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**MEETING REPORT - CONJOINT MEETING OF XX<sup>th</sup> INTERNATIONAL PIGMENT CELL CONFERENCE (IPCC) & V<sup>th</sup> INTERNATIONAL MELANOMA RESEARCH CONGRESS (IMRC) - SAPPORO, JAPAN, MAY 7-12, 2008**

**ES-02 Melanoma prevention and chemotherapy**

**Chairs: Arthur J. Sober and Michael Smylie**

*By Prashiela Manga*

The first presentation of Early Session 2, by Arthur Sober, focused on screening and early detection of melanoma. Improved education of clinicians and new diagnostic aids have resulted in earlier recognition and detection of cutaneous melanoma. New technologies include dermoscopy, digital photography, machine aided visualization and confocal microscopy. For example, Melafind, a hand-held device that emits light and composes images from reflected light, was found to improve sensitivity by 27% (physician sensitivity 71% versus MelaFind 98%). As a result, there has been an increase in identification of smaller, less invasive lesions, although there has been some controversy as to whether the rise is simply due to increased excision of lesions. Screening programs have also been shown to contribute to increased detection rates. For example, a screening program supported by the American Academy of Dermatology performed over 1.8 million screens that resulted in the detection of over 180,000 “suspicious lesions” and over 20,000 “suspected melanomas”.

The second talk, by Michael Smylie covered the use of chemotherapies in the treatment of melanoma. While excision of early stage lesions is a highly effective treatment and prognosis is good for patients, success rates with metastatic disease is by far inferior. Median survival is six to seven months, and five year survival less than 5%. Chemotherapy, while relatively ineffective, has been the standard of care with the single agent Dacarbazine (10-20% response rate). Temozolamide showed about 15-20% efficacy, while several combination therapies, that include Dacarbazine, demonstrate higher response rates, but appear to provide no increase in overall survival and resulting in increased toxicity. Combination with cytokines such as interleukin-2 and interferon have also been tested, however survival data have not been confirmed. Thus melanoma remains refractory to current chemotherapy modalities.

**Keynote Addresses**

*By Zalfa Abdel-Malek*

The Conjoint Meeting of XX<sup>th</sup> International Pigment Cell Conference and the V<sup>th</sup> International Melanoma Research Congress was held in Sapporo Japan, on May 7-12, 2008. The opening session included 4 keynote presentations that were given by Zalfa Abdel-Malek, the IFPCS President, David Fisher, the SMR President, Martin Mihm, who delivered the Thomas B. Fitzpatrick Memorial Lecture, and Kowichi Jimbow, the Meeting organizer.

Professor Abdel-Malek presented on the control of epidermal human melanocyte survival and function by the cutaneous microenvironment. She emphasized the importance of regulation of human melanocyte survival, as reduced survival leads to vitiligo, and genetic mutations in survival factors or their receptors, such as in ET-3 endothelin-B receptor, or c-Kit, result in lack of migration of melanoblasts during embryo development, leading to Hirschprung’s disease and piebaldism, respectively. She reviewed the current evidence for paracrine regulation of

melanocytes, and stimulation of production of paracrine factors by UV radiation. She also provided recent evidence from her laboratory and that of Glynis Scott that melanocytes produce autocrine factors, exemplified by PGE2. Dr. Abdel-Malek then summarized data from her own laboratory showing that in addition to stimulation of melanogenesis and proliferation, alpha-MSH and endothelin-1 (ET-1) also function as survival factors that rescue melanocytes from UV-induced apoptosis, increase repair of DNA photoproducts and reduce generation of reactive oxygen species, effects that inhibit UV-induced genotoxicity. She showed that the survival effect of ET-1 is mediated by increasing the phosphorylation of ERK1/2, which is augmented in the presence of alpha-MSH, and Akt, which is activated by both ET-1 and alpha-MSH. Both kinases lead to phosphorylation of CREB, and Mitf, which in turn increases the expression of the anti-apoptotic Bcl2. She ended by presenting on the regulation of MC1R expression in human melanocytes, namely up regulation of MC1R mRNA levels and cell surface expression by alpha-MSH and ET-1, and down regulation by the MC1R antagonist agouti signaling protein.

The second keynote speaker was Professor David Fisher, who surprised the audience by not presenting on melanocyte signaling in skin and skin cancer, but rather on the detrimental effects of indoor tanning, and the importance of calling for stringent regulation of the use of tanning beds. He emphasized that the melanoma and pigment cell research societies have a responsibility to the public to plead for such regulations. He gave alarming statistics about the usage of tanning booths, especially by young women, between the ages of 16-29, and the frequent visitors to tanning parlors (30,000,000 people/year). He correlated this with melanoma being the leading cause of death of women 25-30 years of age, and the enormous profit of the tanning industry (\$5 billion/year). He emphasized that the UV range used in tanning booths is carcinogenic, and showed that several studies concluded that the use of tanning booths before the age of 35 is associated with 75% elevated risk for melanoma. Importantly, he presented on how the tanning industry advertises the benefits of UV radiation, and how it misinterprets scientific data. "Tanning does not cause cancer", is one example of false advertisement, and the usefulness of UV exposure to prevent Vitamin D deficiency, is another. The reality is that dietary vitamin D is as good as that synthesized in the skin in response to UV exposure. Dr. Fisher called the meeting participants to sign a petition to be sent to the FDA, and emphasized that the scientific body present at this meeting has a responsibility in sending a strong message that there are no safe UV rays, and demanding regulations to discourage the use of indoor tanning devices, and showing that they are as harmful as outdoor tanning. A petition was distributed at the end of this presentation to be signed by participants, and the signed forms were collected to be sent to the FDA by Dr. Fisher.

Professor Martin Mihm presented on malignant melanoma, the sentinel lymph node and the metastatic phenomenon. He reviewed evidence supporting the hypothesis that primary tumors produce immunosuppressing agents, since sentinel node represents an immune tolerant environment, and the seed and soil hypothesis, since tumor cells prepare premetastatic sites, e.g. by enhancing the secretion of fibronectin by stromal cells, and production of VEGF by endothelial cells to provide vasculature to the tumors. Through recruitment of stem cells into potential metastatic site, tumors prepare the niche to which they will spread. He also described that this premetastatic niche can be interrupted at different stages. He also described the anatomical changes in the human lymph nodes, exemplified by reduction in dendritic cells with antigen presenting configuration, and also a marked decrease in paracortical T-cells. Dr. Mihm stated several challenges:

- 1) Understanding the sentinel lymph node immune dysregulation to enhance anti-tumoral immune responsiveness in patients (e.g. to GM-CSF).
- 2) Reducing tolerogenic milieu in the sentinel node.
- 3) Discovering the premetastatic niche to block the arrival of tumor cells in lymph nodes.
- 4) Discovering how to eradicate the minimal tumor burden that is immune resistant.
- 5) Better understanding of the role of stem cells in metastasis.

Professor Kowichi Jimbow ended this session by presenting on translational research strategy based on using melanogenesis and nanomedicine for melanoma-targeted drug delivery system and chemo-thermo-immunotherapy. Dr. Jimbow introduced his talk by describing how melanin biosynthesis, a biological property unique to melanocytes, and highly active in most melanoma tumors, can be exploited to eradicate melanoma tumors. His research group has previously reported that NPrCAP is a good tyrosinase substrate, which is selectively incorporated in melanoma cells and inhibits their growth *in vitro* and *in vivo*. The mechanism of action of NPrCAP involves interacting with an unidentified receptor presumed to be expressed on the melanoma cell surface, and generating oxidative stress when exposed to tyrosinase, resulting in melanoma cell disintegration. This strategy, however, proved to be useful in melanotic, but not amelanotic melanoma tumors. Based on these data, magnetite nanoparticles were conjugated with NPrCAP, and by exposure to alternating magnetic field, there was selective accumulation of this agent in melanosomes, and generation of HSP 70/90 peptide complex, which resulted in necrotic cell death, and prevention of secondary melanoma formation, evidenced by rejection of melanoma rechallenge. This strategy is now used in clinical phase I/II study, and is showing impressive regression of cutaneous metastatic lesion in some stage III-IV patients.

**PS-01 Structural and functional aspects of melanin pigmentation; role of melanin pigmentation in biological behaviour and development**

**PS-02: Hormonal regulation of melanin pigmentation; Function of proopiomelanocortin and melanocortin.**

**Chairs: José-Carlos García-Borrón, Hsin-Su Yu, Noriko Oshima**

*By José-Carlos García-Borrón*

The first conjoined plenary sessions of the 20<sup>th</sup> IPCC and 5<sup>th</sup> IMRC Meeting were entitled “Structural and functional aspects of melanin pigmentation; role of melanin pigmentation in biological behaviour and development” and “Hormonal regulation of melanin pigmentation: function of proopiomelanocortin and melanocortin”. The session was chaired by Drs. Hsin-Su Yu, Noriko Oshima and José-Carlos García-Borrón. Three lectures were presented.

The first talk was delivered by Dr. Hiroaki Yamamoto, and its title was in fact a relevant question whose answer is actively sought by several groups: “What’s the role of melanin pigmentation in visual and auditory senses?” The presence of melanins in extracutaneous locations is indeed a mystery. The main functions of cutaneous melanin pigments are related to location their near the body surface: camouflage and communication between males and females is important in many vertebrates, but in man the main functions are related with protection against UV radiation and radical scavenging. Obviously those functions cannot be extrapolated to the melanins present in the visual and hearing systems. Dr. Yamamoto discussed this issue and presented converging evidence from these two sensory systems pointing to major role for the melanocyte itself as opposed to the melanin pigments. The pigmented cells of the retinal pigmented epithelium form a monolayer of pigmented cells that absorb the light energy impinging the retina and play a key role in the maintenance of photoreceptor excitability. A series of elegant experiments relying on the ablation with lasers of specific pigment cells in tadpoles and assessment of their swimming behavior have demonstrated that the integrity of the eye melanogenic system is essential for correct swimming patterns. Moreover, knockout of the tyrosinase gene impairs the swimming behavior, but not the photic responses of tadpoles, thus indicating that albino animals retain visual ability. On the other hand, photic responses have been assessed by means of electroretinogram recordings in mouse models where specific mutations affecting melanocyte development or function are associated with impaired pigmentation in the RPE. These experiments show that whereas the presence of melanocytes is required for normal responses to light stimuli, pigment itself is dispensable although it may play ancillary roles, probably by providing stress relief mechanisms. These findings suggest that

melanocytes may have functions that not necessarily require pigment production. Further evidence supporting this hypothesis comes from studies of the hearing system, whose function can be analyzed by recording auditory brain stem potentials. Dr. Yamamoto described the location of melanogenically active cells in the stria vascularis, and the finding of hyperpigmentation following exposure to noise. Concerning the function of inner ear melanocytes, these cells are essential for hearing acuity and, moreover, mutations leading to reduced or absent pigment production accelerate the onset of age-related hearing losses. These observations suggest a protective role of the inner ear pigmentary system, whose mechanisms of response to different stresses remain unknown. Interestingly, melanocytes in the stria vascularis express anti-oxidant enzymes, suggesting a detoxification function. In summary, melanocytes do certainly possess pigment-unrelated functions particularly important for the correct development and maintenance of sensory systems. During the discussion of the paper, questions were raised concerning possible differences in pigment structure in cutaneous versus extracutaneous locations, as well as the possible pigment-independent roles of the melanocytes as related to the production of signalling molecules.

The second lecture, presented by Dr. García-Borrón, was entitled “Functional analysis of mahogunin RING finger-1 isoforms”. Dr. García-Borrón’s lab is currently investigating the regulation of melanocortin 1 receptor (MC1R) signalling and the functional differences of the wild type receptor and its natural mutants associated with the red hair phenotype. MC1R signalling is crucial for melanocyte proliferation, differentiation and ultraviolet radiation-induced tanning. MC1R acts as the exclusive effector of melanocortin signalling in epidermal melanocytes, by coupling agonist binding to Gs protein activation and cAMP production. Genetic studies have identified several genes that modulate mouse *Mc1r* signalling. One of them, Mahogunin Ring Finger-1 (*Mgrn1*) encodes a RING domain-containing ubiquitin ligase. Previous work by G. Barsh and T. Gunn has shown that loss-of-function mutations of *Mgrn1* cause a complex phenotype characterized by dark pigmentation similar to *Mc1r* gain-of-function mutations, suggesting that *Mgrn1* is a negative regulator of *Mc1r* signalling. Moreover, mutant mice present serious defects in heart development, a high embryonic lethality and spongiform neurodegeneration. Dr. García-Borrón discussed human MGRN1 isoform distribution and function. Human melanoma cells express 4 MGRN1 isoforms, similar to mouse cells. These isoforms differ in the length of the terminal exon 17, and in the usage of exon 12. This exon contains canonical nuclear localization signals, but when expressed alone MGRN1 isoforms localized to the cytosol, thus showing that the nuclear localization signals in exon 12 are cryptic. Upon co-expression with MC1R in HEK293T cells, all MGRN1 isoforms physically interacted with MC1R as shown by co-immunoprecipitation. MGRNs inhibited MC1R or MC4R receptor signalling, but not cAMP generation following activation of a beta-adrenergic receptor, suggesting that inhibition of receptor function might be restricted to the melanocortin receptor family or other highly related GPCRs. In addition, cAMP generation induced by forskolin or by a constitutively active mutant of the Gs protein remained unaltered in cells expressing the MGRNs. No evidence of MC1R ubiquitination was obtained, either in heterologous systems or in human melanoma cells, and the inhibitory effect on receptor signalling was also observed for a functional ubiquitination-null MC1R mutant obtained by replacing all intracellular Lys residues by Arg. These findings were discussed in terms of a potential mechanism of action of MGRN. Moreover, the cryptic nuclear localization signals in MGRN1 and MGRN2 became active upon co-expression with wild type MC1R, but not upon activation of adenylyl cyclase. This effect was specific for the melanocortin receptor family and was not detected for distantly related GPCRs such as a thromboxane receptor or a beta-adrenergic receptor. The MCR-mediated nuclear targeting of the MGRN suggested the occurrence of nuclear MGRN partners, a possibility that is currently under study. When the paper was open for discussion, the interesting possibility of MGRN effects in organs different from the skin with clinical implications was raised. A differential effect of the MGRNs on wild type and variant MC1R was also considered.

The last lecture in the session, entitled “MC1R dependent human pigmentation responses”, was delivered by Dr. Richard Sturm. Sturm’s lab is actively involved in the study of the genes controlling human skin, hair and eye pigmentation. The talk focused on the phenotypic and functional effects of natural mutations in the MC1R gene expressed in melanocytes. Although marginal expression of the MC1R gene can be detected in a variety of cell types, Dr. Sturm proposed that its physiological actions are basically restricted to the melanocytes. Some of the MC1R natural variants are associated with the so-called red hair colour (RHC) phenotype characterized by red hair, fair skin, poor tanning response, high UV radiation sensitivity and increased skin cancer risk. Dr. Sturm presented the 9 more common variants and discussed their frequency and association with red hair, fair skin and freckling. The variant alleles show significant associations with red hair and freckling, and for some alleles a clear heterozygote effect is detected. On the other hand, there is no clear association of these variants with eye color. In addition to the genetic studies, Dr. Sturm presented data on the functional analysis of the variant alleles. These tend to behave as partial loss of function forms in coupling to the cAMP signalling pathway, but the degrees of functional impairment are different. Loss of function is stronger for I155T and D294H, and intermediate for other variants except for the low penetrance V92M form which seems at least as active as the wild type. The cellular distribution of the variants was also discussed and evidence was presented demonstrating reduced cell surface expression for most of the mutant alleles. Nevertheless, high levels of plasma membrane expression were found for V92M, R142H and D294H. In any case, intracellular retention leading to reduced cell surface expression appeared as a frequent cause of MC1R loss of function, and hence of the RHC phenotype. Moreover, since the MC1R forms dimers, the presence of one mutant monomeric unit may impair the trafficking of wild type monomers, thus accounting for dominant negative effects. The final part of the talk was devoted to describing the MC1R-dependent pigmentation responses in melanocyte/keratinocyte co-cultures. It was shown that MC1R activation leads to higher inductions for DCT than for other melanogenic proteins. This finding was discussed in terms of the relative sensitivity to UV of melanocytes wild type for the MC1R as opposed to melanocytes harboring mutant alleles. The talk was followed by a stimulating and lively discussion on the physiological relevance of the MC1R in non-melanocytic cells.

### **LS-01 Effect of endothelin and stem cell factor on melanocytes and their involvements in skin pigmentation**

**Chair: Shigeki Shibahara**

*By Shigeki Shibahara*

The Luncheon Seminar, sponsored by Kao Corporation, focused on the new findings of the two essential regulators in skin pigmentation, endothelin (ET)-1 and stem cell factor (SCF). Genji Imokawa, who just moved to Tokyo University of Technology, School of Bionics, from Kao Corporation, has been working on the roles of ET-1 and its intercellular signaling in human melanocytes. Imokawa showed that ET-1 or SCF stimulated the phosphorylation of MITF in cultured melanocytes, which was accompanied by the phosphorylation of ERK1/2. The phosphorylation of MITF was completely abolished by a MEK inhibitor PD98059 or by a PKC inhibitor Go 6983, but not by a PKA inhibitor H89 or a p38 MAP kinase inhibitor SB 203580. Moreover, ET1 induced the expression of melanocyte-specific MITF (MITF-M) mRNA at 40-120 min post-incubation with ET-1, which was followed by increased expression of MITF-M protein with a peak at 2-3 h post-incubation. The increase in MITF protein levels was diminished in the presence of a PKA or MEK inhibitor. Moreover, CREB phosphorylation, which leads to MITF expression, was elicited at 5-15 min during ET-1 signaling, but was partially abolished by a MEK or PKA inhibitor. The inhibitory effect of MEK inhibitor was stronger than that of PKA inhibitor in an early phase of ET-1 signaling. Thus, ET-1 appears to induce expression of MITF protein via both cAMP-PKA and MAPK-RSK

signaling pathways. On the other hand, the stimulatory effects of SCF are associated with MAPK-RSK linkage. Imokawa has identified the signaling pathways regulating MITF expression, each of which could be a target for anti-melanogenic agents.

The signaling of SCF and its receptor KIT (membrane bound KIT; m-KIT) plays an important role in melanocyte development, survival, proliferation and melanogenesis. A soluble form of m-KIT (s-KIT) is released from cell surface, but the biological role of s-KIT remains unknown in melanocytes. Ohuchi showed that treatment with 4-aminophenylmercuric acetate (APMA) induced s-KIT production in cultured human melanocytes, which in turn abolished the SCF-induced melanogenesis. Human recombinant s-KIT added to melanocytes inhibited SCF-induced phosphorylation of m-KIT, resulting in suppression of the SCF-induced melanogenesis. Thus, production of s-KIT may be involved in the regulation of human skin pigmentation. Moreover, topical applications of Alkyl-Methoxy-Chromone (AM-Chromone) significantly reduced pigmentation and pigmentation area in lentigo senilis. AM-Chromone specifically suppresses SCF-induced cell proliferation and the differentiation of cultured human melanocytes. Ohuchi has proposed that blocking SCF signaling may be a useful means for enhancing the efficacy of skin-whitening agents.

**CS-01: Chemistry and biophysics of melanin**  
**Chairs: Alessandra Napolitano, Tadeusz Sarna**  
*By Kazumasa Wakamatsu*

Alessandra Napolitano of University of Naples reviewed the knowledge of eumelanin and pheomelanin that has rapidly accumulated over the past few years based on the certain features and properties of melanins relating to their functional significance. This presentation aimed at providing an overview of the latest advances in the structural investigation of eumelanins and pheomelanins.

Tadeusz Sarna of Jagiellonian University summarized the comparison of photoreactivity of eumelanins and pheomelanins and analysis of their spin polarization phenomena. He showed the possibility that the spin photochemistry of melanin determines its observable photoreactivity.

Sunil Kalia of the University of British Columbia presented his study on *in vitro* and *in vivo* melanin analysis via near infrared fluorescence (NIR). NIR autofluorescence of melanin *in vitro* and *in vivo* is directly correlated with melanin content up to a certain concentration, and can be used to some extent to quantify melanin *in vivo*.

Kazumasa Wakamatsu of Fujita Health University presented the reexamination of microanalytical methods for melanin assay. The  $K_2CO_3/H_2O_2$  method has advantage of characterizing eumelanin and pheomelanin simultaneously. With this method, some markers can be used to evaluate levels and ratios of monomer units in various types of melanin pigments.

The last lecture was presented by Dzeneta Nezirevic of Linköping University. She presented GC-MS analysis of chemical degradation products of pheomelanin with HI hydrolysis from the urine of a patient with advanced melanoma and synthetic pheomelanin. The findings of AHPs together with benzothiazinone and two other benzothiazole compounds strongly suggest the incorporation of heterocyclic pheomelanin-type units in the pigment structures.

**CS-03 Animal and experimental models of pigmentary diseases and melanoma****Chairs: Richard A. Spritz and Christine Duval***By Richard Spritz**CS3-1 The genetics of generalized vitiligo; Richard Spritz (University of Colorado Denver, CO, USA)*

Dr. Spritz reviewed the current status of vitiligo genetics, noting that the lack of validated, truly analogous animal models has limited progress on understanding human vitiligo. Vitiligo typically occurs sporadically, with family clustering indicating multifactorial, polygenic causation involving both multiple genes and environmental triggers. Vitiligo shows strong epidemiological association with other autoimmune disorders, especially autoimmune thyroid disease.

Studies of biological candidate genes have been disappointing, most reports based on limited experimental and statistical support and later being refuted. Considering all published candidate gene studies, there currently is fairly consistent evidence for a weak effect of HLA or other loci in the MHC, and strong, consistent evidence for involvement of *PTPN22*; together these findings support a role for autoimmunity in the pathogenesis of vitiligo. Initial reports also indicated involvement of *CTLA4*, but recent studies and a meta-analysis do not support this. In contrast, genome-wide linkage studies are not based on a priori biological hypotheses. Analysis of a single large family localized a gene on chromosome 1p, which appears to represent a transcriptional activating mutation of *FOXD3* in this unique family. Analysis of many additional families localized potential genes on chromosomes 7, 9, and 17p in Caucasian families, and chromosomes 4q, 6, and 22q in Han Chinese. Detailed analysis led to identification of the 17p gene as *NALP1*, a key regulator of the innate immune system that may mediate the inflammatory pathway and apoptosis in response to bacterial pathogens and UV exposure. Positional candidate genes for the chromosome 7 and 9 linkages have also been identified though not yet proven. Similarly, analysis of a large pedigree based on an inbred Romanian village has identified a possible recessive vitiligo susceptibility gene on chromosome 6q.

Major future progress in vitiligo genetics research may come from VitGene, a worldwide consortium of 30 investigators in 13 countries that Dr. Spritz has organized to carry out a genome-wide association study (GWAS) of vitiligo, first in Caucasians and subsequently in patient groups from around the world. These studies offer powerful new approach to identify genes with both large and small effects on disease susceptibility.

*CS3-2 In vitro organotypic models to study skin pigmentation; Christine Duval (L'Oreal Recherche, France)*

Dr. Duval described skin engineering methods aimed at developing a system that would improve on current melanocyte (MC)-keratinocyte (KC) co-cultivation approaches at reproducing the so-called melanin-epidermal unit. Efforts have focused on developing reconstituted epidermis that recapitulate *in vivo* 3-dimensional skin architecture, reproducing ethnic-specific skin pigmentary properties, allow assessment of pharmacological depigmenting agents (especially topical), and allow study of UV-induced hyperpigmentation.

This was accomplished by dispersing cells taken from skin biopsy. Dermal fibroblasts were seeded on collagen, serving as a bed for subsequent seeding of epidermal KC and epidermal MC. After 1 week in submerged culture, the rafts were then elevated and propagated at the air-liquid interface. Initial analyses showed that the resultant "skin" has good histological morphology, with apparent positive DOPA-staining, but negative Fontana-Masson staining, indicating a lack of melanin. To induce melanogenesis, the rafts were first cultured with SCF, with ET-3, with SCF + ET-3, and with bFGF, but without positive result. As an alternative, the rafts were propagated in

KGF-based media, resulting in production of dendritic MC with positive Fontana-Masson staining. Histologic section showed correct localization of cell types, with MCs containing functional melanosomes expressing melanogenic proteins and containing melanin, and these melanosomes are transferred to KCs (resulting in intracellular clusters).

Analysis of rafts derived from MC donors of differing pigmentation phenotypes grossly recapitulate the donor's pigmentation phenotype, with a gradient of pigmentation from light to dark. Functionality of the rafts was assessed by response to external stimuli. 50 nM alpha-MSH made the rafts darker, with MCs exhibiting greater DOPA-staining, increased tyrosinase, and increased melanin content. Exposure of the rafts to 40 microM forskolin (albeit for a longer period than MSH) resulted in even darker color, DOPA-positivity than did MSH. Repeated UV exposure increased MC density and dendricity, and increased pigmentation. These findings provide new opportunity to study the physiology of melanogenesis in a skin model system.

*CS3-3 Inhibition of MEK with AZD6244 is cytostatic as a monotherapy in melanoma, but cytotoxic when combined with docetaxel leading to tumor regression; Nikolas Haass (Centenary Institute of Cancer Medicine and Cell Biology, Sydney, Australia)*

This study addresses anti-melanoma activity of the MEK inhibitor AXD6244 (ARRY-142886). *In vivo*, most melanomas have mutations of the MAPK pathway: ~4% KIT, ~15% NRAS, ~66% BRAF, ~5% CDK4, in most cases resulting in constitutive MAPK activity and constitutively active ERK (regardless of BRAF status). This study used BRAF-V600E melanoma cells in a 3-dimensional melanoma spheroid model, derived by plating melanoma cells in 1.5% collagen matrix, harvest of spheroids, and replating in 0.3% collagen matrix. The spheroids show local invasiveness into the surrounding collagen, though ERK was active only towards the edge of the spheroid (however, one questioner asked whether this might be a staining artifact). AZD6244 was found to inhibit melanoma cell growth via reversible G1 cell cycle arrest, suppressing tumor growth and reducing pERK both *in vitro* and *in vivo* in mice. Likewise, AZD6244 had a similar effect on angiogenesis *in vitro*. Docetaxel enhances the antitumor activity of AZD6244 *in vitro* and in mice results in enhanced tumor regression (dosage 15 mg/kg). A phase 2 trial in several hundred patients with advanced melanoma is about to begin (AstraZeneca). The mechanism of action of AZD6244 is unknown.

*CS3-4 Induction of melanoma in mice: the role of UVR-induced melanocyte proliferation and migration; Graeme Walker (Queensland Institute of Medical Research, Australia)*

Neonatal UV exposure is essentially necessary for melanoma induction in mice, inducing MC proliferation and migration to the basal level of the epidermis. 5 days after neonatal unilateral UVR, hair growth is retarded on the irradiated side and migration of MCs occurs into the basal level of the epidermis on the irradiated side. Proliferation peaks 3-5 days after a single dose of UVR at 27% (versus <1% in skin of adult mice), and continues for 2 weeks or longer. In an NRAS-Q61K mutant RAS the rate of MC migration after UVR was increased, while CDK4 mutation does not affect the basal rate of MC migration. Differential UVR shows that proliferation/migration induction is mediated by UVB, with no effect of UVA; likewise, UVB and not UVA mediates induction of melanoma in mice. The specific mode of action of UVB is unclear; pyrimidine dimers are efficiently removed by the irradiated mice. The site of the proliferating/migrating MCs is not entirely clear. Most seem to be derived from the outer root sheath, though some contribution from basilar MCs cannot be excluded. It was somewhat problematic that the specific genotypes of the mice used in this study were not given.



*CS3-5 Fish models for human melanoma research; Svenja Meierjohann (Biocenter, University of Wuerzburg, Germany)*

Four fish models for study of melanoma were discussed, involving the same signaling pathways that are aberrant in human melanoma. The first model was zebrafish; zebrafish expressing mitfa-BRAFV600E develop melanoma. The second model was a naturally-occurring melanoma line of *Xiphophorus*. Positional cloning identified the causal genes as XMRK, encoding the orthologue of the human EGF receptor, with an activating mutation, C578S. However, the *Xiphophorus* genome has not been sequenced, and these fish are live-bearing, limiting opportunity for genetic engineering. Therefore, for the third model, *Xiphophorus* XMRK was engineered into *Oryziakia latipes* (Japanese Medaka fish), which does not contain this gene normally, under control of the MITF promoter. This resulted in two types of tumors: a) epidermal exophytic chromatophoroma (mixed cell type) and b) extracutaneous invasive melanoma. The XMRK transgene was then crossed onto different Medaka backgrounds. On the “carbio” background (which has reduced pigment cells), principally epidermal exophytic chromatophoromas were seen. On the HB32C background principally extracutaneous invasive melanoma were seen, often arising from the gut. Western blots showed that both *Xiphophorus* and Medaka melanomas expressed elevated XMRK (highest in the invasive melanoma versus the chromatophoromas), elevated PH20, elevated p-MAPK, and elevated p-STAT5 (this last only in the invasive, malignant melanoma), but no apparent change in p-ERK. For the fourth model, the XMRK Medaka was crossed onto p53 mutant Medaka. XMRK-malignant X p53<sup>-/-</sup> mice showed early onset melanoma with much greater proliferation of malignant cells.

### **CS-05 Stress responses and cell signaling in melanocytes and melanoma**

**Chairs: Richard Marais and Stéphane Commo**

*By Caroline Le Poole*

This session was chaired by Drs. Richard Marais from the UK and Stéphane Commo from France. The first lecture by Dr. Marais discussed the importance of BRAF for melanoma genesis. Based on the observation that BRAF mutations are detectable in a large proportion of human melanomas, this group has developed a mouse model where oncogenic BRAF is expressed in melanocytes by driving inducible Cre from a melanocyte-specific promoter. Induction by 4-OHT induces hyperpigmentation stretching beyond areas of original 4-OHT application. Mutant BRAF lesions are stable and do not progress to melanoma without additional cellular changes, such as p16 mutations. The lesions look like benign human nevi. P16 mutations can accelerate the development of full-blown melanoma in about 9 months. This is a great model to study BRAF in melanoma.

In the 2<sup>nd</sup> lecture, Dr. Commo highlighted TRP-2 and its role outside the melanogenic pathway. TRP-2 is not expressed in human hair follicles and is thus dispensable for pigmentation, yet the dopachrome tautomerase function may be important for protecting melanocytes from oxidative stress. In particular, TRP-2 reduces the sensitivity of melanocytes (not: keratinocytes) to H<sub>2</sub>O<sub>2</sub>, hydroquinone and dopamine, as well as to paraquat. Such TRP-2 mediated functions were disrupted in response to site directed mutagenesis. The authors stressed the importance of extramelanosomal TRP-2 expression for its role in antioxidant protection.

Dr. Zhang next addressed the importance of Mcl-1 in melanocyte survival after ER stress. In addition to Mcl-1, ER stress elevated PUMA and Noxa levels in the cell by both p53 dependent and independent mechanisms. The protection offered by Mcl-1 could be reversed by inhibiting PUMA and Noxa-1. ER stress induced apoptosis after Mcl-1 inhibition in melanoma cells may be exploitable for melanoma treatment.

Dr. Le Poole reported the expression of heme oxygenases -1 and -2 by human melanocytes. Both isoforms are involved in heme metabolism, monitor oxygen levels and help counter oxidative stress within the skin. The expression of HO-1 is induced by stress following exposure to 4-TBP or to ultraviolet light, whereas expression of HO-2 is simultaneously downregulated. Both genes were cloned from human cDNA and introduced into mammalian expression vectors to study their protective roles against UV-mediated cell damage and death. The authors speculated on the role of HO-1 overexpression for discriminating responders from non-responders to UV treatment in vitiligo.

Finally, Dr. Abdel-Malek discussed MC1R allelic variants. Melanocyte cultures homozygous or compound heterozygote for R151C, R160W or D294H were refractory to alpha-MSH and sensitive to UVB compared to wildtype. Several receptor variants are associated with melanoma and skin cancer risk, with CDK2a and BRAF involved in penetrance. The involvement of receptor variants on melanoma risk was studied using agouti analogs ASIPYY and by transfecting wildtype MC1R. Differential responses to UV or alpha-MSH as mediated by wildtype versus variant MC1R were studied by microarray analysis. A functional MC1R is required to observe responses to alpha-MSH antagonizing responses to UV. Curiously, DCT (TRP-2) is upregulated in response to either treatment.

#### **CS-06 Development and differentiation of pigment cells**

**Chairs: Kyoung-Chan Park and Emi Nishimura**

*By Tom Hornyak*

Concurrent Session 6, devoted to the “Development and differentiation of pigment cells”, was held late Thursday afternoon, May 8, 2008, in the Empress Room of the Royton Sapporo Hotel. This session was chaired by Kyoung-Chan Park of Seoul National University, Korea, and Emi Nishimura of Kanazawa University, Japan.

The first talk, entitled “Hypopigmenting strategies based on signal regulation of melanogenesis”, was delivered by Dr. Park. His talk began with the presentation of a striking clinical image, showing hypopigmentation of the region of a Mongolian spot surrounding a superimposed café-au-lait patch on the buttocks. This observation suggests that signaling factors associated with the café-au-lait patch induced hypopigmentation of the nevus. Dr. Park referenced the review by Solano et al., *Pigment Cell Res.* 19, 550 (2006), describing the two classes of agents shown to have biological activity as depigmenting agents, biologic agents and chemical agents. His data included a comparison of the activities of hydroquinone, a direct tyrosinase inhibitor and the “gold standard” of depigmenting agents that is available clinically, and sphingosine-1-phosphate (SIP), not a direct tyrosinase inhibitor and unavailable clinically, which was found previously (Kim et al., *J Cell Sci.* 116, 1699-1706 (2003)) to inhibit melanogenesis in the highly melanogenic, spontaneously immortalized mouse melanocyte line Mel-Ab. SIP was found to induce ERK phosphorylation, an effect possibly linked to its effects upon melanogenesis, and it was also found to protect cells against UVB-induced melanocyte cell death. Two other strategies were described to downregulate melanogenesis. One involved the use of terrein, a fungal extract, which, on its own, does not inhibit tyrosinase activity in a cell-free system while activating ERK and inhibiting MITF phosphorylation. In combination with KI-063, a resorcinol derivative and a tyrosinase inhibitor, it exhibits additive effects. Heat treatment, which activates the p38 MAP kinase pathway, in combination with p38 MAP kinase inhibition was also mentioned as a potential hypopigmenting strategy.

Dr. Nishimura spoke about the “Role of transforming growth factor beta (TGF-beta) in melanocyte stem cell (MSC) renewal”, alternatively titled “Role of stem cell niche-derived TGF-beta in MSC renewal”. She reviewed previous work that has characterized *Dct-lacZ* cells in the bulge area of the murine follicle as slow-cycling, immature cells that demonstrate self-renewal and produce differentiated progeny, all characteristics of stem cells. The focus of this presentation was to describe molecular mechanisms, especially those contributed by the niche, the surrounding follicular and extrafollicular environment of the MSC, that maintain stem cell dormancy. The importance of Bcl-2 at the early anagen stage upon initial MSC division was described. A consideration of candidate factors released by niche cells that may regulate the behavior of MSCs at this stage led to TGF-beta, which is expressed by hair follicle bulge region keratinocytes at stage 7-8. Smad2, a TGF-beta signal transducer, was also expressed by *Dct-lacZ* melanocytes at a similar hair follicle stage. In addition, treatment of melanocytes with TGF-beta decreased the fraction of cells in S/G2M, rendered them DOPA-negative, and resulted in a smaller, rounder cell shape. *In vivo*, targeted knockout of a TGF-beta receptor in melanocytes resulted in early hair graying associated with ectopic differentiated melanocytes in the follicle, findings similar to the *Bcl2*<sup>-/-</sup> phenotype. Along these lines, treatment of Bcl-2<sup>-/-</sup> mice with an antibody blocking TGF-beta reverted the gray hair phenotype, linking TGF-beta and Bcl-2 activity in the maintenance of MSC quiescence in the stem cell niche.

Dr. Ha-Young Hwang, from the laboratory of Dr. Thomas Hornyak (NIH), discussed a new model for the identification and characterization of quiescent melanocytes in a presentation entitled “Characterization of melanocyte label-retaining cells (LRCs) by microarray analysis”. In this model, *Dct-tTA* (“Tet-Off”) transgenic mice are used in conjunction with the Tet-regulated *TRE-H2BGFP* transgenic mouse line to drive inducibly the expression of a stable, nuclear-localized histone 2B-GFP (H2BGFP) fusion protein. Although bitransgenic mice exhibit H2BGFP expression in cells throughout the outer root sheath of murine hair follicles, extended administration of doxycycline results in a marked reduction of the number of cells expressing H2BGFP, termed Dct-H2BGFP LRCs. Their expression is restricted to the bulge region of the hair follicle. Dct-H2BGFP LRCs colocalize with BrdU label-retaining cells, indicating their quiescence. Microarray analysis of gene profiles obtained from Dct-H2BGFP LRCs, in comparison with cells expressing H2BGFP constitutively in these bitransgenic mice, revealed 234 genes whose expression was significantly different in Dct-H2BGFP LRCs. Further analysis of these genes may reveal specific markers of MSCs.

A presentation by Karine Schouwey, from the laboratory of Dr. Friedrich Beermann (ISREC, Epalinges, CH) was entitled “Notch1 and Notch2 signaling RBP-Jk is essential for proper hair pigmentation and RPE development in the mouse”. In this presentation, results of experiments in which floxed *Notch1*, *Notch2*, or *RBP-Jk* alleles were ablated in the melanocyte lineage at E10.5 by the use of *Tyr-Cre* mice were described. As a larger number of alleles of either *Notch1*, *Notch2*, or *RBP-Jk* were deleted, mice experienced more progressive graying. For example, deletion of 2 alleles resulted in scattered gray hairs, but deletion of 3 or more caused more extensive graying. These observations suggest a role for Notch signaling in MSC maintenance. Interestingly, mice with 4 deleted Notch alleles also showed a decreased number of melanoblasts at E14.5 and E15.5, suggesting that severe loss of Notch signaling can impair melanocyte development during embryogenesis as well. In a complementary gain-of-function experiment, the expression of constitutively active NotchIC under control of the *Dct* promoter was able to rescue the *Notch* knockout phenotypes, but not the *RBP-Jk* phenotype. Hence *Notch1* and *Notch2* may be partially redundant in MSCs. *Hes1* overexpression in melanocytes could rescue the hair graying phenotype caused by loss of *Notch*, but *Hes1* deletion has no hair graying phenotype. Additional studies were focused upon the role of Notch signaling in the RPE during eye development. *Tyrl-Cre* was used to delete

*RBP-Jk*, yielding a small eye phenotype associated with a thinner RPE, whereas overexpression of Notch1C in the RPE resulted in RPE proliferation and a completely closed eye. Loss of *Hes1* in the RPE caused no ocular phenotype.

Irina Pshenichnaya, also from the laboratory of Dr. Friedrich Beermann (ISREC, Epalinges, CH), presented on the topic “c-Myc is required for melanocyte development”. Similar to the previously described work, *Tyr-Cre* mice were used to delete floxed *Myc* specifically from the melanocyte lineage. This deletion resulted in gray mice with markedly fewer melanocytes in the hair follicle bulb. Unlike deletion of Notch, no age-related graying is observed. Melanoblasts in whole-mount embryos were analyzed to determine whether melanocyte loss in adult mice reflected a developmental phenotype. There was a progressive loss of melanoblasts from E11.5 to E16.5, implicating *Myc* in the production of normal melanoblast numbers during this critical developmental window. Additional experiments were performed to establish the mechanism behind the severe melanocyte loss in adult hair follicles. At P8, a detailed count revealed 1/3 the number of normal cells in the bulge region, but only 1/10 the normal number in the bulb, implying that the activity of *Myc* may be critical for promoting the proliferation and/or survival of melanocytes in transition from an immature to a more differentiated state, regardless of whether that is during embryogenesis or during adult hair follicle cycling.

This session provided new insights into the regulation of melanocyte development, differentiation, and stem cell quiescence. Certain signaling pathways may exhibit quite selective effects on these distinct stages of the melanocyte life cycle; others may exhibit manifold effects throughout the life cycle. Further work in this area should reveal the nature of elegant mechanisms linking the transitions of melanocytic cells as they progress from initial specification and migration and, in post-natal life, a period of quiescence to eventually reach the fully differentiated state.

#### **ES-04 Hormones and pigments in fungus, plants, animals and humans**

**Chairs: Jan Borovanský and Shosuke Ito**

*By Jan Borovanský*

Early Bird Breakfast Seminar “Hormones and pigments in fungus, plants, animals and humans” brought together three classics of melanin and melanogenesis research – Prof. A. Slominski, Prof. S. Ito and Prof. K. Wakamatsu.

Prof. Slominski, not unexpectedly, devoted his contribution to his favorite topics – hormonal regulation of melanogenesis and biological implications of the pigmentary system. He characterized melanogenesis as a precisely regulated system exhibiting a high degree of functional diversity and described various principles of the regulatory control. He emphasized that melanogenesis regulating hormones may modify the overall homeostasis through endocrine, metabolic immune and behavioral actions, while the melanogenic activity itself would function as a molecular sensor and transducer of noxious signals and a regulator of the cutaneous homeostasis in the local response to stress.

Until the end of the XX<sup>th</sup> century research activities concerning pigmented bacteria and fungi were limited mostly to Russia (see e.g. SP Ljach: Microbial melanogenesis and its functions. /*In Russian*/, Nauka, Moscow 1984, 274 pp). They demonstrated the radioresistance of the pigmented microbes in the soil of high altitude, e.g. in the Pamir range, as well as the survival of pigmented fungi in the cooling system of the Chernobyl powerplant. Due to its radioresistance, thermotolerance and heavy metal binding, the bacterial and fungal melanins offer a chance of technological exploitation and hence have become an object of intense investigation.

Prof. Ito summarized the up-to-now known precursors of bacterial and fungal pigments. Since only some of them are derived from tyrosine and DOPA, the identification of new pigments is still to be established while the common degradatory analytical procedures cannot be routinely exploited.

Prof. Wakamatsu from Prof Ito's Department, where microanalytical methods based on the chemical degradation of melanins followed by HPLC analysis were developed and optimized, presented an extensive list of eu- and phaeomelanin contents in various pigmented tissues of humans, mice, birds, fish and Crustaceans. Such data represent a foundation for any future thorough thoughts on the function of the melanin pigments. It is of interest to add that while there have been many studies performed on the quality and quantity of melanins, reports on the cell and tissue concentration of the functional units of melanin (=melanosomes) have been rather scarce (cf. *Pigment Cell Res.* 4, 1991, 222-224).

Although the seminar included just three contributions, the overall amount of the new information was enormous. The seminar was sponsored by Novartis Pharma K. K.

### **PS-03 Developmental biology and genetics of melanin pigmentation and**

### **PS-04 Biosynthesis, trafficking and transfer of melanosomes**

**Chairs: Toyoko Akiyama, Chung-Hsing Chang, Heinz Arnheiter and Erling Koppang**

*By Andrzej Slominski*

#### *PL-6 The role of MITF isoforms during pigment development; Dr. Heinz Arnheiter (MDS, NINDS, NIH)*

Dr. Arnheiter discussed the fascinating topic of alternative promoter use, alternative splicing, alternative translation initiation and a variety of post-translational modification of the MITF. He outlined the data from his group showing that in mice the expression profiles of MITF isoforms generated by alternative promoters use differ between neuroepithelial- and neural crest-derived pigment cells, and that the genetic elimination or upregulation of the isoforms have different phenotypic effects. He then focused on the importance of alternative splicing of the exon 2B, which is critical in the regulation of melanocyte behavior but is less important for the retinal pigment epithelium. He further discussed possible crossregulatory mechanisms between spliced enhancer proteins, MITF splicing, and cell cycle regulation. Dr. Arnheiter concluded that alternative splicing of the MITF may affect melanocyte development and malignant transformation and therefore the mechanism underlying this process requires further investigation.

#### *PL-7 Protein-protein interaction in melanosome biogenesis; Dr. Vijay Selaturi (University of Wisconsin)*

Dr. Selaturi presented the data generated in his laboratory on the melanosome biogenesis. Using sophisticated methodology he has studied a complex process of series of protein sorting and vesicular trafficking that are mediated by protein-protein interactions. In his elegant study, he used TRP1 as a marker to explore cytoplasmic protein-protein interactions involved in trafficking of melanosomal proteins and he has discovered novel interactions that participate in early steps of the trafficking of the TRP1. He presented detailed and convincing molecular and biochemical studies on those interactions and the regulatory mechanism(s). His data suggested that interactions of TRP1 with GIPC and APPL provide a potential link between melanosomal protein trafficking and regulation of melanin synthesis by PI3 kinase signaling.

**CS-07 Neural crest and melanocyte differentiation**

**Chairs: Bernhard Wehrle-Haller and Tomohisa Hirobe**

*By James Lister*

The seventh concurrent session on neural crest and melanocyte differentiation covered a broad range of topics, from biochemistry and structural biology to developmental and stem cell biology. Bernhard Wehrle-Haller (University of Geneva, Switzerland) began the session with his presentation entitled “Synergies between integrin receptors and Kit-signaling regulate melanocyte migration and differentiation”, in which he discussed the relationship between two signaling pathways vital to survival of melanocytes in the epidermis. Clustering of integrin receptors is necessary for adhesion and signaling, and mutation of acidic residues in the beta-3 integrin causes a loss of clustering, which can be restored by making reciprocal mutations in a loop of basic residues in the talin protein. In closing, Dr. Wehrle-Haller presented an elegant animation of his proposed model of interaction between the beta-integrin and talin molecules during signaling events in conjunction with the Kit pathway.

Tomohisa Hirobe (National Institute of Radiological Sciences, Chiba, Japan), in a talk entitled “Ferrous ferric chloride stimulates the proliferation and differentiation of cultured keratinocytes and melanocytes in the murine neonatal epidermis”, described experiments from his laboratory in which the effects of iron compounds on cells in culture were explored. Co-culture of newborn mouse epidermal melanocytes with keratinocytes induced proliferation of the pigment cells, an effect which correlated with the production of GM-CSF by the keratinocytes. Addition of ferrous ferric chloride (FFC) to these cultures in the form of ceramic microspheres stimulated proliferation and differentiation of both keratinocytes and melanocytes by about twofold, and stimulated the differentiation of melanoblasts grown in media lacking mitogens. Skin cells thus show similar response to these compounds as had been previously seen with blood cells.

James A. Lister (Virginia Commonwealth University, Richmond) discussed his lab’s research on zebrafish development in his talk “*foxd3* regulates specification of pigment cell types in the zebrafish neural crest”. Loss of function mutations in the zebrafish *Mitf* gene, *mitfa*, and the forkhead transcription factor *foxd3*, have opposite phenotype with regard to a pigment cell type known as an iridophore: numbers of these cells are increased in *mitfa* mutants and decreased in *foxd3* mutants. Double mutants show significant rescue of iridophore numbers, suggesting that *mitfa* acts downstream of *foxd3* in this cell type. Evidence was presented to support the notion that Foxd3 directly represses *mitfa* transcription in a bipotent melanocyte/iridophore precursor to control the choice between these two cell fates.

William J. Pavan (National Human Genome Research Institute) presented an update on an ongoing forward genetic screen in the mouse in his talk, “A sensitized mutagenesis screen identifies modifiers of *Sox10* neurocristopathies”. The basis of the approach is the interaction previously reported by the Pavan lab, that mice heterozygous for loss of function of *Sox10* show an enhanced phenotype when combined with single-hit mutations in other genes in pigment cell development pathways, such as *Mitf* or *Kit*. The Pavan lab has been trying to identify *Modifier of Sox10 neurocristopathy (Mos)* loci which may represent new pathways or new components of known pathways. The presentation focused on the identification of the *Mos1* gene, which was found to be allelic to *Gli3*, a transcription factor that functions in the hedgehog signaling pathway. *Gli3* is not required for melanocyte differentiation but *Gli3* homozygotes have a reduction in melanoblast number. Intriguingly, melanoblasts can be rescued in mutant mice by expressing a form of Gli3 protein that functions as a constitutive transcriptional repressor.

Tsutomu Motohashi (Gifu University, Japan) presented thought-provoking results regarding the developmental potential of mammalian melanoblasts in his presentation “Multipotent cell fates of melanocyte precursors isolated from embryonic and neonatal skin”. In this series of studies, post-migratory melanoblasts were isolated from mice by flow cytometry for c-Kit-positive, CD45-negative cells, followed by culture on ST2 stromal cells. After three weeks in culture, a variety of neural crest derivatives were observed, including neurons, glia, and smooth muscle. Cells isolated from as late as P6 neonates produced melanocytes, neurons, and glia. Surprisingly, plating at limiting dilutions revealed that single Kit<sup>+</sup>/CD45<sup>-</sup> cells were able to produce multiple cell types at high frequency. Addition of the Kit blocking antibody ACK2 to these cultures prevented the appearance of these colonies of multiple cell types. Dr. Motohashi speculated that interactions with the stromal cells were somehow inducing plasticity in these purified melanoblasts.

### **CS-08 Pigmentary disorders; albinism**

**Chairs: Yasushi Tomita and Lluís Montoliu**

*By Lluís Montoliu*

The concurrent session on “pigmentary disorders: albinism” included four presentations ranging from clinical observations, epidemiology aspects, animal models of these congenital hypopigmented diseases and molecular and cellular biology approaches.

First, Yasushi Tomita, from Nagoya University, delivered an interesting talk on the “oculocutaneous albinism and dyschromatosis symmetrica hereditaria, DSH”. He provided a most updated review of human cases diagnosed with different types of albinism in Japan highlighting the abundant OCA4 patients, after the most prevalent OCA1 cases. He also explained a systematic review of current knowledge of DSH, at the molecular level, associated with mutations in the adenosine deaminase acting on RNA1 (ADAR1) gene, indicating the types of mutation and locations within the gene of the genetic alterations.

Second, Lluís Montoliu, from the Centro Nacional de Biotecnología (CNB-CSIC) in Madrid (Spain), summarised their work done over the past years with several animal models, using a variety of transgenic mice (artificial chromosome-type transgenes, inducible transgenes, biochemical substituting transgenes, etc.), to study the known visual and the most recently recognised hearing deficits associated with oculocutaneous albinism type I in mice.

The session continued with a very interesting presentation from Prashiela Manga, from the New York University Medical Center, who reported a potential role of the pink-eye diluted protein in tyrosinase folding. She described how diverse mutations in genes associated with different types of albinism (OCA1, OCA2, OCA3) resulted in reduced maturation and retention of the corresponding affected proteins in the endoplasmic reticulum (ER). In the case of mutations in the p locus it appears that they impact on the redox state of the intracytoplasmic vesicles, thereby altering the ER process of the melanogenic proteins.

Finally, the session concluded with a much enjoyable talk delivered by Robert Aquaron, from School of Medicine, Université de la Méditerranée, Marseille, France, who greatly summarised their findings of specific mutations in the OCA2 gene, the most prevalent type of albinism in Africa. His work and collection of epidemiological data from affected individuals in different African countries enable him to reproduce and trace, at the molecular level, the major population migrations historically associated in this continent, nicely correlating molecular with anthropological data.

**LS-03 A new biphenyl derivative, Magnolignan; its effects on skin pigmentation with a new mechanism**  
**Chairs: Prasad Kumarasinghe and Hidemi Nakagawa**

*By Li Ni Komatsu*

Luncheon Seminar LS3-1, sponsored by Kanebo Cosmetics Inc., focused on the mechanisms and effects of a newly identified pigmentation inhibitor, Magnolignan, which is a biphenyl derivative. The first half of the presentation given by Dr. Minoru Sasaki focused on the identification and mechanism of action studies of Magnolignan. Screening of a group of phenolic derivatives in mouse B16 melanoma cells results in the identification of Magnolignan as a potent pigmentation inhibitor with  $IC_{50}$  value of 4.0  $\mu\text{g/ml}$ , which is more effective than the well-known pigmentation inhibitors such as kojic acid, arbutin and hydroquinone. In contrast to some of the well-known pigmentation inhibitors, Magnolignan has very little effect on the enzymatic activity of mushroom tyrosinase. Immunoblotting analysis suggests that in cultured normal human melanocytes, Magnolignan down-regulates tyrosinase at the protein level, but not the mRNA level. Pulse-chase assay indicates that Magnolignan inhibits tyrosinase maturation at the posttranslational level and leads to a decreased amount of tyrosinase via acceleration of degradation. Taken together, Magnolignan inhibits melanin synthesis by decreasing the amount of matured tyrosinase in melanosomes.

The second half of the presentation given by Dr. Shinichi Watanabe focused on the clinical aspects of Magnolignan. A double-blind test was performed using 43 Japanese subjects with UV-induced hyperpigmentation. A lotion formulated with Magnolignan significantly lightens the UV-induced hyperpigmented area after 3 week application. A separated test was conducted on 51 Japanese female patients with hyperpigmentation disorders for 6 months, which results in a significantly improved pigmentation in 77% of the tested subjects. Additional tests conducted on Asian women indicated that over time Magnolignan lightens the skin color with no obvious unfavorable reactions. All the results indicate that Magnolignan is a novel agent for cosmetic skin lightening and treatment of hyperpigmentation disorders.

**CS-10 Pigmentary disorders; depigmenting diseases (vitiligo & related disorders)**

**Chairs: William A. Gahl and Raymond E. Boissy**

*By Gisela F. Erf*

This session was chaired by Dr. A. William Gahl, NHGRI, NIH, Bethesda, MD and Dr. Raymond E. Boissy, University of Cincinnati, College of Medicine, Cincinnati, OH. The session started with Dr. Gahl's report on "Human Disorders Involving Melanocyte Organelles". The focus of his talk was on three distinct, relatively rare, human hypopigmentation disorders involving aberrant intracellular vesicles within melanocytes: Hermansky-Pudlak syndrome (HPS), Griscelli syndrome, and Chediak-Higashi syndrome (CHS). Melanosome abnormalities observed in these disorders are also reflected to varying degrees in the function of leukocytes and platelets as well as neuronal cells. In the past 10 to 12 years, advances have been made in the identification of genes and gene products responsible for the observed syndromes. For HSP, eight genes associated with 8 disease subtypes (HSP1-8) have been identified. The products of these genes interact with each other in biogenesis of lysosome related organelle complexes (BLOCs); BLOC-1 contains HPS7 and 8, BLOC-2 contains HPS3, 5, and 6, and BLOC 3 contains HPS1 and HPS4. Subtypes of HSP are based on certain BLOC alterations. In Griscelli syndrome, mutations in the genes encoding the small GTPase Rab27a, the molecular motor protein Myosin Va, and melanophilin have been reported. Lastly, for CHS, diagnosis involves mutations of the *LYST* gene, now called *CHS1*. The CHS1 product appears to be involved in either vesicle fusion or fission and the nature of the mutation



can predict the severity of the disease. Hence, in an effort to address rare disorders like HP, Griscelli and CH syndromes, much knowledge has been gained in understanding basic mechanisms that govern vesicle trafficking and their roles in pigmentation, hematologic, immunologic and neurologic processes.

The next presentation was by Dr. Boissy on the role of presenilin-1 (PS1) in melanogenesis. PS1 is needed for efficient targeting of cargo vesicles to melanosomes. Inhibition of the gamma-secretase activity of PS1 resulted in suppression of melanization plus an extra 7 KD fragment for tyrosinase, Tyrp-1 and Tyrp-2, suggesting incomplete processing of the C-terminal portions of these proteins. PS1 inhibition and co-localization studies conducted using cultured human melanocytes support a role of PS1 in melanogenesis in humans. Furthermore, examination of skin from patients with Alzheimer's Disease revealed abnormal tyrosinase trafficking in two of the three patients examined. These studies demonstrate the role of PS1 in pigmentation and may find application in the diagnosis and prognosis of certain types of Alzheimer's Disease.

The third speaker was Dr. Caroline Le Poole, Loyola University, Chicago, IL. She reported on "Differential mechanism of cell death induced by topical depigmentation agents 4-TBP and MBEH". While 4-TBP has been primarily studied in light of occupational vitiligo, MBEH serves as a FDA approved depigmentation treatment. Studies on 4-TBP have clearly established its ability to induce apoptosis in melanocytes. It is important to also examine the mechanism of MBEH-induced cell death. Side by side comparisons revealed that 4-TBP and MBEH induce different modes of cell death, with MBEH inducing necrosis. Fibroblasts and melanocytes were similarly affected by these compounds, while keratinocytes appeared to be relatively resistant, suggesting that MBEH is best applied topically. Information gained from this study is critical for the proper use of MBEH as a depigmenting agent in vitiligo and potentially in melanoma.

The fourth speaker, Gisela F. Erf, University of Arkansas, Division of Agriculture, Fayetteville, AR, reported on the Smyth line (SL) chicken model for autoimmune vitiligo. The talk focused on examples demonstrating the many unique opportunities this animal model offers for the study of autoimmune vitiligo and autoimmune diseases in general. The combination of the predictably high spontaneous incidence of SL vitiligo, together with the easy, repeatable access to the target tissue (the feather), allows for time course studies examining events prior to and throughout the development of vitiligo in the same individual. On the other hand, the predictably low incidence of SL vitiligo without a previously identified environmental trigger (routine live herpes virus of turkey (HVT) administration at hatch) provides opportunity to examine vitiligo-precipitating factors in genetically susceptible individuals. Hence, many opportunities exist to study the etiology, pathology, treatment and prevention of autoimmune vitiligo in this animal model.

The final speaker of this session was Dr. Mauro Picardo, San Gallicano Dermatological Institute, Rome, Italy, who reported alterations in membrane lipid profiles and function in cells from vitiligo patients. Specifically, membrane lipid profiles were examined in melanocytes, fibroblasts and peripheral blood mononuclear cells from vitiligo and healthy subjects. Lipid dependent signal transduction was also evaluated. Key observations included high cholesterol content, altered fatty acid arrangement and lipid peroxidation in membranes of cells from vitiligo patients. These changes were reflected in down-stream lipid dependent MAPkinase pathways, including increased phosphorylation of CREB and ERK. By increasing the membrane cholesterol content of normal fibroblasts, the membrane impairments observed in vitiligo cells could be simulated. This approach provides an important tool to gain insight into the role of membrane alterations in vitiligo.

**CS-11 Pigmentary disorders; hyperpigmentation (melasma, senile freckles and other related diseases)**

**Chairs: Mauro Picardo, Miri Seiberg and Rashmi Sarkar**

*By Miri Sieberg*

Mauro Picardo started the session with a review on the inhibition of the melanogenic process via multiple and different pathways. Historically, the inhibition of tyrosinase activity was the only target for depigmenting agents. With increasing knowledge, we now look at the inhibition of multiple processes, at the melanocyte, keratinocyte, and even the fibroblast level. At the melanocyte level, depigmenting agents can 1) act before melanin synthesis, e.g. inhibit transcription or glycosylation of melanogenic enzymes, 2) act during melanogenesis, e.g. inhibit tyrosinase or act as ROS scavengers, and 3) act after melanin is produced, e.g. to inhibit melanosome transfer. At the keratinocyte level, the inhibition of the PAR-2 pathway reduces melanosome uptake. Recent data suggest that fibroblasts-secreted factors like KGF could also serve as targets for depigmenting agents. Depigmenting agents may affect multiple pathways, and a single agent could induce multiple effects on skin cells.

Miri Seiberg described the work of Nannan Chen and Connie Lin on the role of KGF and IL-1alpha in the initiation of hyperpigmentary lesions. A Senile Lentigo (SL) is a UV-induced hyperpigmented lesion, characterized histologically with hyperpigmented basal layer and elongated rete ridges. The team hypothesized that SL is a keratinocyte-induced pathology. Topical treatments of pigmented epidermal equivalents or of human skin explants with KGF, or with the combination of KGF and IL-1alpha resulted in increased melanin deposition in the basal layer. Topical treatments of swine skins with KGF, IL-1alpha and their combination resulted in visual skin darkening. Moreover, these treatments led to the creation of histological features similar to SLs, namely a hyperpigmented basal layer and elongated rete ridges. Finally, KGF and IL-1alpha induced pigment deposition and rete ridges elongation in human skins transplanted onto SCID mice. These data suggested that KGF and IL-1alpha play a role in the initiation of hyperpigmentary lesions. The team hypothesized that once a hyperpigmentary status and rete ridges elongation are established, KGF is no longer required for maintaining the lesion.

Hirofumi Aoki described studies of established SLs, documenting lower frequency of cell division in the melanin-containing keratinocytes within the hyperpigmented lesion. Gene expression (array) analysis of SLs and adjacent skins of 16 individuals revealed an increase in markers of inflammation and a reduction in differentiation markers. Histological staining of SLs with Ki67 revealed that the accumulation of melanin within the keratinocytes correlates with the lack of Ki67 expression, suggesting a suppression of cell division by the accumulated melanin. Cultured keratinocytes were then incubated with isolated melanosomes or fluorescent microspheres to document phagocytosis and nuclear cap formation *in vitro*. When the keratinocytes were incubated with increasing concentrations of melanin, a lower frequency of cell division was documented *in vitro*, in correlation with the increase in melanin ingestion by the keratinocytes. It was noted that cell division is not affected by the high content of melanin in keratinocytes of darker skins. A mosaic model was suggested, with hyper-proliferation of non-pigmented keratinocytes and slow division of pigmented keratinocytes.

**CS-13 MITF in melanocytes and melanoma**

**Chairs: Shigeki Shibahara and Eirikur Steingrimsón**

*By Keren Bismuth*

Dr. Shigeki Shibahara from Tohoku University, Sendai, Japan first presented an overview on MITF history. He next presented the characterization of Mitf-black eyed white (*Mitf-bw*). The mouse has a white coat and black

eyes. At the molecular level this mutation is characterized by the insertion of a L1 element in intron 3. By doing microarrays analyses comparing skin derived from *WT* or *Mitf<sup>bw</sup>*, the group found a new melanocyte marker, Lipocalin-type prostaglandin D2 synthase (L-PDGS). L-PDGS is an enzyme involved in the biosynthesis of prostaglandin and is expressed in hair follicle melanocyte. This enzyme was specifically absent from *Mitf<sup>bw</sup>* skin and is also not expressed in human melanoma cell line. Enzymatic products of L-PDGS, PDG2 may inhibit the growth of human melanoma cells. This suggests a possible link between MITF and the inhibition of catalytic activity.

Dr. Eirikur Steingrímsson from the University of Iceland, Reykjavik, Iceland presented the results of an extensive BAC rescue experiment meant to test the *in vivo* role of MITF post-translational modifications. First, he showed that a BAC containing the whole *Mitf* gene, except for exon 1A, is able to rescue the phenotype of the *Mitf<sup>vga9</sup>* null allele, indicating that exon 1A is not needed for pigment cell development. Second, Steingrímsson and colleagues mutated several phosphorylation sites in the *Mitf* BAC including Ser73, Ser409 and Ser307 into an Alanine. Of all of the BAC transgenic mice made only BAC Ser307A was not able to fully rescue the *Mitf<sup>vga9</sup>* null phenotype, indeed the Ser307A transgenic mouse is white with black eyes, suggesting that this amino acid is important for MITF function in neural crest derived melanocytes. Third, he presented various BAC transgenic mice carrying deletion within exon 2 and/or its surrounding introns. The absence of exon 2A and exon 2B are not deleterious to melanocyte development, which seems to suggest that exon 2 is dispensable for MITF function in melanocyte.

Dr. Keith Hoek from University Hospital of Zurich, Switzerland presented a talk on identifying new MITF target genes. Dr. Hoek and colleagues over-expressed M-Mitf cDNA in SK-Mel-28 cells that are of melanocytic origin but express little Mitf. Microarrays analyses from transfected and non-transfected cells identified as much as 6910 potential targets. Using the striking variability in Mitf expression observed among different published melanoma microarray studies, Dr. Hoek correlated the expression of Mitf with other genes and used this to filter out false positives from the transfection results. This re-identified thirteen of forty published targets and an additional 71 novel candidate targets. He finally underlined the necessity to give appropriate attention to the statistical relevance of the raw data obtained in this kind of high throughput studies.

Dr. Akiha Kawasaki from Tohoku University, Sendai, Japan gave an oral presentation on the role of *Mitf* in melanophore dendricity and melanosome distribution. She injected WT or dominant negative (dn) form of Mitf in *X. laevis* embryos then did neural crest culture from the injected embryos. She showed that the level of dendricity and the dispersion of melanosomes was higher in WT Mitf injected embryos compared to non-injected, dn Mitf resulted in low level dendricity and aggregated melanosomes. Immunostaining revealed that the levels of Rab27a were slightly increased in melanophores that over expressed WT MITF while it is decreased in dn-Mitf. These results suggest that *Mitf* is implicated in melanosome transport and in melanophore dendricity.

Dr. Keren Bismuth from Pierre and Marie Curie University/INSERM, Paris, France presented work done in H. Arnheiter's laboratory on the generation of an *Mitf<sup>Ser73A</sup>* knock-in mouse model. First, she reported the generation of a highly unstable *Mitf<sup>Ser73A</sup>* allele, which has the Ser73A mutation in the Mitf gene along with a large internal duplication of the WT *Mitf* gene. This allele showed a high degree of somatic and meiotic reversions, which eventually led to the exclusion of the duplicated *Mitf* sequence. Second, she showed that the reverted *Mitf<sup>Ser73A</sup>* allele is black, surprisingly the Ser73A mutation lead to the preferential exclusion of exon 2B which contained the Ser73A residue. Expression of MITF protein lacking exon 2B increases the number of differentiated melanocytes. Exon 2B may have a role in the control of melanocyte proliferation.

**CS-14 Molecular and surgical pathology**

**Chairs: Dirk J. Ruiter and David E. Elder**

*By Yuji Yamaguchi*

Dirk Ruiter discussed the difficulties pathologists face in diagnosing melanocytic tumors based on histology. One method to discriminate between benign and malignant melanocytic lesions and Spitz nevi may be through characterization of B-RAF, H-RAS and N-RAS mutations. B-RAF and N-RAS mutations are commonly found in melanocytic lesions other than Spitz nevi, while H-RAS mutations found frequently in these lesions. A number of new detection techniques have improved the ability to rapidly and efficiently detect mutations, including Multiplex Ligation-dependent Probe Amplification (MLPA) to monitor copy number and mutation specific MLPA probes for mutation detection in tumors with a low number of cells. In addition, CDKN2A and TP53 mutations may be useful in the characterization of melanocytic lesions.

David Elder reported on molecular and histopathology of melanoma. Histology is still the primary method for the classification of tumors. The combination of histology and mutation detection proved a more useful approach, with potential for increased accuracy and correlation to pathogenic mechanisms. However, the histogenetic classification of tumors has not been used extensively for the selection of treatment protocol, and may be a new avenue to determine the best therapeutic approach.

Yuji Yamaguchi described a study aimed at determining the effects of tanning on Caucasian skin. UV-induced pyrimidine (6-4) pyrimidone photoproduct formation, cyclobutane pyrimidine dimer formation and p53 nuclear accumulation were observed in Caucasian versus African American skin, while relatively more cells were seen to undergo apoptosis in African American skin. These data suggest that decreased UV-induced skin cancer seen in African-American skin may result from a combination of the decreased DNA damage and the more efficient removal of UV-damaged cells.

Tobias Hohenauer reported on a novel marker for human melanoma, Brn3a that was found to enhance melanoma cell survival via suppression of p53 activity. Brn3a, previously shown to be increased in neuroectodermal tumors, is expressed at low levels in normal tissue. When expression is knocked-down in melanomas, cell viability is reduced and G0/G1 arrest increased leading to apoptosis. Brn3a inhibition resulted in upregulation of p21cip/waf, which is regulated by p53. Furthermore, p53 was found to be stabilized suggesting that expression of Brn3a in human melanomas promotes cell proliferation and survival by targeting p53.

Gilles Landman discussed the use of sentinel lymph node in determining melanoma prognosis. Cell cycle proteins: Cyclin D1, CDK4, p16ink4 and p21WAF1, cell adhesion protein avb3 integrin and metalloproteinases-2 and -9 were investigated in cutaneous melanoma with and without metastasis to the sentinel lymph nodes using immunohistochemistry. Cyclin D1 was found to be an independent variable that could be used to predict nodal metastases.

**LS-06 UVA sunscreen protection - how much is enough???**

**Chair: Miri Seiberg**

*By Hideya Ando*

Recent studies have revealed that UVA can cause photoaging (dermal damage such as solar elastosis) through indirect DNA damage via ROS (reactive oxygen species) generation even in the absence of UVB. Therefore,

sunscreen products require balanced UVB and broad spectrum UVA protection. The UVA protection is mainly measured by MPPD (minimum persistent pigment darkening) reaction in Fitzpatrick skin phototype II, III, IV individuals when irradiated with only UVA, with comparison of protected and unprotected skin area, however, regulation on sunscreen labeling for UVA are not globally harmonized and should be addressed. In addition, long time exposure to sunlight can degrade UVA-sunscreen product that leads to the decrease of UVA protection factor (UVA-PF), therefore, photostability of sunscreen product is required.

### **ES-07 Sun light and epigenetics of melanoma**

**Chair: Barbara A. Gilchrest**

*By Toshikazu Ushijima*

Epigenetic modifications are defined as modifications that are associated with DNA and faithfully replicated into daughter cells upon somatic cell replication, and include DNA methylation at CpG sites and histone modifications. It is also known that DNA methylation of a CpG island in a gene promoter region can completely repress transcription of the downstream gene. Once aberrant epigenetic modification is established in a cell, it is inherited almost forever, and can cause disease conditions. In fact, it is well established that epigenetic alterations, along with genetic alterations, are causally involved in various human cancers by inactivating tumor-suppressor genes, such as *CDKN2A (p16)*, *MLH1*, and *CDH1*.

Application of aberrant DNA methylation is now at the stage of clinical application. It is now known that aberrant DNA methylation in normal-appearing tissues can be used as a cancer risk marker<sup>1,2</sup>. DNA methylation patterns in cancer tissues are often associated with clinicopathological characteristics. For example, methylation of multiple CpG islands in neuroblastomas is a very accurate prognostic marker<sup>3</sup>.

Although melanomas have been generally believed to be a disease of genetic alterations, predominantly due to UV irradiation, it is now recognized that epigenetic alterations are present in melanomas. For example, when methylation of 19 tumor-suppressor genes was analyzed in 13 melanoma cell lines, nine genes were methylated in at least one cell line<sup>4</sup>.

To identify methylation-silenced genes in melanomas, we first performed methylation-sensitive-representational difference analysis (MS-RDA) using three melanoma cell lines (MeWo, WM-266-4 and MMac) and human embryonic melanocytes (HEMs). Promoter methylation of 34 genes was identified, and, especially, promoter methylation of *PRDX2*, a negative regulator of PDGF signalling, was considered to be important in melanomagenesis<sup>5</sup>.

Gene expression-based screening for methylation-silenced genes were also performed. Three melanoma cell lines (HMV-I, MeWo, and WM-115) were treated with a demethylating agent, 5-aza-2'-deoxycytidine, and genes whose expression was induced were screened by oligonucleotide microarrays. Eighteen genes methylation-silenced in melanoma cell lines were identified, and, especially, silencing of *TFPI-2*, an invasion suppressor gene, was found to be associated with melanoma metastasis<sup>6</sup>. These findings showed that epigenetic mechanisms are also involved in melanoma development and progression.

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4. Furuta et al. *Cancer Sci* 2004;95:962-96
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#### **LS-08 The evolution of sun protection: from SPF to IPF**

**Chair: Masamitsu Ichihashi**

*By Mary S Matsui*

Mary Matsui, from the Clinique Laboratories in New York presented LS-08. The goal of this presentation was to describe current issues in sun protection so that a better understanding of “non-sunscreen” photoprotection and its potential benefits can be gained. To this end, mechanisms important in the initiation and development of UVR-induced skin cancer and photoaging were reviewed, and recently obtained data were presented to demonstrate that supplemental materials such as anti-oxidants may provide further protection when added to chemical and physical sunscreens.

Despite improvements in sunscreen formulation, which include increased spectral coverage and product transparency, there is evidence that the public is still not adequately protected by conventional commercially available sunscreens. Full spectrum (or “real-life” sun exposure) UV-induced skin damage includes direct DNA damage, cutaneous and systemic immune suppression and oxidative stress.

It is well accepted that ultraviolet radiation (UVR) is the most important etiologic factor in basal and squamous cell carcinomas and although not entirely straightforward, epidemiologic studies also implicate UVR as an etiologic agent for the pathogenesis of melanoma. Sunburn is reasonably well correlated with UVB and direct DNA damage, the initiating event in mutagenesis and carcinogenesis. However, it has been demonstrated that suberythemal doses of UVR cause a variety of molecular changes including DNA damage and immune suppression. This means skin damage occurs even in the absence of erythema, suggesting that new measures of photo-protection should be developed, and these new measures should be used to inform prevention of this “unseen” damage.

Because UV-induced immune suppression is a critical arm of damage leading to skin cancer, it is desirable that topical sun protection products have ingredients proven to maintain cutaneous immune function. It has been established that sunscreens are variably immuno-protective, and this may contribute to variability in their ability to reduce the risk of skin cancers. In addition, it has been suggested that UVA induced *oxidative* DNA damage is more important than originally thought, as UVA penetrates deeper into the skin and these lesions are found in the basal layer, where mutations can occur in stem cells.

Non-sunscreen photoprotective agents are those that act with different mechanisms than chemical or physical sunscreens. Most commonly they are either antioxidants or DNA repair enzymes. For example, topically applied green tea polyphenols have been shown to inhibit UVR-induced erythema, decrease CPD, reduce levels of 8-hydroxydeoxyguanosine, inhibit Langerhan’s cell depletion (a surrogate endpoint for immune suppression) and in an *in vivo* model (-)–epigallocatechin-3-gallate (EGCG) prevented UVR-induced inhibition of cutaneous immune function. In addition to its anti-oxidant property, EGCG has also been shown to induce IL-12, leading to enhanced DNA repair and also inhibits NFkB. Other photoprotective materials include polyphenols/flavonoids, grape extract/resveratrol, proanthocyanidins, quercetin, genistein, pomegranate extract, red clover, pycnogenol and the combination of vitamins C and E.

Topically applied DNA repair enzymes have also shown promise in terms of ameliorating the damage done by UVR. In clinical studies, when applied after sun exposure, they prevented UVR-induced suppression of local cutaneous immune function and can reduce the accumulation of cutaneous neoplasms in xeroderma pigmentosum patients.

Supplemental photoprotection will benefit all consumers and the “natural” aspect of these materials may encourage greater use. New measurement paradigms should be predictive of both acute UV damage such as erythema and pigmentation, and long term consequences such as skin cancer and photoaging. Because botanical extracts can be unstable in commercial formulation, human efficacy studies using meaningful endpoints should be performed on finished products.

### **CS-21 Genomics and proteomics of melanoma**

**Chairs: Alan Spatz and Nicholas Hayward**

*By Graeme Walker*

Alan Spatz (Gustave Roussy Institute, France) described investigations into why survival from melanoma is worse in men than in women. The difference does not seem to stem from hormonal or sun exposure differences. They hypothesized that a gene on a sex chromosome may be involved. Strategies to find such genes are different than for autosomal genomic changes, because both males and females have only one active X that could carry a tumor suppressor or oncogene. Dr. Spatz used a clever system involving expression of the *XIST* gene as a marker of loss of the active X chromosome, in combination of array CGH and *in vitro* studies, and confirmed the frequent loss of the active Xp22 associated with poor survival. qRT-PCR studies on candidates revealed loss of *PPP2R3B* (involved in DNA replication) on Xp22. Intriguingly, this gene is also present as a single copy on the Yp11 and was commonly lost in males with melanoma associated with poor survival. They hypothesized that as random genomic loss would be more likely to target the small Y chromosome than the much larger X chromosome, this may largely explain the worse prognosis for melanoma in males. This represents a fascinating addition to the catalogue of changes that occur during melanoma progression.

Nicholas Hayward (QIMR, Australia) outlined analysis of melanoma cell lines using Agilent array CGH and Affymetrix microRNA chips. MicroRNAs (MiRs) are small (~22 nucleotide) non-coding RNAs that regulate the expression of other genes. Dr. Hayward's group looked for MiRs whose expression changes correlated with CGH copy number changes. MiR-211 had the largest mean expression difference between melanocytes and melanoma cells and mapped within a commonly deleted region. MiR-211, located within an intron of *TRPM1* (Melastatin, a transcriptional target of MTF), was one of a handful of miRNAs that discriminated between melanomas and other cancers (Gaur *et al*, 2007). Bioinformatic analysis predicts several MiR211 targets: *JUN*, *PRKCA*, *POU3F2* (BRN-2), *SMAD3*, *RUNX1*, *RUNX2*, *ATF7*, *FGF2*, and *WNT5A*. Some of these targets have been validated. Thus MiR-211 may play a central role in tumorigenesis through modulating the expression of these growth regulators. Dr. Hayward discussed the difficulty of overlaying CGH and expression array data of single samples, because of the randomness of genomic instability in an individual tumor. Instead, a large number of samples and intensive bioinformatics capability is necessary. MiR functional analysis is complicated by the fact that each may have many targets.

Mayumi Fujita (University of Colorado, USA) discussed the utility of blood melanoma biomarkers, which have some advantages over tissue-based markers (e.g. blood sampling is less invasive and can be performed repeatedly

during tumor progression). To discover markers that may assist with melanoma diagnosis and prognosis they performed microarray gene expression profiling of whole blood from melanoma patients and controls. From the top five genes that differentiated the two groups, they found that only two, a complement component gene (C1QB), and Pleckstrin (PLECK2) were sufficient to predict with high probability whether the blood was from a melanoma patient or controls. They are confirming these results on a bigger pool of melanoma patients.

David Easty (St. James's Hospital, Ireland) discussed the role of tyrosine phosphorylation in melanoma cells. Melanomas show loss of function of tyrosine phosphatases and gain of function of receptor tyrosine kinases (RTKs). His laboratory has used phospho-RTK arrays to examine global RTK activity in melanoma cell lines and found that only specific RTKs are activated, and that the particular RTK activated may depend on type of melanoma and/or the stage of progression. He presented an intriguing model, where loss of phosphatases accompanies the transition for melanocyte to melanoma, and RTK gain of function is associated with progression to advanced melanoma. RTK phosphorylation seems important in driving melanoma tumorigenesis, and this work should increase our understanding of which phosphatases and kinases are deregulated at discrete times during progression. This information will be critical for potential therapeutic targeting of these pathways.

With the aim to find out how keratinocytes might influence melanocytes after UV exposure, Chong Jin Loy (Johnson & Johnson Asia Pacific, Singapore) used the HCAT cell line to examine the response of keratinocytes to UVR. They used a combination of expression microarrays and 2-D gel protein electrophoresis/mass spectrometry to examine gene expression at 6h and 24h after UVR. Predictably, many classes of genes were deregulated after UVR exposure, with those involved in inflammatory and oxidative stress response significantly upregulated. For the genes tested, they found good concordance with results from protein and expression analyses. The genes most significantly upregulated were 14-3-1, involved in cell cycle control, and Hsp 27, a heat shock protein that can regulate the production of inflammatory cytokines. Dr. Loy's group is continuing to try to integrate this large amount of data from two platforms, and hope to shed light on mechanisms by which keratinocytes control pigmentation responses to UVR.

### **CS-24 New melanoma risk markers and prognosis**

**Chairs: Joost Van Den Oord and Georg Weinlich**

*By Lester Davids*

*CS 24-1 Melanoma gene expression profiling: prognostic markers and insight in tumor progression; Van Oord, J. et al. (University Hospitals, KUL, Leuven, Belgium)*

The novelty of this paper was that gene expression profiling in primary cutaneous melanoma is more commonly obtained by using cell lines. This group presented profiling using melanoma tissue which allowed them to correlate expression profiles with histological and survival data. Out of the 11,043 genes used in the array, they found 361 up or down-regulated genes of which 254 were regarded as genes with a prognostic signature to predict the 4-yr distant metastasis free-survival. Upregulated genes were found to be those involved in DNA damage (TYMS), nuclear transport (KPNA2) and unwinding of DNA (MCM proteins). Under-expressed genes included those encoding proteins that inhibit several serine proteases (SPINT2) and spindle assembly (RANBP1). In summary, this approach allows the identification of novel differentially expressed genes that can be employed as useful immunohistochemical markers and have an overall impact on improved patient survival.



*CS 24-2 Histological and serological new risk markers in melanoma; Weinlich, G. (Medical University of Innsbruck, Innsbruck, Austria)*

This paper centered on prognostic versus progression markers in highly aggressive melanoma with the aim being to establish risk as early as possible in patients. One promising new marker is Metallothionein (MT). This small, cysteine-rich protein protects melanoma against UV and chemotherapeutics and is found to be overexpressed in melanoma cells. In a large study, it was the second best risk marker beside tumor thickness and was already predictive in low-risk melanomas. Another marker studied by this group was the serological measurement of tryptophan degradation and neopterin concentration. In summary, lower tryptophan and higher neopterin concentrations correlated to predict a shorter survival.

*CS 24-3 Matricellular proteins produced by melanocytes and melanomas : potential role of tenascin-C as a key component in the melanoma stem cell niche; Fukunaga-Kalabis, M. et al. (The Wistar Institute, Philadelphia, PA, USA)*

Matricellular proteins are modulators of cell-matrix interactions and cell functions and although over the past few years numerous papers have been presented on proteins such as osteopontin, SPARC and tenascin being upregulated in melanoma, very little has been presented on their roles in tumor growth, survival and metastasis. In summary, this paper from the Herlyn group, focused on the role of tenascin C in the stem cell niche and backed up by microarray experiments showed that melanoma progression correlated with an increase in tenascin C mRNA in vertical growing melanomas and metastatic melanoma cells. Interestingly, TN-C is not expressed in melanocytes. Moreover, melanoma cells grown in stem cell medium grew as spheres and TN-C knockdown experiments revealed increased attachment of these cells compared to control cells – suggesting that these cells are good candidates for dormant tumor/stem cells. The melanoma spheres were also resistant to doxorubicin treatment, further showing that tenascin-C plays a critical role in drug resistance of melanoma cells by contributing to the niche for stem cells.

*CS 24-4 Lack of cytoplasmic ERK activation is an independent adverse prognostic factor in primary cutaneous melanoma; Hansson, J. et al. (Karolinska Institutet, Stockholm, Sweden)*

The aim of this study was to estimate the impact on survival of NRAS and BRAF mutations and activation of Akt and ERK in primary cutaneous melanomas. Using a cohort of 57 primary cutaneous T1-2 melanoma tumors obtained with a Laser capture dissection method and a wide variety of statistical analyses, they found that shorter overall survival was associated with the presence of ulceration and BRAF exon 15 mutations. They suggested that the absence of cytoplasmic ERK activation in poor prognosis T1-2 melanomas may be associated with some other uncharacterized pathway leading to tumor progression. Overall, this group showed that cytoplasmic p-ERK could potentially be used as a prognostic marker in T1-2 melanomas.

*CS 24-5 Differential cell adhesion within an isogenic model of melanoma progression under shear flow conditions using a microfluidic cell-based assay; Gremel, G. et al. (University College Dublin, Dublin, Ireland)*

As extravasation of melanoma cells from the tissue through the endothelial layer and into the bloodstream is a characteristic of metastasis, modeling its process is pertinent in melanoma research. This paper presented a model of melanoma adhesion to endothelial cell-derived proteins using a Microfluidic Platform. This is a mechanical model which mimics the *in vitro* microenvironment of tumors. Cells are moved along channels which is then captured by a camera. Primary melanomas were used and cultured to become increasingly metastatic – these cells were then sent along 8 micron channels in parallel which were coated with cell adhesion proteins fibronectin, ICAM1, VCAM1 and BSA. Significant cell adhesion was found only on the VCAM1 coated channels in the highly metastatic 1205Lu lung metastatic cells. Although more work is planned, this model provides unique insight into the extravasation process of melanomas of different metastatic potentials.

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