosis, basement membrane disruption, and increased vascularity. From these histological findings, melasma can be regarded as one of the phenotypes of photodamaged skin, rather than just an epidermal pigmentation disorders. Therefore, therapeutic approaches for melasma have to focus on improving the damaged and photo-aged skin condition, not just removing the epidermal pigmentation.

We evaluated the effect of hydroporation with GHR formulation composed of copper-GHK, oligo-hyaluronic acid, Rhodiola extract, tranexamic acid, and b-glucan on melasma. Results showed that MI and EI significantly decreased after procedure. Skin biopsy showed that collagen fibers increased in upper dermis after treatment. Immunohistochemical staining revealed that increased collagen type IV and procollagen in the basement membrane area and upper dermis, respectively. Furthermore, the number of p63 positive cells increased along the basement membrane. To confirm the effect of hydroporation with GHR formulation on epidermal stem cells, we analyzed co-expression pattern of p63 and histone deacetylase (HDAC) 1, a potential new specific marker for stem cells in interfollicular epidermis. Results showed that p63-positive and HDAC1-negative cells significantly increased after procedure.

In conclusion, hydroporation with GHR formulation may have depigmenting and erythema decreasing effects by enhancing the microenvironment of skin and epidermal stemness.

P.101 | The ultrastructural integrity of melanosome is required for its photo-protection against DNA damages in human keratinocytes exposed to UVB or UVA irradiation

Tiechi Lei

Department of Dermatology, Renmin Hospital of Wuhan University, Wuhan, China

Background: The main function of melanocytes (MCs) is to produce melanins and distribute them to neighboring keratinocytes (KCs) via carrier organelles termed melanosomes. As keratinocytes undergo terminal differentiation and migrate upward from the basal layer to the stratum corneum, melanosomes are properly degraded by hitherto unappreciated mechanism(s). In this study, we analyzed whether the degradation of melanosome affects its photo-protection capacities.

Methods: 1) Primary human KCs and MCs were cultured from human foreskin tissue and HaCaT cells were subcultured for comet assay; 2) Human melanosomes were isolated from human eyes post-mortem, the rupture in the outer membrane of melanosomes was induced by using the physical approach of multiple freeze-thaw cycles and manually grinding using a glass Dounce homogenizer; 3) KCs were incubated with intact or broken melanosome samples, respectively, and treated with UVA or UVB irradiation; 4) the ultrastructural changes of

melanosomes were observed by electron microscopy; 5) DNA damages of KCs were immunostained using anti-CPDs and anti-8oxo-dG antibodies as well as evaluated using comet assay.

Results: 1) Comet assay results showed that there was a statistically significant increase in tail length of UVB-exposed KCs treated with broken melanosomes as compared with intact melanosomes, no difference was found in UVA-exposed KCs treated with either broken or intact melanosomes. 2) UVB-induced DNA damages (anti-CPDs staining) were dramatically suppressed in UVB-exposed KCs treated with intact melanosomes as compared with broken melanosomes, no difference of UVA-induced DNA damages (anti-8oxo-dG staining) was visualized between both groups.

Conclusion: The ultrastructural integrity of melanosome is required for its photo-protection against DNA damages, particularly resulting from UVB irradiation.

Acknowledgements: This work was supported by grants from the National Natural Science Foundation of China (NSFC Grants 81371717 and 81573028).

P.102 | Epidermal melanocytes of segmental and non-segmental vitiligo subtypes show altered expression of E-cadherin but not P-cadherin

Veronique Delmas[1]; Christine Grill[1]; Laila Benzekri[2]; Alain Rubod[1]; Khaled Ezzedine[3]; Alain Taieb[3]; Yvon Gauthier[3]; Lionel Larue[1]

[1]CNRS UMR3347, INSERM U1021 Instiut Curie, Orsay, France; [2]Mohammed V University in Rabat, Rabat, Morocco; [3]Department of Dermatology and Pediatric Dermatology INSERM U1035, Bordeaux, France

The loss of melanocytes from the skin is the main clinical feature of patients with vitiligo, resulting in depigmentation macules. We have recently shown that E-cadherin, which mediates the adhesion between melanocytes and keratinocytes in the epidermis, is discontinuously/heterogeneously distributed within (or lost from) melanocyte membranes of non-segmental vitiligo (NSV) patients (Wagner et al., 2015). This alteration of E-cadherin distribution on melanocytes is observed in biopsies distant from the depigmentation macules, before clinical lesions appear, indicating that the alteration of E-cadherin distribution is an early event in the pathogenesis of vitiligo. Here we assessed whether alteration of E-cadherin is a common first step in the various subtypes of vitiligo by studying the distribution of E-cadherin on epidermal melanocytes of frozen skin biopsies from Segmental Vitiligo (SV) patients. Our results show that melanocytes of both vitiligo subtypes, NSV and SV, share the same altered distribution pattern for E-cadherin and β-catenin, which is different from that of melanocytes of healthy subjects. This favours the cell-adhesion defect theory, called melanocytorrhagy, not only for the initiation step of NSV, as originally proposed, but also for the SV subtype. Furthermore, we studied the distribution of P-cadherin, the other type I cadherin expressed in the basal layer of the epidermis. We found that there was no statistically significant difference between controls and the two vitiligo subtypes, indicating that P-cadherin distribution is not altered in vitiligo. This observation may indicate that alteration of E-cadherin distribution is an intrinsic defect of this protein and not a general defect affecting cell-cell adhesion molecules.

P.103 | Smyth Line Chicken Model for Autoimmune Vitiligo: Relevance and Importance for Basic and Translational Research

Gisela F. Erf

University of Arkansas, Department of Poultry Science, Fayetteville, AR, USA

Multifactorial, non-communicable disorders such as autoimmune vitiligo involve interplay of genetic, environmental, metabolic, and immune system components. The individual alterations, contributions, and interrelationships of factors underlying autoimmune diseases are difficult to dissect. Animal models that spontaneously develop the autoimmune disease are rare and particularly valuable when addressing activities leading to loss of tolerance and disease expression. In recent years, a variety of murine models with induced autoimmune vitiligo have become available that play an important role in dissecting mechanisms involved in melanocyte loss and in development of treatment-strategies. Before clinical trials, it is however important to also include testing in animals with spontaneously occurring autoimmune vitiligo. The best-established animal model for spontaneously developing autoimmune vitiligo is the Smyth line (SL) of chicken. Over the past 25 years, many parallels between human- and SL-vitiligo have been clearly demonstrated, supporting the importance of this model for basic biomedical research and underlining the unique opportunities for treatment testing. The SL-model consists of three MHC-matched lines of chickens: the vitiligo-susceptible SL with a predictably high incidence of vitiligo, the parental vitiligo-susceptible Brown line (BL) that rarely expresses the disorder, and the Light-brown Leghorn chicken which is vitiligo resistant. In SL-vitiligo, the target tissue is the living portion (a column of dermis enveloped by epidermis) of growing feathers. This skin derivative allows for minimally invasive and repeated access to the target-tissue prior to and throughout vitiligo development. In vitro and in vivo studies demonstrated the central role of cell-mediated immunity in melanocyte loss, identified an environmental trigger, and revealed aberrant melanocyte-/melanogenesis-related activities during induced cellular stress. Like in human patients, SL-vitiligo is also associated with other autoimmune disorders. Hence, this animal model is ideally suited for incorporation as an intermediate model in collaborative biomedical research on mechanisms, treatment, and prevention of vitiligo.

P.104 | Hollow microneedles for cell transplantation in vitiligo

Benedetta Gualeni[1,6]; Sion A. Coulman[1,6]; Dev Shah[2]; Pey F. Eng[3]; Huma Ashraf[4]; Paul Vescovo[5]; Gareth J. Blayney[3]; Laurent-Dominique Piveteau[5]; Owen J. Guy[3]; James C. Birchall[1,6]

[1]Cardiff University, Cardiff, UK; [2]The Hillingdon Hospital, Uxbridge, UK; [3]Swansea University, Swansea, UK; [4]SPTS Technologies, Newport, UK; [5]Debiotech SA, Lausanne, Switzerland; [6]Extraject Technologies Ltd, Cardiff, UK

Autologous cell suspension grafting is now an established technique for the treatment of vitiligo in eligible patients. The technique relies on laser abrasion or dermabrasion to prepare the recipient skin site for topical application of healthy cells as a solution or a spray. Preparation of the treatment area is time-consuming and invasive and therefore carries the risk of scarring, skin discoloration, infections and bleeding. After application of the cell therapy, the treated areas needs to be dressed to enhance cell survival and attachment, and to protect from infection and further trauma.

Microneedles (microscopic needles with lengths of less than 1 mm) have been widely used for the pain-free intradermal delivery of therapeutics and vaccines, and the extraction of biological fluids for real-time monitoring of biomarkers. We have developed and tested a hollow microneedle device capable of minimally-invasive cell delivery for the treatment of vitiligo. This novel method of cell delivery negates the need for skin pre-treatment and post-procedural immobilization, making this technique particularly attractive for the treatment of mobile areas such as finger joints, lips and eyelids. Our studies have investigated the intradermal delivery potential of microneedles comprised of different materials, configurations, dimensions and lumen diameters.

Cell viability and functionality of both keratinocytes and melanocytes were preserved after extrusion through silicon microneedles with a bore size ≥75 μm (cell survival ≥90%). Fluorescently labeled cells were also effectively delivered to the upper dermis in an excised human skin organ culture model, using microneedles with a bore size ≥80 μm.

In conclusion, hollow microneedles provide an innovative minimally-invasive method for delivering functional cells into the skin. This provides a potential new treatment option for vitiligo. We are currently evaluating this concept in a pilot clinical study in vitiligo patients.

P.105 | Measuring treatment response in vitiligo using suction blistering

Jorge A. Hinojosa; Andrea Tovar-Garza; Luis Juarez; Amit G. Pandya

University of Texas Southwestern Medical Center, Dallas, TX, USA

There is a need to establish a biomarker of vitiligo disease activity that can not only help predict and prevent worsening skin depigmentation but can also serve as a surrogate maker of treatment response in clinical trials of novel therapies. CXCL9 has been shown to be important in the pathogenesis of vitiligo and is highly sensitive and specific in predicting disease activity when measured in the blister fluid of active lesions. However, changes in lesional CXCL9 levels in response to therapy has not been thoroughly studied. Thus, we compared levels of CXCL9 and CXCL10 in the blister fluid of active lesional and non-lesional skin of vitiligo patients before and after treatment in order to correlate chemokine levels with treatment response.

Ten untreated patients with active vitiligo were recruited into this prospective study. Epidermal blisters were created in active lesional and non-lesional skin at the initial visit and then again after 4-9 weeks of combination therapy. Serum samples were also obtained at each visit. CXCL9 and CXCL10 protein concentrations within blister fluid and serum were then quantified via ELISA.

CXCL9 and CXCL10 levels were significantly elevated in the blister fluid of lesional skin of all patients when compared to non-lesional skin at baseline. After treatment, levels of CXCL9 moreso than CXCL10 within lesional skin decreased significantly. CXCL9 and CXCL10 levels within non-lesional skin did not change significantly with treatment. CXCL9 but not CXCL10 showed treatment response within serum.

CXCL9 levels within the active lesional skin of patients with vitiligo were elevated compared to non-lesional skin but decreased to levels similar to that of non-lesional skin after treatment. Measuring CXCL9 levels directly in the skin using suction blistering may be an effective method of detecting early treatment response in clinical trials and can help overcome some of the limitations of serum markers.

P.106 | HSP70 enhances the production of interferon-alpha by plasmacytoid dendritic cells: Relevance for vitiligo pathogenesis

Clément Jacquemin[1]; Jerôme Rambert[2]; Guillet Stéphanie[3]; Khaled Ezzedine[4]; Alain Taieb[3]; Katia Boniface[1]; Julien Seneschal[1]

[1]INSERM U1035 BMGIC, ATIP-AVENIR Immuno-Dermatology, University of Bordeaux, Bordeaux, France; [2]INSERM U1035, AQUIDERM, University of Bordeaux, Bordeaux, France; [3]Department of Dermatology, University of Bordeaux, Bordeaux, France; [4]Department of Dermatology, University of Paris-Est, Créteil, Créteil, France

Human plasmacytoid dendritic cells (pDCs) are a subset of DC specialized in the production of type I interferon (IFN-α/β) under cognate activation, and have been involved in a wide range of cutaneous inflammatory and autoimmune disorders, including vitiligo and cutaneous lupus erythematosus (CLE). Heat-shock proteins (HSP) are molecular chaperones essential for maintaining cellular functions and can be released extracellularly upon cellular injury or necrosis, acting as a danger signal as shown in vitiligo. Therefore, we sought to characterize the interplay between HSP70, pDCs and IFNα production in vitiligo. We confirmed a significant increased production of HSP70 in the epidermis of progressive vitiligo. Importantly, pDCs were primarily localized in the epidermis, in close proximity to keratinocytes expressing HSP70. Additional in vitro experiments revealed that pDCs were

able to aggregate HSP70. pDCs cultured with exogenous HSP70 underwent maturation and activation by expression of the costimulatory molecule CD80. Moreover HSP70 increased the uptake of exogenous DNA by pDCs. Lastly, HSP70 potentiated the production of IFNα by activated pDCs, which in turn induced the secretion of chemokines ligands CXCL9 and CXL10 by keratinocytes important for the recruitment of CXCR3 + T cells in vitiligo skin. All together these data demonstrate that overexpression of HSP70 in progressive vitiligo could promote activation of pDCs and potentiate the production of IFNα by activated pDCs. Targeting HSP70 could be an interesting approach to improve vitiligo disease.

P.107 | RNASET2 promotes NB-UVB induced melanocytes apoptosis through activating P53-P21 signaling in vivo and in vitro

Yuli Kang; Leihong Xiang

Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China

Background: Vitiligo is a common acknowledged pigmentary disorder characterized by depigmented lesions because of the destruction of functioning melanocytes. Our previous study demonstrated that the expression of RNASET2 was highly upregulated in vitiligo. NB-UVB irradiation is a useful way for the therapy of vitiligo. However, the impact of RNASET2 on therapeutic effect of NB-UVB for vitiligo remains unknown.

Objective: To investigate the impact of RNASET2 on therapeutic effect of NB-UVB for vitiligo in zebrafish model and in vitro.

Methods: The response of melanocytes in the surface of zebrafish with different irradiation dose of NB-UVB were analyzed by microscope; apoptosis and melanin synthesis of melanocytes were assessed by RT-PCR, laser confocal microscopy, western blot, immunohistochemical technique, and flow cytometry.

Results: Suitable dose irradiation of NB-UVB could induce the synthesis of melanin, while high-dose and accumulated low-dose could significantly promote melanocyte apoptosis. The expression of rnaset2-mRNA and rnaset2 protein in the surface of zebrafish was up-regulated after exposure to NB-UVB and it was correlated with the dose of NB-UVB. Overexpression of rnaset2 suppressed NB-UVB induced melanin synthesis and promoted melanocytes apoptosis in zebrafish model. The expressions of Caspase8, caspase3 and H2AX were higher in rnaset2 overexpression zebrafish, compared with control group. In vitro, RNASET2 promoted NB-UVB induced melanocyte apoptosis through enhanced nucleus translocation of P53 and activation of its downstream signaling pathways.

Conclusion: Narrow-band UVB induced biphasic regulation effects on melanocyte in zebrafish. The over-expression of rnaset2 promoted NB-UVB induced melanocytes apoptosis through activating P53-P21 signaling in vivo and in vitro. High expression of RNASET2 in patients of vitiligo may predict bad therapeutic effect of NB-UVB treatment.

P.108 | MicroRNA-211 regulates oxidative phosphorylation and energy metabolism in human vitiligo

Anupama Sahoo[1]; Bongyong Lee[1]; Sanjaya K. Sahoo[1]; Tatsuya Seki[2,1]; Katia Boniface[3]; Julien Seneschal[3]; Soumen Das[4]; Xianlin Han[1]; Michael Steppie[5]; Sudipta Seal[4]; Alain Taieb[3]; Ranjan J. Perera[1]

[1]Sanford Burnham Prebys Medical Discovery Institute, Orlando, FL, USA; [2]Medical and Biological Laboratories, Nagoya, Japan; [3]Institut National de la Santé Et de la Recherche Médicale, Bordeaux, France; [4]University of Central Florida, College of Medicine, Orlando, FL, USA; [5]Florida State University, College of Medicine, Orlando, FL, USA

Vitiligo is a common, chronic skin disorder characterized by loss of epidermal melanocytes and progressive depigmentation. Vitiligo has a complex immune, genetic, environmental, and biochemical etiology, but the exact molecular mechanisms of vitiligo development and progression, particularly those related to metabolic control, are poorly understood. Here we characterized the human vitiligo cell line PIG3V and the normal human melanocyte cell line HEM-l by RNA-sequencing, targeted metabolomics, and shotgun lipidomics. Melanocyte-enriched miR-211, a known metabolic switch in non-pigmented melanoma cells, was severely downregulated in vitiligo cells and biopsies from vitiligo patients, while its novel predicted targets transcriptional co-activator PGC1-α (*PPARGC1A*), ribonucleotide reductase regulatory subunit M2 (*RRM2*), and serine-threonine protein kinase TAO1 (*TAOK1*) were reciprocally upregulated. miR-211 binds to and represses PGC1-α at the 3'UTR locus. Melanogenesis, cell cycle, apoptosis, and oxidative stress genes and pathways were significantly dysregulated in PIG3V cells. Although mitochondrial numbers were constant between normal and vitiligo cells, mitochondrial complexes I, II, and IV and respiratory responses were defective in vitiligo cells. Nanoparticle-coated miR-211 partially augmented the oxygen consumption rate in PIG3V cells. The lower oxygen consumption rate, changes in lipid and metabolite profiles, and increased reactive oxygen species production observed in vitiligo cells appear to be partly due to abnormal regulation of miR-211 and its target genes. The turnover of respiratory substrates might be more important than mitochondrial numbers in energy utilization in vitiligo cells. These genes represent potential biomarkers and therapeutic targets in human vitiligo.

P.109 | Deciphering the role of skin effector and resident memory T cells in vitiligo

Katia Boniface[1]; Clément Jacquemin[1]; Christina Martins[1]; Nesrine Boukhedouni[1]; Khaled Ezzedine[2]; Alain Taieb[3]; Julien Seneschal[1,3]

[1]INSERM U1035, BMGIC, ATIP-AVENIR Immuno-dermatology, University of Bordeaux, Bordeaux, France; [2]Department of Dermatology, University of Paris-Est, Créteil, Créteil, France; [3]Department of Dermatology, University of Bordeaux, Bordeaux, France

Vitiligo is a chronic auto-immune depigmenting skin disorder that results from a loss of melanocytes. Multiple combinatorial factors have

been involved in disease development, with a prominent role of the immune system, in particular T cells. After repigmentation, vitiligo frequently recurs in the same area, suggesting that vitiligo could involve the presence of resident memory T cells (T_{RM}). Yet, the phenotype and contribution of effector memory T (T_{EM}) cell subsets in the skin of vitiligo patients remain to date unclear and controversial due to the lack of extensive analysis in human and animal models reproducing the complexity of the disease. Therefore, we sought to perform a thorough characterization of the phenotype and function of skin memory T cells in vitiligo. Stable and active vitiligo perilesional skin is enriched with a population of CD8 T_{RM} expressing both CD69 and CD103 compared to control healthy skin. Expression of CXCR3 is observed on the majority of CD8 T_{RM} in vitiligo and these cells displayed increased production of IFNγ and TNFα. Our study highlights the presence of functional CD8 T_{RM} in both stable and active vitiligo, reinforcing the concept of vitiligo as a skin memory disease. Remaining CD8 T_{RM} in stable disease could play a role during disease flares, emphasizing the interest to target this cell subset in vitiligo.

P.110 | Dysregulation of circulating T cells anergy might be involved in autoimmune disorder of vitiligo

Atsushi Tanemura; Ling Yang; Madoka Takafuji; Danbee Ha; Atsushi Tanaka; Shimon Sakaguchi; Ichiro Katayama

Osaka University Graduate School of Medicine, Osaka, Japan

Background: Vitiligo is the most common pigmentation disease. Whereas various pathogenetic mechanisms have been suggested, the exact mechanism is still unclear. Recently, the presence of melanocyte-specific T lymphocytes with naïve characteristics in HLA-A2 healthy donors was reported. Furthermore, high frequency of circulating Melan-A-specific, HLA-A*0201*-restricted cytotoxic T lymphocytes was observed in patients with vitiligo, these cells were also observed with high levels of the skin homing receptor, cutaneous lymphocyte-associated antigen.

Objective: To further clarify the mechanism in vitiligo by evaluating the phenotype of peripheral melanocyte-specific T cells and their anergy status in patients with vitiligo.

Methods: 13 HLA-A*0201* vitiligo patients and 10 genotype-matched healthy donors were randomly selected for this study. PBMCs were separated by centrifugation over Ficoll-Paque, and then purified $CD14^+$ APCs, $CD14^-CD8^+$ lymphocytes and $CD8^-CD25^+$ Treg cells were cultured and expanded over 10 days under Melan-A-peptide stimulation. Melanocyte-specific cytotoxic T cells (CTLs) were detected using fluorescent HLA-A*02:01 tetramers containing the immunodominant Melan-A (ELAGIGILTV) antigen, so called Tet^+ cells, and the immunophenotyping and function of both the cytotoxic T cells and Treg cells were analyzed by flow cytometry. The activated and anergy status of Tet^+ cells were examined by expression of CCR7 and CD45RA or CTLA-4, respectively. Finally, we also examined whether they were altered before and after monthly steroid half-pulse therapy to progressive vitiligo patients.

Results: Compared to healthy donors, high frequency of melanocyte-specific T cells and disturbing phenotype of Treg cells were observed in vitiligo patients. We found the recovery of disturbance of T cell anergy along with halt of disease progression after steroid therapy.

Conclusions: There might be a certain contribution of T cell anergy in autoimmune vitiligo. We suppose that steroid half therapy could halt disease progression via modulating Treg and melanocyte-specific CTLs' function.

P.111 | Melanocyte-specific knockout of TSC2 in mice induces skin depigmentation

Fei Yang; Lingli Yang; Mari Wataya-Kaneda; Atsushi Tanemura; Ichiro Katayama

Department of Dermatology, Course of Integrated Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan

Hypopigmented macules is known as the earliest sign in tuberous sclerosis complex (TSC) patients, which is a disease resulting from constitutive activation of the mammalian target of rapamycin (mTOR) pathway. We previously reported that hypopigmentation macules in TSC patients were recovered by topical mTOR inhibitor rapamycin treatment. However, the precise underlying mechanism remains uncertain.

Here, we generated mice with conditional deletion of TSC2 in melanocytes ($mcTSC2^{ko/ko}$) using flox-cre system. TSC2 protein expression level and TORC1 pathway activation were evaluated in mice skin epidermal melanocytes, by western blotting analyses and immunofluorescence staining. As expected, these mice exhibited undetectable TSC2 protein expression and constitutive hyperactivation of mTORC1 in melanocytes, indicating successful deletion of TSC2 in melanocytes. Very interestingly, strikingly reduced pigmentation was observed in mice tail skin starting at 4 weeks of age. Moreover, topical rapamycin treatment completely reversed the depigmentation in tail skin.

These results suggest that constitutive mTOR activation might be involved in hypopigmentation of TSC, and furthermore, this mouse model provides clearer evidence for the involvement of mTORC1 in pigmentation regulation.

P.112 | A pilot study to determine vitiligo severity using computer image analysis

Steven Thng[1,2]; Sai Yee Chuah[2]; Emily Gan[2]; Anjali Jhingan[2]; Adams Kong[3]

[1]Skin Research Institute of Singapore, Singapore, Singapore; [2]National Skin Centre, Singapore, Singapore; [3]Nanyang Technological University, Singapore, Singapore

Currently, qualitative measures like Vitiligo Area and Scoring Index and the Vitiligo European Task Force are used to score vitiligo severity. These scores, however, are qualitative and subject to intra

and inter-assessor variability. Reliable outcome measures are essential to document treatment outcomes and to make cross-study comparisons.

We have successfully developed an algorithm that calculates BSA involvement for trunk and limbs of patients with vitiligo using digital image analysis. This algorithm was piloted on 20 patients.

Patients with generalised vitiligo of trunk and limbs were recruited for this study. For each patient, a set of 10 standardized photographs were taken focusing on trunk, upper limbs, and lower limbs. The 10 photographs were sent for digital image analysis to determine the total body surface area (BSA) involvement. The algorithm calculated percentage BSA was then compared with both the VASI score and the scoring of percentage BSA by independent dermatologists.

Pearson's correlation coefficients were used to evaluate the correlation between our calculated BSA assessment scores versus clinical VASI scores and human BSA assessment scores. To determine the accuracy of algorithm BSA assessment against the standard human BSA assessment, t-test was used to test the null hypothesis that the variance between both assessment scores is more than 5%.

Results show that our automated algorithm was able to accurately map out all vitiligo lesions, regardless of size and was able to express the area involved as a percentage of total body surface area of that region. Computer BSA assessment was significantly correlated with the clinical VASI and human BSA assessment, with correlation coefficient of above 0.9. Variance between computer and human BSA was significantly within 5% (p-value <.01).

This pilot study demonstrates the promise of our program to objectively quantify total body surface involvement in patients with vitiligo vulgaris for the trunk and limbs.

P.113 | Characteristics, treatment and disease progression of late-onset vitiligo

Yan L. Kong[2]; Sai Yee Chuah[2]; Steven Thng[1,2]

[1]*Skin Research Institute of Singapore, Singapore, Singapore;* [2]*National Skin Centre, Singapore, Singapore*

Background: Late-onset vitiligo is poorly characterised, and little is known about the disease characteristics, progression and treatment response in this group of patients.
Objectives: This study aims to describe the clinical characteristics, treatment response and the clinical course of patients with late-onset vitiligo.
Methods: We retrospectively reviewed the case records of all patients diagnosed with late-onset vitiligo, defined as being aged 50 years and above at the point of clinical onset, from 1st January 2010 through 31st December 2014. Information obtained included patient demographics, characteristics of vitiligo, as well as the treatment response.
Results: Out of a total of 3128 patients diagnosed with vitiligo over the 5-year period, 461(14.7%) patients had late-onset disease. There were more females (n = 260, 56.4%), with an average onset of 59.4 ± 7.4 years. Most patients were Chinese (n = 308, 66.8%), and had Fitzpatrick skin types III (n = 151, 32.8%). Almost 1 in 10 patients had a history of other autoimmune disease. The majority of patients had non-segmental vitiligo (n = 436, 94.6%), of which focal vitiligo was the most common subtype (n = 209, 45.3%).

Treatment received included topical creams, phototherapy and/or surgical grafting. Patients who were treated with a combination of topical creams and phototherapy, yielded the best clinical response (p < .001), with more than half of the patients achieving good epidermal repigmentation (n = 81, 56.6%). The choice of phototherapy did not significantly affect clinical response (p = .851).
Conclusion: Late onset vitiligo is not uncommon and tends to be of the focal vitiligo subtype. Treatment response is fair and combination therapy is more effective.

P.114 | Treatment outcomes of vitiligo in Asian children

Evelyn Tay[2]; Sai Yee Chuah[3]; Steven Thng[1]

[1]*Skin Research Institute of Singapore, Singapore, Singapore;* [2]*Changi General Hospital, Singapore, Singapore;* [3]*National Skin Centre, Singapore, Singapore*

Background: Childhood vitiligo is common. However, studies on the treatment outcomes in this group of patients are scarce.
Objectives: We aim to identify factors that predict for treatment response in a cohort of Asian patients with childhood vitiligo.
Methods: A retrospective review of electronic medical records of patients with childhood vitiligo was conducted from 2006 to 2015. Data such as patient demographics, disease characteristics, treatment modalities and treatment responses were recorded.
Results: 205 patients were included. Patients who presented within 1 year of disease onset were more likely to achieve >75% repigmentation (p < .01). With the use of topical agents alone, twenty-eight percent achieved >50% repigmentation and 20% achieved >75% repigmentation. Patients with focal vitiligo (p < .01), a short time to presentation ≤6 months (p < .01) and the absence of unstable vitiligo (p < .01) were more likely to achieve >50% repigmentation with the use topical agents alone.
Limitations: This is a retrospective study in a small cohort of patients.
Conclusions: A shorter duration of vitiligo is associated with better repigmentation. Patients with focal vitiligo of short duration have a good chance of achieving repigmentation with topical agents alone.
Key words:
vitiligo, childhood, pediatric, early treatment, treatment outcomes:

P.115 | The use of antioxidants in the treatment of vitiligo: A systematic review and meta-analysis of randomized controlled trials

Jung Min Bae; Han Mi Jung; Yu Seok Jung; Ji Hae Lee; Gyong Moon Kim

The Catholic University of Korea, Suwon, Korea

Background: Accumulating evidence suggests that melanocytes from vitiligo patients have intrinsic defects to manage oxidative stress. However, the use of antioxidants remained controversial in the treatment of vitiligo.

Objectives: To assess the additional effects of antioxidants during phototherapy in patients with vitiligo.

Methods: Manual searches of reference lists and computerized searches of the MEDLINE, EMBASE, Cochrane Library, and Web of Science (from inception through April 23, 2017) were conducted to identify randomized controlled trials (RCTs) that compared the efficacy of phototherapy alone or in combination with systemic antioxidants for vitiligo. The outcome of interest was treatment response (≥50% repigmentation) in individuals with vitiligo.

Results: We analyzed 4 RCTs comprising a total of 128 patients. The combination of systemic antioxidants and phototherapy showed the significantly higher response rates comparing to the phototherapy only in the treatment of vitiligo (RR 1.49, 95% CI 1.06-2.09).

Conclusions: The use of antioxidants should be encouraged to enhance the treatment response during phototherapy for patients with vitiligo.

P.116 | Perifollicular repigmentation on poliosis

Jung Min Bae; Ji Hae Lee; Sung Hye Eun; Gyong Moon Kim

The Catholic University of Korea, Suwon, Korea

Background: Poliosis, an absence of functional melanocytes in a hair follicle, is considered a poor prognostic factor in treatment of vitiligo because the melanocytes of hair follicles are the major source of repigmentation.

Objectives: We sought to evaluate the clinical outcome of poliosis in treatment of vitiligo.

Methods: A retrospective study was performed on 21 patients with vitiligo who have poliosis in head and neck and were treated with the combination therapy of 308-nm excimer laser, topical tacrolimus, and intralesional triamcinolone injection for more than 3 months. Dermoscopy was used to evaluate the lesions thoroughly.

Results: Perifollicular repigmentation associated with poliosis was observed in 11 patients (52.4%). Five patients were segmental vitiligo and the others were non-segmental vitiligo. It occurred even after 6 months of the combination treatment, whereas most cases of perifollicular repigmentation without poliosis started within 1 month.

Conclusions: Hair follicles are considered the reservoir of melanocytes in vitiligo, and the treatment is not easy where poliosis is present. However, we observed the perifollicular repigmentation associated with poliosis with the continuous treatment. These findings would be explained with both activation of inactive amelanotic melanocytes and differentiation of melanoblast, and melanocyte stem cells in hair follicle.

P.117 | Protective effects of phototherapy against cardiovascular and cerebrovascular events in patients with vitiligo: A population-based cohort study

Jung Min Bae; Hyuck Sun Kwon; Han Mi Jung; Ji Hae Lee; Gyong Moon Kim

The Catholic University of Korea, Suwon, Korea

Background: Narrowband UV-B (NB-UVB) phototherapy has been the mainstay of treatment for vitiligo. Although excessive UV exposure is often associated with adverse effects including premature photoaging and increased risks of skin cancers, additional benefits of UV exposure have also been raised.

Objectives: To determine the impact of NB-UVB phototherapy on the cardiovascular and cerebrovascular risks in patients with vitiligo.

Methods: A population-based cohort study was performed utilizing the Korean National Health Insurance Claims Database from 2007 to 2016. All patients with vitiligo aged ≥ 40 years were identified and categorized into four groups based on the treatment sessions of NB-UVB phototherapy (≤2, 3-49, 50-99, and ≥100) during the period. Outcomes of interests were myocardial infarction and stroke. Cox proportional hazards models were used for multivariable analyses.

Results: A total of 41,766 patients with vitiligo were enrolled. Comparing to patients with ≤2 phototherapy sessions (n = 20,497), patients with ≥100 phototherapy sessions (n = 2,765) showed significantly decreased risks of cardiovascular (HR 0.602, 95% CI 0.371-0.976) and cerebrovascular events (HR 0.817, 95% CI 0.679-0.985) after adjustment for potential confounders.

Conclusions: NB-UVB phototherapy can lower the risks of myocardial infarction and stroke in patients with vitiligo.

P.118 | Skin seeding technique using 0.8-mm motorized punch for refractory vitiligo

Jung Min Bae[1]; Jiehoon Kim[2]; Dong Seok Kim[3]

[1]*The Catholic University of Korea, Suwon, Korea;* [2]*Dr. Kim's Skin & Laser Clinic, Suwon, Korea;* [3]*Eureka Skin & Laser Clinic, Seoul, Korea*

Background: Punch grafting is a simple used technique for the treatment of stable vitiligo, resistant to medical therapy. However, the time-consuming nature and frequent cobblestoning remains the limitation of the procedure.

Objectives: To evaluate the efficacy of a novel surgical method for refractory vitiligo: skin-seeding technique (SST) using 0.8-mm motorized punch for refractory vitiligo.

Methods: A total of 24 lesions in 18 patients (13 to 66 years old) with vitiligo, stable for at least 3 months, were included. The stainless steel punch of 0.8 mm in diameter loaded into the hand piece of micromotor, was used for skin graft from both donor and recipient region. After the punched skin was extracted from the recipient region, donor

grafts that were harvested from the retroauricular skin were seeded into the recipient chambers. Each seeding site was less than 0.5 cm apart. After 1-week of hydrocolloid dressing, the lesions were treated with excimer laser for 3 months.

Results: Overall, 22 of 24 lesions (91.7%) achieved > 75% repigmentation. Most of the patients (88.9%) were very satisfied with this technique, and no major systemic or local complications were reported. The common side effects were cobblestone appearance (54.2%) and color mismatch (66.7%) in the recipient region, however, most of them were rated acceptable by the patients.

Conclusions: In our procedure, 0.8-mm punches were used to minimize side effects, and the use of motorized punch markedly reduced the operating time as well. SST using 0.8-mm motorized punch could be a convenient and simplified surgical modalities for refractory vitiligo.

P.119 | Identifying gaps in the narrowband ultraviolet B phototherapy guidelines for vitiligo

Bhavnit K. Bhatia; Iltefat H. Hamzavi

Henry Ford Hospital, Detroit, MI, USA

Phototherapy has been shown to be a safe, effective method of treatment for vitiligo. Although widely utilized, until recently, there were no consensus guidelines regarding protocol for phototherapy. An original article by Madigan et al. in 2016 and subsequent consensus article from the Vitiligo Working Group in 2017 have produced a protocol for phototherapy. This protocol covers frequency, initial dosing and subsequent modifications, how to define course of treatment, and assess response, among other points. This is a very large step in the direction of establishing a universal standard of care for phototherapy of vitiligo. Still, key gaps exist. At the forefront of these gaps is the lack of randomized controlled trials, as most recommendations are based upon expert consensus. In addition, as we move toward new systemic treatment options for vitiligo, including pulse-dose steroids, minocycline, and JAK inhibitors, many questions arise. Questions that remain open for discussion include the role of phototherapy as an adjuvant to systemic treatment, the duration of treatment including active and maintenance therapy, and whether there is a need for adjustment of maximum or cumulative dose UV dose based upon skin type.

P.120 | Application of melanin index and L*a*b* color space for the evaluation of vitiligo and chemical leukoderma

Masahiro Hayashi; Ken Okamura; Yuko Abe; Tamio Suzuki

Yamagata University, Yamagata, Japan

Background: Acquired skin hypopigmentation is caused by many etiologies. Among them, vitiligo is an autoimmune disorder targeting skin melanocytes, appearing as multiple irregular white patches on the skin. Rhododendrol (4-(4-hydroroxyphenol)-2-butanol)-induced leukoderma is caused by rhododendrol-containing cosmetics. It is important to assess the disease severity of vitiligo/leukoderma accurately to conduct unbiased clinical evaluation. however, no gold standard assessment method been reported.

Objective: We aimed to evaluate the color of vitiligo/leukoderma and surrounding normally pigmented skin quantitatively.

Methods: We investigated whether the digital instruments Mexameter® MX18 and CM-700d spectrophotometer can quantitatively distinguish vitiligo/leukoderma from normally pigmented skin with melanin index. CM-700d was also used to quantify the color of vitiligo/leukoderma lesions and surrounding normally pigmented skin in L*a*b* color spaces. Additionally, ΔE^*ab ($\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$), which indicates the color difference between two measured points, was calculated.

Results: Melanin index measured by using MX-18 and CM-700d, and L^* and b^* values measured by using CM-700d consistently distinguished vitiligo/leukoderma from normally pigmented skin. The ΔE^*ab value well correlated with the clinical color contrast between vitiligo/leukoderma and surrounding normally pigmented skin.

Conclusion: These methods can be useful tools for assessment of vitiligo/leukoderma lesions, disease severity, and patient quality of life.

P.121 | 2% topical tofacitinib cream in the treatment of vitiligo vulgaris

Sara Li; Victor Huang

Brigham and Womens Hospital, Boston, MA, USA

Vitiligo is an autoimmune disorder of pigmentation resulting from the destruction of melanocytes affecting 1-2% of the world's population. Recent work has demonstrated the profound impact that vitiligo can have on those affected including higher rates of personality disorders, lower self-esteem, limitations to interpersonal relationships, and depression. The current mainstays of treatment are topical agents and phototherapy. Topical steroids have been shown to only elicit benefit in 50-60% of patients. Calcineurin inhibitors such as pimecrolimus and tacrolimus have similar reported efficacy rates. Phototherapy, especially narrow band ultraviolet B (NBUVB), has been shown to be efficacious treatment for vitiligo being effective in inducing >75% repigmentation in ~70% of patients. Phototherapy, however, is not universally accessible, and is associated with the need for frequent visits that limit its practical application.

With the implication of the interferon-gamma pathway in the pathogenesis of vitiligo, several case reports have demonstrated clinical efficacy of systemic treatment with JAK inhibitors, tofacitinib and ruxolitinib. Herein, we report the successful treatment of facial vitiligo with a topical preparation of tofacitinib which promises a therapeutic option that does not require frequent office visits, carries low risk of systemic adverse effects, and is more cost effective than systemic treatment.

P.122 | Autologous cell suspension grafting in segmental vitiligo and piebaldism: a randomized controlled trial on the recipient site preparation

Janny E. Lommerts; Arne Meesters; Lisa Komen; Marcel Bekkenk; Menno De Rie; Rosalie Luiten; Albert Wolkerstorfer

Netherlands Institute for Pigment Disorders, Academic Medical Centre, Amsterdam, Netherlands

Introduction: Autologous non-cultured cell suspension transplantation is an effective treatment for repigmentation in segmental vitiligo and piebaldism. The optimal depth of ablation and other laser settings of the recipient-site before cell suspension transplantation is unknown. The objective of this study was to assess the efficacy and safety of less invasive recipient-site preparations.

Methods: We compared different recipient-site preparations before cell suspension transplantation in patients with segmental vitiligo (n = 3) and piebaldism (n = 7). In each patient, we randomly allocated four CO_2-laser recipient-site preparations (i.e. standard, superficial, fractional and control site) to four depigmented lesions. We assessed repigmentation and side-effects 6 months after the grafting procedure.

Results: We found significant higher repigmentation in the standard (median 68.7%, p = .011) and superficial (median 58.3%, p = .007) full surface CO_2-laser ablated recipient sites when we compare them to the control site, but not for fractional (median 0.0%, p = .144) CO_2-laser ablation. Persistent erythema was less frequently present in superficial than standard full-surface recipient-site preparation. No other differences in side-effects were seen.

Conclusion: Superficial full surface CO_2-laser ablation is an effective recipient-site preparation before cell suspension transplantation in segmental vitiligo and piebaldism. Fractional CO2-laser recipient-site preparation with the settings used in this study was not effective.

P.123 | Vitiligo Cosmetic Acceptability Scale (VICAS): Preliminary results on the validity and reliability of a new measurement tool in vitiligo

Janny E. Lommerts[1]; Marcel Bekkenk[1]; Reinhart Speeckaert[2]; Nanja Van Geel[2]; Albert Wolkerstorfer[1,2]

[1]Netherlands Institute for Pigment Disorders, Academic Medical Centre, Amsterdam, Netherlands; [2]Department of Dermatology, Ghent University Hospital, Ghent, Belgium

Background: Cosmetic acceptability of repigmentation is marked as a core outcome domain in vitiligo. Nevertheless, no tool is available to measure this important domain. In this study, we tested the validity and reliability of a new tool to measure the cosmetic acceptability: the Vitiligo Cosmetic Acceptability Scale (VICAS). The VICAS is comprised of one question 'How satisfied are you with the cosmetic aspect of the repigmentation?' answered on a 5-point Likert Scale (very dissatisfied to very satisfied).

Methods: We included patients with non-segmental vitiligo and followed them during NB-UVB therapy. After 26 weeks of treatment patients scored the VICAS, SA-VES, Skindex-29 and Vitiligo Noticeability Score (VNS). The validity of the VICAS was based on hypotheses testing as a gold standard measuring cosmetic acceptability is lacking. Intra-observer reliability was measured by assessing the intra-observer agreement of the VICAS at week 26 (test) and week 28 (retest).

Results: Up to this date, 20/30 patients (median age: 42 yr; 45% male) were seen for follow-up. Median affected body surface area at start of treatment was 5.7% (IQR 1.8–11.7) and median repigmentation was 9.7% (IQR -24.0%–47.2%). Seventy percent showed satisfaction with the cosmetic aspect of the repigmentation, resulting in a median VICAS score of 3 (IQR 2-3). Reliability was moderate (kappa 0.313, measured in 55%). None of the validation hypotheses were confirmed up to this date.

Conclusion: Our preliminary results suggest that the VICAS is a moderately reliable tool to measure the cosmetic acceptability of repigmentation. However, the validity of the VICAS remains unclear. Hypothetically, different psychological, social, cultural and physical factors are contributing to cosmetic acceptability which makes validation with construct validity of the tool more difficult. Possibly, the outcome domain cosmetic acceptability needs to be subdivided in other outcome domains and further research is needed on the subject.

P.124 | Prospective measurement of the responsiveness of the Vitiligo Extent Score (VES) and self-assessment VES (SA-VES) during NB-UVB therapy in vitiligo patients

Janny E. Lommerts[1]; Nanja Van Geel[2]; Reinhart Speeckaert[2]; Marcel Bekkenk[1]; Albert Wolkerstorfer[1]

[1]Netherlands Institute for Pigment Disorders, Academic Medical Centre, Amsterdam, Netherlands; [2]Department of Dermatology, Ghent University Hospital, Ghent, Belgium

Background: The VES and SA-VES are valid and reliable measurement tools to measure the extent of vitiligo. In a previous retrospective study we showed that the VES is a responsive instrument. However, in a prospective setting the responsiveness of the VES has not yet been measured. Furthermore, the responsiveness of the self-assessment of the VES by patients (SA-VES) has never been investigated. In this study, we measured the responsiveness of the VES and SA-VES prospectively during 6 months of NB-UVB therapy in 30 vitiligo patients.

Methods: We included 30 patients with vitiligo and followed them during 6 months of NB-UVB therapy. Before and 6 months after start of NB-UVB therapy, patients were asked to fill in a questionnaire comprising questions concerning baseline characteristics, SA-VES and improvement after treatment. One independent vitiligo expert assessed the VES on the photographs taken before and 6 months after start of NB-UVB treatment. Measurement of the responsiveness was based on hypotheses testing; when 75% or more of the hypotheses were confirmed the VES and SA-VES were marked as responsive.

Results: In total, 30 patients were included and the preliminary results presented here are based on 13 patients (43.3%). The median age of those patients was 42 years and 31% were males. The affected body surface area (VES) at start of treatment was 2.5% (median, IQR 0.8-8.4%). The percentage of repigmentation of all patients was 20.7% (median, IQR 2.7-39.2%) and 32.2% (median, IQR 10.9-46.3%) in patients marked as improved (n = 10, 77%). Both VES and SA-VES were marked as responsive as ≥ 75% of the hypotheses were confirmed after treatment.

Conclusion: Our preliminary results suggest that the VES and SA-VES are valid, reliable and responsive measurement tools to measure the extent of vitiligo.

P.125 | Monobenzyl Ether of Hydroquinone (MBEH) in vitiligo management: A comprehensive clinical evaluation

Medhat El Mofty[1]; Yasmin Armanious[2]; Wedad Z. Mostafa[1]; Samia Esmat[5]; Laila Gadelrub[3]; Nesrine Samir[1]; Solwan I. El Samanoudy[1]; Marwa El Mesidy[5]; Sarah I. Ismail[5]; Dina G. Saadi[5]; Michael Nageh[4]; Hager El-Sayed[5]

[1]Cairo University, Cairo, Egypt; [2]EVA Cosmetics, Giza, Egypt; [3]EVA Pharmaceuticals, Giza, Egypt; [4]EVA Pharmaceuticals, Giza, Egypt; [5]Faculty of Medicine Kasr Al Ainy, Cairo University, Cairo, Egypt

Background: Depigmentation presents a final alternative in patients with widespread vitiligo, those failing to attain satisfactory pigmentation after therapy and having a compromised quality of life (QOL). Monobenzyl Ether of Hydroquinone (MBEH) is the most widely employed topical preparation but not much is known regarding specific features of its utilization.

Objective: Our aim was to determine the relation between disease characteristics in different patients; extent, activity, pattern, history of previous depigmentation and the final clinical response to MBEH. Our goals included measurement of patients' QOL, evaluation and comparison of efficacy and tolerability of both concentrations.

Methods: In a double blind prospective study, forty consecutive patients with extensive facial and hand vitiligo were randomly divided into 2 groups; A and B treated with MBEH 20 and 40%, respectively. All patients applied physical sunscreens. Detailed clinical evaluation was performed. Patients' response was assessed bimonthly along one year by; clinical evaluation, photography, point counting, percent color dilution and biometric measurement of melanin by a colorimetric tool *dermacatch*. Dermatology Life Quality Index (DLQI), Visual Analogue Scale (VAS), side effects and relapses were assessed.

Results: Thirty nine patients completed the study; 19 (A), 20(B). Upon completion, a significant dilution of color, reduction of the pigmented area and decrease of melanin content were recorded. DLQI and VAS scores improved significantly. No significant difference was found in efficacy between both groups, but a significantly higher degree of irritation was noted in group B. Relapse was not significantly different either.

Conclusion: Depigmentation of exposed pigmented skin in vitiligo patients has a marked positive influence on patients' QOL. Physical sunscreen application is essential for maintenance of depigmentation. MBEH 20% offers a cost effective modality for all patients with fewer side effects. However, 40% may be chosen in poor or non responders.

P.126 | Autologous non cultured cellular grafts versus hair follicle outer root sheath cell suspension

Myint M. San; Wai Y. Phyo; Yin H. Win; Sabai Chain

KEMBANGAN UHC Hospital, Yangon, Myanmar

As global awareness on vitiligo increases in this information technology age, medical professionals are faced with seeking the best option in managing vitiligo as treatment varies with individual needs.

Vitiligo surgery has become a more effective way for a better and faster repigmentation outcome and both doctors and patients alike opt for these treatments.

This particular report looks into the principle, technical procedures and the advantages and disadvantages over Autologous non cultured epidermal cell suspension and Hair follicle outer root sheath cell suspension in the surgical treatment of vitiligo.

P.127 | Marked repigmentation on intractable vitiligo skin by novel 311 nm UV laser irradiation and external application of KS lotion or castor oil

Takakazu Shibata

Shibata Clinic of Dermatology, Osaka, Japan

We found marked perifollicular and/or peri sweat pore repigmentation on intractable vitiligo patients' skin by novel gain 311 nm titan-sapphire ultra violet(uv) laser irradiation and by combination external application of KS lotion or castor oil.

Gain 311 nm titan-sapphire uv laser has much more power and reaches deeper skin zone than ordinary excimer light and is suitable for irradiation on corrugated skin lesions for example inner eye angle area or inter fingers area.

KS lotion is made from roots of eatable plants which had been used for treatment of vitiligo skin in ancient China. The mechanism of repigmentation by KS lotion isn't unclarified, but I found very much marked follicular repigmentation on intractable vitiligo skin by this uv laser irradiation and application of KS lotion.

Castor oil includes ricinin which can stimulate *wnt* signal. *Wnt* signal can change melanocyte stem cells in niche of follicle to melanocytes. But this melanocyte moves only to hair papilla. Skin damages caused by this uv laser irradiation or by Co_2 laser skin abrasion may cause melanocyte's epidermotropism.

P.128 | Differential diagnosis and outcome of childhood vitiligo: Clinical and immunohistochemical analysis

Madoka Takafuji; Atsushi Tanemura; Mari Kaneda; Aya Takahashi; Ichiro Katayama

Osaka University, Suita-city, Japan

Introduction: While vitiligo can involve a wide age range of patients, a couple of previous studies stated that most patients have been acquired at the age of less than 20 years old. In case of vitiligo in childhood, the discrimination between complete and incomplete hypopigmentation is important to make a correct diagnosis of vitiligo differentiated from Ito's hypomelanosis, depigmented nevus, and piebaldism. Recent review archived in PCMR 2012 describes for detail clinical characteristics of vitiligo in childhood rather than in adolescent as follows; 1) easy to show Koebner's phenomenon 2) more frequent in segmental type 3) more frequent in association with Sutton's nevus, and so on.

Purpose: Our aim in this study is to understand the character of vitiligo in childhood and to perform a correct differential diagnosis of hypopigmented diseases.

Methods and Results: We assessed the type of leukoderma, clinical course, response to treatment for 94 cases less than 18 years old visiting our institute from 2010 to 2017. Although it was difficult to discriminate between depigmented nevus and segmental vitiligo during infancy, segmental vitiligo showed favorable outcome of repigmentation irrespective of any treatment. In addition, we performed histopathological and ultrastructural analysis to compare between complete and incomplete hypopigmented lesions of the cases without correct diagnosis.

Conclusion: The present study supposes the usefulness to perform daily clinical examination for pediatric patients with hypopigmented lesions and advocate the importance for pediatric dermatologists to pay attention to patients' clinical course and to provide optimized treatment options for various type of vitiligo patients.

P.129 | The majority of patients with vitiligo have a clinical sign of activity

Andrea Tovar-Garza; Nader Aboul-Fettouh; Jorge Hinojosa; Amit G. Pandya

University of Texas Southwestern Medical Center, Dallas, TX, USA

Background: Disease progression in vitiligo patients' is unpredictable and characterized by periods of stability interspersed with periods of slow or rapid depigmentation. Signs of activity may be indications for more aggressive therapies in an effort to halt disease progression.

Objective: Determine the prevalence of activity signs, such as confetti-like lesions, trichrome and Koebner phenomenon in patients with vitiligo.

Methods: This was a prospective cross-sectional study of new patients with non-segmental vitiligo attending the outpatient dermatology clinic between September 2015 and December 2016 at the University of Texas Southwestern Medical Center. Clinical examination of vitiligo lesions was performed by a dermatologist and the presence of confetti-like depigmentation, trichrome depigmentation and the Koebner phenomenon were recorded. Photographs of the lesions were taken and reviewed by the same dermatologist for confirmation.

Results: A total of 200 patients were included, out of whom 178 (89%) had generalized vitiligo, 14 (7%) had acrofacial type and 8 (4%) had mixed type. One hundred and twenty-three patients were female (61.5%). The mean age was 38.6 years (range of 3-80). The mean disease duration was 9.2 years (range 4 months to 40 years). One hundred and thirty-three patients (66.5%) were not receiving any treatment at the time of visit. The mean body surface involvement was 9.4%. Of the 200 patients, 121 (60.5%) displayed at least one of the three vitiligo markers of activity. Confetti-like depigmentation, trichrome lesions and Koebner phenomenon were identified in 92 (46%), 54 (27%) and 59 (29.5%) patients, respectively. Nineteen (9.5%) patients had all three markers of activity on clinical exam.

Conclusion: While a search for a reliable biomarker continues, these clinical signs are helpful in predicting disease activity. The results of the current study indicate the majority of patients presenting for evaluation and treatment of vitiligo have a clinical sign of activity.

P.130 | Six month outcome of non-cultured epidermal suspension grafting using suction blisters

Andrea Tovar-Garza; Jorge Hinojosa; Amit G. Pandya

University of Texas Southwestern Medical Center, Dallas, TX, USA

Background: Surgical interventions for vitiligo are considered in stable patients who have not responded to previous treatments. Non cultured epidermal suspension grafting using suction blisters for harvesting epidermal cells was recently described.

Objective: We sought to determine the efficacy and safety of non-cultured epidermal suspension grafting in our Pigmentary Disorder Clinic at UT Southwestern Medical Center.

Methods: Retrospective review of all patients who underwent non-cultured epidermal suspension grafting in our clinic from August 2014 to December 2016 and who had at least a 2-6 month follow up visit. Disease stability was determined as: absence of new lesions and/or worsening of existing lesions for at least 12 months before surgery. Narrow-band UVB phototherapy was restarted a week after the transplant. Repigmentation during a 2-6 month follow up period was assessed categorically as: excellent (>75% repigmentation), moderate (51-75%), good (25-50%) and minimal (<25%).

Results: Of the 10 patients who underwent 17 procedures, 8 patients who underwent 13 procedures had a 2 to 6 month follow up period. Four patients (50%)had generalized vitiligo, three (37.5%) had

segmental type and one (12.5%) had leukoderma from laser tattoo removal. The recipient sites were as follows: 3 (23%) on the head/neck, 1 (7.7%) on trunk, 9 (69.3%) on upper and lower extremities and 1 (7.7%) on the hand. Data from this 13 procedures show an excellent to moderate repigmentation in 23%, good repigmentation in 30% and minimal in 46%. None of the patients with fingertip involvement had moderate to excellent repigmentation. Adverse events presented in only 2 patients, consisted mainly of infection at the recipient site and a hypertrophic scar on the lower extremity. Using trypan blue and a hemocytomer, the mean percent of living keratinocytes in three patients was 76%.

Conclusion: NCES grafting using suction blisters has an overall good to excellent repigmentation response.

P.131 | Treatment of active vitiligo using oral dexamethasone, NB-UVB and clobetasol

Andrea Tovar-Garza; Jorge Hinojosa; Amit G. Pandya

University of Texas Southwestern Medical Center, Dallas, TX, USA

Background: Pulse dosing of oral dexamethasone has been reported to be effective to arrest disease activity in patients with vitiligo, however, further studies are needed.

Objective: Evaluate the efficacy, safety and tolerability of oral dexamethasone pulse therapy, NB-UVB and topical clobetasol for active vitiligo with 1 year follow-up.

Methods: Retrospective review of all patients with active vitiligo seen in the pigmentary disorders clinic at UT Southwestern Medical Center seen in 2014-2015. Active disease was defined as new lesions or extension of existing lesions confirmed by photography, as well as Koebner, confetti-like or trichrome lesions. Patients received the following regimen: dexamethasone 4 mg PO on 2 consecutive days for a maximum of 6 months, NB-UVB 3 times a week and clobetasol 0.05% QD. We also measured body surface area (BSA) involvement at the end of dexamethasone therapy and one year after the baseline visit.

Results: A total of 24 patients were enrolled. The mean treatment duration was 3.5 months (range 2-6 months). Disease activity was arrested in 22/24 (92%). Confetti-like lesions were present in 19 (79%) patients, trichrome in 15 (63%), Koebner in 7 (29%) and new lesions in 5 (21%). At the end of dexamethasone therapy only 2 of 24 patients (8%) had confetti-like depigmentation while the remainder had no signs of active disease. The mean BSA involvement with vitiligo at baseline was 16%, which decreased to 11% after treatment, which is an improvement of 31%. A total of 29% had side effects, including insomnia, weight gain and acne. At 12 months, the mean BSA decreased from 11% to 7%. Eight (35%) patients had active disease at 12 months, however, 7 of these patients were noncompliant with treatment.

Conclusion: OMP dexamethasone, NB-UVB and topical clobetasol achieved arrest of disease in 92% of active patients with mild side effects.

P.132 | Development of a mobile application technique for the follow up of target lesions in vitiligo

Sanne E. Uitentuis[1,2]; Marcel W. Bekkenk[1,2]; Albert Wolkerstorfer[1,2]

[1]Academic Medical Center, Amsterdam, Netherlands; [2]Netherlands Institute for Pigment Disorders, Amsterdam, Netherlands

There is a current need for validated outcome measures in vitiligo. To follow up change of specific vitiligo lesions over time a simple and valid measurement technique is still needed. Current techniques use overhead sheets or photos combined with image analysis software to calculate the lesion size and its change overtime. These techniques are time consuming and have not been completely validated. A golden standard for the assessment of target lesions remains to be determined.

The development of a mobile application (app) that can measure the size of vitiligo lesions can make the follow-up easier, less time consuming and possibly more accurate. The aim of this paper is to describe how a new app technique could fill the need for an accurate and easy to use outcome measure to assess target lesions in vitiligo.

We will propose and discuss an app where patients or physicians use their mobile phones to make a picture of a target lesion. A reference sticker with a standard size and color is placed next to the lesion and serves for correction of the angle, lighting and distance to the skin. The app uses software that can recognize borders of lesions as well as repigmentation islands within the lesions. Subsequently the app will use the reference sticker to calculate the exact size.

We are currently validating the app technique as an outcome measure for target lesions by using it on different patients in different settings. We will compare outcomes with measurements of the existing techniques.

The use of this app seems promising for the day-to-day clinic. Physicians can assess the results of therapy and patients can measure their own disease more accurately and monitor their lesions at home. Furthermore this would be an ideal outcome measure in vitiligo research, especially when specific target lesions are treated.

P.133 | How to define the cut off points for severity and extent in vitiligo: Interpretation from the patients' point of view

Nanja van Geel[1]; Cecilia A. Prinsen[2]; Albert Wolkerstorfer[3]; Marcel Bekkenk[3]; Reinhart Speeckaert[1]

[1]Department of Dermatology, Ghent University Hospital, Ghent, Belgium; [2]Department of Epidemiology and Biostatistics Amsterdam Public Health research institute, VU University Medical Center, Amsterdam, Netherlands; [3]Department Dermatology AMC/University of Amsterdam and Netherlands Institute for Pigment Disorders, Amsterdam, Netherlands

Background: Grading of severity is a major factor in the management of patients with vitiligo. So far, no thresholds for limited, moderate,

severe and very severe/extensive vitiligo have been defined. The patients' perception is utmost important when defining these cut off points.

Objectives: The aim was to identify the cut off scores for "extent" and "severity" from the patients' point of view and to investigate the role of several factors on patients' interpretation (e.g. disease impact, skin type, location of lesions…).

Methods: Consecutively included vitiligo patients at the Ghent University Hospital completed a questionnaire including a 5-point assessment scale for severity and extent, ranging from not severe/no involvement to very severe/very extensive. Disease impact was scored by a global rating scale, ranging from 0 to 10. Location of lesions and total affected Body Surface Area (BSA) were based on the Self Assessment Vitiligo Extent Score (SAVES). Fitzpatrick skin type was assessed by patients and physicians.

Results: Based on a preliminary evaluation, median BSA value per category for disease extent was 0.50% for limited, moderate 2.25%, extensive 5.15% and very extensive 9.33% (n = 270). Cut-off value (BSA) based on ROC analysis between limited and moderate extensive was 1.46%, between moderate and extensive 3.92%, and between extensive and very extensive 7.65%. The median BSA value for limited disease severity was 0.25%, moderate 0.61%, severe 1.57% and very severe 5.42% (n = 285). Cut-off value (BSA) based on ROC analysis between limited and moderate severe was 0.21%, between moderate and severe 1.87% and between severe and very severe 5.4%.

Conclusion: Patients' perception of disease severity and extent seem to vary within the first 10% of BSA affected. Patients' interpretation of vitiligo severity and extent will probably vary amongst patients worldwide. Future studies are therefore necessary to get further insight into this variation.

P.134 | Corticosteroids decreasing serum CXCL10 in a female chinese patient of vitiligo, thymoma with MG and autoimmune thyroiditis

Li Zhang; Leihong Flora Xiang

Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China

Vitiligo is a common acquired pigmentary disease caused by the destruction of epidermal melanocytes with underlying autoimmune response. There is rare report of vitiligo concomitant with thymoma-myasthenia gravis (MG). Nowadays, the association between vitiligo and autoimmune diseases has not yet been fully explained. The objective of this study was to report a rare case of a concomitant presentation of vitiligo associated with thymoma with MG and autoimmune thyroiditis. We report a rare case of a 62-year-old female was referred for treatment of a one-year history of vitiligo and 3-mouth history of thymoma with MG and autoimmune thyroiditis. After thymoma resection and three month on prednisone 20 mg-30 mg per day, the muscle weakness was resolved and halometasone cream for topical use per day, the vitiligo lesions were well controlled. Serum CXCL10, sCD25 and sCD27 decreased. We suggest that dermatologists should pay attention to the possible concomitance of thymoma with MG and autoimmune thyroiditis with vitiligo. CXCL10 may be a potential biomarker for predicting vitiligo and its concomitant disease. Further studies are needed to explore the potential mechanisms in the concomitance of these diseases.

P.136 | Leukotrichia in vitiligo: A clinical cross-sectional study

Wedad Z. Mostafa; Maha F. Elmasry; Rania M. Mogawer

Faculty of Medicine Kasr Al Ainy, Cairo University, Cairo, Egypt

Background: Leukotrichia has been considered a predictor of poor outcome in vitiligo. However, few studies focused on its value.

Objectives: Our aim was to conduct a thorough appraisal of leukotrichia providing insight into its relevance and associations in the context of vitiligo.

Methods: In this cross-sectional study, adult vitiligo patients attending the Dermatology Clinic and Phototherapy Unit from April to September 2016 were included. Family history, demographic data, clinical examination, VGICC classification, Vitiligo Area and Severity Index (VASI), Vitiligo Extent Score (VES), Vitiligo Disease Activity (VIDA) Score, Dermatology Life Quality Index (DLQI), and Vellus Score were implemented and measurements correlated with leukotrichia which was evaluated by clinical examination, dermoscopy and Wood's light.

Results: Leukotrichia was recorded in 48.6% of patients (52/107); vellus hair was involved in 78.8% (41/52), terminal in 59.6% (31/52) and both in 38.5%(20/52). The incidence of vellus hair and scalp leukotrichia was significantly higher in generalized symmetrical than multifocal symmetrical or acrofacial vitiligo; p values: .002 and .001 respectively. Vellus scores showed significant correlation with VASI, VES grade and DLQI (p values: .005, .001, .025 respectively), but not with disease duration nor activity. Patients with negative Koebner's phenomenon showed significantly higher incidence of leukotrichia (P value: .023). In few patients regression of leukotrichia was observed and hairs regained pigmentation under the influence of nb-UVB phototherapy and/or topical calcineurin inhibitors.

Conclusion: Although leukotrichia in vitiligo is considered an ominous sign denoting depletion of follicular stem cells, it is quite common, more so in stable than active vitiligo and its distribution is likely to differ among various types and to influence disease progression. Both vellus and scalp leukotrichia occur more frequently in generalized symmetrical vitiligo. The phenomenon of repigmenting leukotrichia should be attended to meticulously and needs further evaluation and investigation.

P.137 | Analysis of repigmentation in vitiligo using the mouse model with rhododenol-induced leukoderma (RIL)

Yuko Abe; Yutaka Hozumi; Ken Okamura; Tamio Suzuki

Faculty of Medicine, Yamagata University, Yamagata, Japan

We have established and characterized the mouse model with RIL, which was topically applied Rhododenol® (RD) solution on the skin of hairless hk14-SCF transgenic (Tg) mice (Abe Y et al. J Dermatol Sci, 2016). The histological characteristics were closely similar to vitiligo.
In order to search effective treatments for vitiligo, we investigated methods to promote repigmentation using the RIL mouse model. And also, we found a few white spots remained in repigmented lesions after the treatment. Then, we investigated the reason why melanogenesis would not occur in these white spots.

We tested tacrolimus and vitamin D3. These ointments were topically applied on the back of the RIL mouse model respectively. We failed to find any effect for repigmentation on the back applied with tacrolimus ointment. On the other hand, apparent promotion effect was found with vitamin D3 ointment. This effect was increased by a combination with UVB radiation. Furthermore, we found that expression of *c-kit* was increased in vitamin D3 treatment site compared with control by immunohistochemical and qRT-PCR methods.

In some of white spots, the number of melanocytes and an expression of tyrosinase were found to be similar to those in repigmented lesions, however, melanocytes in the white spots revealed negative for the dopa reaction, indicating some troubles in tyrosinase synthesis or maturation.
These results suggested that the RIL mouse model can be good tools to investigate the pathomechanism and establish new treatments for vitiligo.

P.140 | Modulation of ion transporter proteins may regulate pigmentation

JàNay K. W. Cooper[1]; Toral Vaidya[2]; Tomohiro Hakozaki[3]; Ana Lusia Kadekaro[2]; Raymond E. Boissy[2]

[1]James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH, USA; [2]Department of Dermatology, University of Cincinnati College of Medicine, Cincinnati, OH, USA; [3]Procter & Gamble Company, Cincinnati, OH, USA

Background/Objective: Galactomyces ferment filtrate (GFF, Pitera™) is a yeast derived extract currently used as a moisturizing agent in cosmetics. GFF demonstrates anti-aging and hypopigmenting effects on skin within 14 days. The mechanisms of action underlying GFF are relatively unknown and therefore are the focus of our study.
Methods: In vitro human pigment cell models, including foreskin derived normal human epidermal melanocytes (NHEM) and human melanoma (SK MEL), were treated with or without GFF 0-10%. Melanin content was quantified by spectrophotometric assay at 490 nm. NHEM dendrites were evaluated by electron microscopy. Tyrosine hydroxylase activity was determined with tritiated tyrosine and assayed by liquid scintillation. RNA-seq transcriptome profiling was completed with RNA extracted from NHEM. Protein expression was quantitated by Western Blot and densitometric analyses. Localization of ion transporter and melanogenic proteins were assessed by indirect immunofluorescent microscopy.
Results: GFF suppressed constitutive pigmentation in NHEM and SK MEL and reduced tyrosine hydroxylase activity in NHEM and protein lysate. GFF did not significantly alter the RNA nor protein levels of melanogenic proteins required for melanin synthesis (MITF, PMEL, TYR, TYRP1, TYRP2/DCT). Notably, GFF did modify the RNA expression of ion transporters including V-ATPases and H^+, Na^+, K^+, Ca^{2+}, Cl^- specific pumps, carriers, and channels known to affect both intracellular and organelle pH.
Conclusions: GFF inhibits melanin synthesis by deterring tyrosine hydroxylase activity. The new working hypothesis is that GFF significantly alters the cisternal environment of the melanosome counter to optimal tyrosine hydroxylase activity.