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## HAPPY NEW YEAR 2011

### CONTENT

#### Discussion, Letters to the editor, Reviews, Short communications, ...

Meeting report. Hinxton-Cambridge, UK  
History of pigment cell research in Europe (Prof. J. Borovansky)

#### Review of the literature

1. Chemistry of Melanins and other pigments  
(Prof A. Napolitano)
2. Biology of pigment cells and pigmentary disorders  
(Dr M. Picardo)
3. MSH, MCH, other hormones (Prof M. Böhm)
4. Photobiology (Dr N. Smit)
5. Neuromelanins (Prof M. d'Ischia)
6. Genetics, molecular and developmental biology  
(Dr F. Beermann)
7. Tyrosinase, TRPs, other enzymes  
(Prof JC. Garcia-Borron)
8. Melanosomes (Prof J. Borovansky)
9. Melanoma experimental, cell culture (Dr R. Morandini)

#### Announcements and related activities

- Calendar of events
  - Official List of ESPCR 2010 members
-



**LETTER TO THE EDITOR  
DISCUSSION, REVIEW,  
SHORT COMMUNICATION, ...**

## **ESPCR Meeting Report September, 4-7, Hinxton-Cambridge, UK**

### **Session 1 : Development biology of pigment cells and MSCs**

Chair: Véronique Delmas and Takahiro Kunisada

We see many reports describing multipotential stem cells possibly derived from neural crest cells found in the various part of the body. “Chromatoblast” concept reported by **Robert Kerlsh** (University of Bath, UK) may hopefully bring some order in the discussion about these neural crest-related stem cells. He found that ablation of *ltk* kinase mostly affect iridophore cell fate, however, *ltk* expression marks all pigment cell lineages and Schwann cells. Using this kind of lineage markers in Zebrafish in which precise lineage analysis is possible during embryogenesis, dynamics of “chromatoblasts” (or any kind of neural crest stem cells) during progressive fate restriction of neural crest stem cells could be clarified. Melanocyte stem cells were functionally classified by **Stephen Johnson** (Washington Univ. Med. Sch., USA) in Zebrafish. Addition of melanocytotoxic MoTP to the early embryo ablate matured melanocytes in trunk region but the regenerated melanoblasts appeared within 4 days after MoTP washout, indicating the presence of adult-type melanocyte stem cells. While the addition of ErbB3 inhibitor AG1478 or *erb3b* defective *picasso* mutant strain develop normal melanocyte, MoTP-induced melanocyte regeneration was abolished by the addition of ErbB3 inhibitor or in *erb3b* mutant. He therefore suggested that embryonic melanocytes develop directly, without proceeding through the ErbB-dependent adult type melanocyte stem cells. These analytical studies might eventually be combined to elucidate whole melanocyte cell lineage picture in which experimentally-tested real stem cells were nicely imposed. The requirement of BRAF and CRAF signaling during melanoblast development and homeostasis was presented by **Agathe Valuet** (Institut Curie, France). The double *Braf* and *Craf* knockout (KO) mice displayed normal pigmentation at birth, however following the first hair molting, the double KO mice exhibited progressive hair graying. After several hair cycles, analysis of the double KO mice revealed a disappearance of melanocyte stem cell in the bulge. It was previously show that the emergence of the melanocytes that produce the first hair requires KIT signalling whereas the presence of melanocyte stem cells in the bulge become independent of it. Taken together, the authors proposed an uncoupling between KIT and RAF signaling during melanocyte lineage development. To understand stem cells, quantitative analysis of the particular cell lineage seems to be essential and **Lionel Larue** (Institut Curie, France) presented mathematical models of melanocyte development during early embryogenesis in mouse skin. His model explained different behavior of dermal and epidermal melanocytes during development. Interestingly, beta catenin that profoundly affect proliferation of epidermal melanocyte precursors did not affect dermal ones, therefore a new key to the regulation of early melanocyte development must be held by the beta catenin function in developing melanocytes. Another mathematical model was presented by **Richard Mort** (MRC, United Kingdom) to analyse melanoblast behavior during embryonic development. The goal of the study was to explain how individual cellular properties and tissue expansion can contribute to the dispersal of the melanoblast population. The authors presented live imaging of EYFP-melanoblast migration in embryonic skin in an ex vivo system. Analysis of time lapse sequences revealed that melanoblasts are highly mobile and allowed measurement of the dynamics of their migration.

## Session 2: Genetics of pigment cell transcription

Chair: Vittoria Schiaffino and Eirikur Steingrímsson

Session 2 had 3 invited speakers and 3 selected presentations, which discussed various transcriptional regulatory pathways operating in the melanocyte lineage and in particular involving MC1R, Mitf, beta-catenin and Notch signalling.

**Zalfa Abdel-Malek (Cincinnati, USA)** started the session by discussing the central role of MC1R in regulating human pigmentation and the UV response. She showed that human beta-defensin 3 (HBD3) acts as an antagonist of MC1R by blocking the effects of alpha-MSH in human melanocytes. As an alternative possibility based on mouse model phenotypes, Dr. G. Barsh in the audience underlined that HBD3 might actually act as a neutral agonist for MC1R, enhancing its constitutive activity. Zalfa also showed how transcription of MC1R can be affected by either alpha-MSH, ACTH, by cAMP signaling or by Endothelin 1 and bFGF. She also presented evidence, which suggest that MC1R may undergo different levels of desensitization in different melanocyte strains. This, however, was independent of the MC1R genotype and to the expression level of G protein-coupled receptor kinases (GRKs).

**Eirikur Steingrímsson (Reykjavik, Iceland)** described the structure of the MITF protein and how it has elucidated important aspects of dimerization and DNA binding properties of the protein. The structure has also provided information on the biochemistry of the Mitf Mi-Wh mutation, a mutation with interesting phenotypic and genetic characteristics. Eirikur also discussed the acetylation of MITF and how it affects function and may lead to an oncogenic protein, in particular when modifying residues K243/248 within the helix-loop-helix domain.

**Francesca Pignoni (Syracuse, USA)** described how she has used the powerful *Drosophila* genetic system for modeling the oncogenic activities of MITF in the *Drosophila* wing epithelium. In order to do this she has used the Gal4-UAS system to express MITF in different cells of the wing epithelium and then analyse effects on various aspects of cell function including proliferation, apoptosis, cell adhesion and migration. Her results show that in combination with activated RAS, MITF can act as an oncogene in the fly epithelium. In addition to this she has used deletion strains, siRNA strains, ENU mutagenesis and the yeast-2-hybrid screen to search for modifiers and mediators of MITF function. Several genes were found in more than one of the screens, suggesting that they are true modifiers or mediators of MITF.

**Claudia Wellbrock (Manchester, UK)** has investigated the controversy regarding the role of beta-catenin in melanoma. In contrast to other kind of cancers, nuclear beta-catenin in melanoma has been associated with both reduced overall survival as well as with good prognosis. Using melanoma cell lines with high and low levels of nuclear beta-catenin, she showed that in melanoma, high expression of nuclear beta-catenin is indicative for a non-invasive phenotype whereas low beta-catenin levels results in invasive cells. These effects of beta-catenin are at least partially mediated through effects on MITF gene expression. Thus, the activation of the same signaling pathway in different types of cancer might result in opposite effects, depending on cancer-type specific downstream targets.

**Genevieve Aubin-Houzelstein (Paris, France)** discussed her analysis of the role of Strawberry Notch homolog 2 (Sbno2) in melanocytes and melanocyte stem cells. She used transgenic mice to overexpress Sbno2 in the melanocyte lineage and studied both coat color phenotype and fate of the melanocyte stem cells. Mice overexpressing Sbno2 are largely normally pigmented at birth but gradually lost pigment starting at 3 months of age. There was a reduction in melanocyte precursors from the bulge region with successive hair cycles, suggesting that Sbno2 overexpression affects stem cell maintenance. Interestingly, they also observed a ring of beta-galactosidase positive cells around several hair follicles, suggesting a cell fate switch of the melanocyte precursors.

Finally, **Bhushan Sarode (Lausanne, Switzerland)** presented studies on the role of Notch1 and 2 receptors in melanoblast and melanocyte stem cells maintenance. Notch signaling plays critical role in many biological processes. Deletion of Notch1 and Notch2 receptors results in dose dependent hair graying, due to an elimination of melanocytes and melanocyte stem cells. The expression of a NotchIC transgene rescued this deficiency, however, not the deletion of RbpJK, thus proving further evidence

that Notch signaling acts via RbpJK transcription factor in pigment cells. The identification of targets in melanocytes might indicate why melanocyte stem cells disappear in the absence of Notch. For that purpose he undertook a microarray strategy in order to identify downstream factors involved in this process.

### **Session 3 : Pigmentary Disorders**

Chair: Prasad Kumarasinghe and Lluís Montoliu

Sunday 5, September 2010, 8.45 AM-10.15 AM

Albinism is characterized by hypopigmentation and affects 1 in 17000 human beings. It is a heterogeneous group of rare genetic conditions. Severe visual deficiencies are common among albinos therefore albinism affects the quality of life significantly. **Lluís Montoliu** spoke on the topic ‘Towards a universal genetic diagnosis of all types of albinism’. He described the various types of oculocutaneous albinism and ocular albinism, including Hermansky – Pudlak and Chediak Higashi syndromes. Up to 14 genes have been described to be associated with albinism. These genes encode proteins that are necessary for normal melanogenesis, melanin synthesis, melanosome function, and lysosome related organelle development and function. Over 500 mutations have been reported in genes associated with albinism. However, in most medical institutions or laboratories, genetic diagnostic tests are not available for definite categorization of types of albinism. CIBERER, the Biomedical Network Research Centre on Rare Diseases in Spain has attempted to develop a universal genetic diagnosis for all types of albinism. This approach uses Sequenom iPLEX methodology which combines automated array processing of DNA samples with mass spectrometry. This method can be used to differentiate up to 1000 alleles with known mutations from a given DNA sample. DNA obtained from 2 ml of saliva is sufficient for this analysis. It was an interesting and thought provoking presentation highlighting one of the relatively neglected pigmentary disorders.

**Rudolph Happle** spoke on Patterns and mechanisms of pigmentary mosaicism. It was interesting analysis of various mechanisms of clinically evident pigmentary mosaicism. He explained the mosaicism patterns of narrow band Blaschko lines, broad band types such as McCune Albright syndrome as well as checkerboard pattern and phylloid patterns. Patchy pattern without midline separation is postulated for giant melanocytic nevi. He also spoke of genomic versus epigenetic mosaicism. Genomic mosaics following Blaschko’s lines include pigmentary mosaicism of Ito’s type. Cutis tricolor is a peculiar genomic mosaic characterized by paired hyper and hypopigmented macules reflecting twin spotting or allelic didymosis. Epigenetic mosaics include X inactivation patterns as observed in incontinentia pigmenti. Monoallelic expression (autosomal epigenetic mosaicism) has been proposed to explain an unusual familial constellation of pigmentary mosaicism following Blaschko’s lines. Canine autosomal trait ‘brindle’ was also described and its possible analogy to this syndrome was also debated. Happle’s vast experience in cases of pigmentary mosaicism was clearly evident in the presentation. This presentation was particularly useful for the clinicians and clinical geneticists.

**Caroline Le Poole** spoke on HSP70i as the sole requirement for activating depigmentation in vitiligo prone mice. Previously, they had reported the ability of HSP70i secreted by vitiligo melanocytes in increased amounts, to activate DC mediated cytotoxicity. HSP70i is also accelerated by TRP2 DNA vaccine induced T cell mediated depigmentation in C57BL/6 mice. They had performed a study to challenge the redundancy of HSP70i in vitiligo, using knockout mice lacking constitutive or inducible HSP70. Pmel1 mice transgenic for a T cell receptor reactive with gp100 were subject to vaccination with an expression vector encoding HSP70i versus empty vector DNA. HSP70i knockout mice depigmented in response to TRP2, the response to antigen challenge was impaired in HSP1 knockout mice. Their study suggests a crucial and non redundant role for inducible HSP70i in accelerating vitiligo. The study also demonstrated that vaccination with HSP70i was necessary and

sufficient to induce depigmentation in *Pmel1* transgenic mice. Le Poole and others suggest a crucial role of stress induced HSP70i in vitiligo, and in interfering with this may have significant impact on progress of depigmentation.

**Elisabeth Minder** talked on the use of afamelanotide for treating patients affected by erythropoietic protoporphyria (EPP). The title of the talk was 'Afamelanotide in erythropoietic protoporphyria, a randomized, placebo controlled multicentre phase III trial'. It was a study sponsored by the Clinuvel pharmaceutical company. Phototoxicity and intolerable pain in the skin following exposure to sunlight are main symptoms of EPP. Afamelanotide is a melanocortin 1 receptor agonist and an analogue of alpha MSH, and it has shown beneficial effects in reducing symptoms in EPP. The investigators of their study had administered afamelanotide during a long term clinical assay and collected clinical and laboratory data and evaluated adverse events. The study was a placebo controlled trial. Controlled release controlled release implant was administered every 60 days. The patients were asked to record daily, any adverse events, the time spent in the sun, and EPP related pain level on a standard pain scale (Lickert). Melanin density measurements and laboratory safety controls were done bimonthly. During afamelanotide treatment, EPP patients tolerated sun exposure for several hours a day. They remained pain free for most of the time and severer pain episodes were uncommon. Prolonged use of afamelanotide was well tolerated by all the patients according to the investigators. Overall it was an interesting presentation. If similar results are obtained in larger studies as well, the findings may pave the way to reduce or minimize pain on sun exposure in EPP patients world over.

## **Session 4 : Melanocyte cell biology**

Chair: Graça Raposo and Miguel Seabra

Session X was chaired by M. Seabra from Imperial College London and IGC, Portugal, and G. Raposo from the Department of Cell Biology (CNRS UMR144) in Institut Curie . The session consisted of 3 invited lectures, including those of the two chairs and 1 oral presentation selected from the abstracts. The goal of the session was to give an overview of current topics on the cell biology of the melanocyte and also on the biology of melanocyte interactions with keratinocytes and epithelial cells. Graça Raposo presented studies carried in her group in close collaboration with the group of Michael Marks at UPenn in Philadelphia. The studies presented aim to get novel insights onto the biogenesis of immature and mature melanosomes in melanocytic cells, models of eumelanogenesis. In particular she showed unpublished data revealing that proteins of the Tetraspanin family are involved in the endosomal sorting of *Pmel17*, a process required for the formation of the amyloid fibrillar sheets of premelanosomes. She also showed that late steps of melanogenesis are regulated by protein components whose encoding genes are mutated in the Hermansky Pudlak Syndrome. These late steps of melanosome maturation and melanin synthesis require the specialization of early recycling endosomes through the coordinated action of molecular Adaptors and a microtubule motor. The dialogue between endosomes and melanosomes could be potentially regulated by melanocyte-keratinocyte interactions. Following the first talk on melanocytes, Miguel Seabra presented very recent studies performed to investigate the mechanisms of melanosome transfer from melanocytes to keratinocytes and the fate of melanosomes within keratinocytes. Using high resolution electron microscopy of human skin, he proposed that melanosomes are exocytosed by melanocytes into the extracellular space and the melanosome core is subsequently taken up by keratinocytes. Within keratinocytes, the melanosome core particles were concentrated onto vesicles that rapidly gain late endosomal markers such as LAMP1, suggesting that keratinocyte uptake share similarities to phagosome maturation in macrophages. Janice Brissette (State University of New York Downstate Medical Center, New York City) presented studies of a novel class of epithelial cells, termed 'pigment recipients.' She showed that pigment recipients play an important role in the patterning of pigmentation, as they attract melanocytes and stimulate pigment transfer, thereby engineering their own pigmentation. Pigment recipients acquire their abilities via the *Foxn1* transcription factor, which

induces the recipients to transmit signals to the melanocytes. The signals appear to include diffusible proteins, such as Fgfs, and cell-bound proteins, such as Notch activators.

Finally Mireille van Gele (Dept. of Dermatology, Ghent University Hospital, Belgium) presented a newly developed in vitro reconstructed skin model containing keratinocytes and melanocytes. This model is suitable to study the effect on pigmentation after knockdown of tyrosinase (key-enzyme in melanogenesis) or molecular components (MyoVa ; Rab27 ;melanophilin) involved in melanosome transport.

## **Session 5 : Melanoma biology and therapeutics**

Chair: Richard Marais and Kyoung Chan Park

Not Available

## **Session 6 : Melanocytes, migration and melanoma**

Chair: Lionel Larue and Elisabeth Patton

**Erik Sahai** (London Research Institute, UK) presented evidence of reversible phenotype switching in melanoma using intravital imaging for melanosomes combined with a Brn-2-GFP reporter construct. Sahai and colleagues identified a sub-population of cells containing little or no pigment and high levels of Brn2::GFP expression that are motile in the primary tumour and enter the vasculature. Significantly, the less differentiated state of motile and intravasated cells are not maintained at secondary sites, implying switching between states as melanoma cells metastasize. Thus, a subset of less differentiated cells exits the primary tumour but subsequently give rise to metastases that include a range of more differentiated and pigment producing cells.

**Corine Bertolotto** (INSERM, France) presented evidence that long-term depletion of MITF in melanoma cells, triggers activation of the DNA damage response signaling pathway associated with an up-regulation of p53, and ultimately ends into cellular senescence-like phenotypes. Our findings uncover the existence of a lineage-restricted DNA damage response/p53 signaling pathway in melanoma that is inhibited by MITF to prevent senescence and favor melanoma cell proliferation.

**Liz Patton** (MRC Human Genetics Unit, UK) presented the results of a series of small molecule screens to identify compounds that control melanocyte development in zebrafish. One class of compounds, the nitrofurans, selectively targeted differentiated and melanocyte stem cells (MSCs) in zebrafish development. Patton and colleagues also screened for suppressors of the nitrofuran MSC phenotype and identified the PRL3 phosphatase a new regulator of MSCs. Thus, small molecule screening in zebrafish has identified a series of chemical regulators of melanocyte development, that point to new molecular and therapeutic pathways.

**Marina Mione** (IFOM, Milan) presented a new model of melanoma developed in zebrafish using the Gal4/UAS system to express oncogenic HRAS under the control of the kita promoter. In this model, melanoma developed very early (at 2 weeks) and did not require inactivation of tumor suppressors like p53 or PTEN. The study of melanoma initiating cells, aided by the presence of a GFP tagged oncogene and the use of chemicals to ablate a subpopulation of erbB3+ melanocyte stem cells (Hultman et al., 2009), showed that, in this model, melanoma originates mostly from differentiated larval melanocytes.

**Anna Golovko** (Uppsala University, Sweden) presented evidence that STX17 is upregulated in Grey horse melanocytes and melanomas, with the full-length being the predominant form. Immunofluorescence studies showed a complete co-localization of the STX17 isoforms and their partial co-localization with the ER. Additional work from the laboratory has shown that STX17 mediates ERK signaling in Grey horse melanoma cell cultures (Jiang et al, submitted). Golovko and colleagues now show that ERK1/2 is activated already in melanocytes of Grey horses, which, together with the co-expression of high levels of STX17 in these cells, further strengthens STX17's role in ERK activation

and/or signaling.

## Session 7 : Senescence

Chair: Robert Ballotti and Dorothy C Bennett

Session 7 was centred on cell senescence (permanent growth-arrest following extensive proliferation or oncogene activation), in melanocytes and melanoma. **Daniel Peeper**, who gave the EMBO lecture, focused on oncogene-induced senescence (OIS) caused by BRAF<sup>E600</sup>, as proposed to occur in nevi. He reported the presence of IL6 and IL8 (part of the “senescence messaging secretome”) in benign lesions in vivo. The CDK inhibitor p16<sup>INK4A</sup> has previously been implicated in melanocyte senescence, is upregulated by BRAF<sup>E600</sup> and is strongly expressed in naevi, but not by all naevus cells, suggesting a requirement for other factor(s) for the induction and maintenance of senescence in these cells. p15<sup>INK4B</sup>, another CDK inhibitor, was found to be greatly increased in abundance by BRAF<sup>E600</sup>, but neither p16 nor p15 (nor both) seemed to account fully for BRAF<sup>E600</sup>-induced senescence in human fibroblasts or melanocytes. Interestingly, studies of melanoma arising from a contiguous naevus led to the identification of PTEN as lost in the melanomas, suggesting the PI kinase pathway as regulating senescence. Indeed, BRAF<sup>E600</sup> inhibited AKT3 expression in culture, and PTEN inhibition (resulting in AKT activation) prevented BRAF<sup>E600</sup> induced senescence. Candidate gene and large-scale genomic approaches have been undertaken to identify new signalling networks involved in senescence bypass in BRAF<sup>E600</sup> induced cancers.

**Maria Soengas** gave a very innovative lecture dealing with the role of protein degradation pathways in melanoma development and resistance to chemotherapeutic treatments. Melanoma cells have an overactive metabolism and need efficient mechanisms of clearance of aggregate-prone proteins and damaged organelles. Sequestosome 1 (SQSTM1/p62), a multifunctional protein that links the proteasome to the autophagic machinery, was found to be overexpressed in melanoma compared to nevi. SQSTM1 silencing induced a senescence-like phenotype in melanoma and blocked tumour growth in mice. Furthermore, SQSTM1 localization was modified by treatment with chemotherapeutic agents. Interestingly, SQSTM1 silencing did not affect normal human melanocyte growth. Therefore, these data point to protein degradation pathways and more specifically to SQSTM1 as a potential target for new anti-melanoma drugs.

**Dot Bennett** spoke about a network project with four other British groups and Cancer Research Technology, to develop methods for rapidly screening libraries of chemicals, siRNAs etc, for the ability to induce senescence in cancer cells. This might provide a therapeutic route for cancer types resistant to conventional treatments. Screening methods were initially developed using normal human fibroblasts, and then for A375P melanoma cells, using an Arrayscan digital reader. A screen of a bioactive chemical library with the fibroblasts yielded a number of “hits”, confirmed with biological and protein markers, and three of these also gave stable senescence-like arrest in melanoma cells and also in other types of cancer cells, giving encouraging support for this approach.

Lastly, **Susanne Schiffner** from the group of Anja Bosserhoff gave an oral presentation. She reported on the diffusible factor MIA (melanoma inhibitory activity/protein), often secreted by melanoma cells. They found that it is also produced by most nevus cells, and by normal human senescent melanocytes. When overexpressed in normal human melanocytes, MIA was able to induce markers of senescence including  $\beta$ -galactosidase, p21 and p15. Likewise, knockdown of MIA in human melanocytes apparently delayed senescence. KO of MIA in mice could accelerate the appearance of melanomas. It was proposed that MIA might have some properties of an oncogene, being associated with melanoma cells, but could also contribute to senescence, perhaps as with oncogene-induced senescence.

## Session 8 : Signalling pathways in melanocytes and melanoma cells

Chairs: Colin Goding and Anja Bosserhoff

The session opened with a talk from Alain Mauviel (Paris) on the role of Gli2, a mediator of Hedgehog signaling, in melanoma. He demonstrated that Gli2 expression is transcriptionally upregulated by TGF- $\beta$  signaling, in the most aggressive melanoma cell lines. GLI2 promoted melanoma migration and invasion by controlling a mesenchymal transition characterized by loss of expression of E-cadherin and increased MMP secretion. *GLI2* knockdown in melanoma cells that express high levels of GLI2 significantly inhibited their invasive capacity and the development of experimental bone metastases in mice. *GLI2* expression was shown to be heterogeneous within human melanoma lesions, and associated with tumor regions in which E-cadherin is lost, and was increased in distant metastases as compared to primary lesions. Consistent with this, GLI2 staining by immunohistochemistry of 37 primary melanoma tumors, demonstrated that GLI2 expression was associated with Clark level and with reduced distant metastasis-free survival. Also, GLI2-positive distant metastases were associated with dramatically reduced overall survival. The data presented provided support for a direct role for GLI2 in driving melanoma invasion and metastasis and indicate that GLI2 expression in melanoma tumor lesions may be a marker for poor prognosis.

Vittoria Schiaffino (Milan) discussed the protein product of the ocular albinism gene, OA1, a G protein-coupled receptor localized to intracellular organelles, melanosomes and lysosomes. Recently Lopez et al., 2008, provided evidence for an OA1-mediated signaling pathway triggered by L-DOPA at the plasma membrane and suggested that tyrosine in the medium act as a kind of ligand, able to promote the constitutive internalization and downregulation of the receptor. However, the results from Vittoria's laboratory indicated that amino acid starvation, including cell culturing in tyrosine-free medium, induces the general upregulation of exogenous transgenes, most likely by means of epigenetic modifications. Therefore, the effects of low tyrosine in the medium on the total amount of exogenous OA1 are neither OA1 specific nor tyrosine specific, and this amino acid does not appear to play any role on the intracellular localization of the receptor. Further work will therefore be necessary to identify the ligand for the OA 1 receptor.

Jose Carlos Garcia Borrón (Murcia) discussed the functional properties of natural human MC1R mutants. The major R151C, R160W and D294H *MC1R* alleles associated with red hair, fair skin (the RHC phenotype) and increased skin cancer risk encode for forms with decreased signaling through cAMP, yet they were shown activate the ERKs at least as effectively as wild-type MC1R. This disparate effect of the mutations on the two major signaling pathways originating from the MC1R was unexpected, since it was generally assumed that cAMP is responsible for ERK activation in MSH-stimulated melanocytic cells. After confirming that MC1R-mediated ERK activation in melanocytes and melanoma cells is independent on cAMP by a series of pharmacological studies, an alternative pathway linking MC1R to the ERKs was sought. Pharmacological interference, siRNA and functional reconstitution experiments showed that MC1R activation led to transactivation of the cKIT receptor, thus pointing to an unexpected link between MC1R and KIT signaling in melanocytes and melanoma. Preliminary data suggested that Src is also activated following MSH binding to the MC1R and is located upstream of cKIT in the pathway mediating MC-dependent ERK activation in melanocytic cells. Thus, the cAMP and ERK pathways are not activated sequentially by the MC1R, but rather they are triggered independently. Both pathway display different sensitivity to many natural mutations, with an imbalanced signaling in major RHC alleles.

Ian Jackson spoke about his collaboration with MRC Harwell through which they have identified new mouse mutations that affect the switch between eumelanin and pheomelanin synthesis during hair growth. One of them is a recessive mutation that increases the amount of pheomelanin. The phenotype is very similar to that of a knockout of the *Corin* gene described recently by Enshell-Seijffers, Bruce Morgan and colleagues and the mutation maps to the *Corin* locus. It appears to be a loss of function caused by a genomic duplication leading to an exon duplication, and frame shift, in the



mRNA. The second new mutation was dominant, and had the opposite phenotype; that is it had increased eumelanin. Mapping and sequencing revealed a point mutation in the G-protein alpha stimulatory subunit.

Finally Yoko Funasaka spoke on the role of the metabotropic glutamate receptor subtype 1 (mGluR1), a G protein coupled receptor activated by glutamate that is functionally expressed in the central nervous system. She had shown previously that ectopic expression of mGluR1 in melanocytes is essential for both development and *in vivo* growth of melanoma in transgenic mice. Upon stimulation by glutamate, mGluR1 can couple to multiple signaling pathways through different G proteins, such as activation of phospholipase C (PLC)  $\beta$  that triggers IP<sub>3</sub>-mediated Ca<sup>2+</sup> release and activation of PKC, an increase cytoplasmic cAMP, and activation of ERK1/2 MAP kinase. To understand the contribution of each signaling pathway to melanoma, she studied the effects of inhibitors of phospholipase C, PKC, MEK1/2, and cAMP phosphodiesterase, antagonists to Ca<sup>2+</sup> and calmodulin, and an activator of adenylyl cyclase that were administered to transgenic mice engineered to develop melanoma. With the exception of the cAMP inducers, each inhibitor of PLC, PKC, Ca<sup>2+</sup> release, calmodulin, and MEK1/2 inhibited melanoma development. Although administration of a glutamate release inhibitor suppressed melanoma growth, these signal transduction inhibitors only suppressed melanoma growth partially. The results indicate that for development of melanoma, activation of multiple signals is required, but for melanoma growth, inhibition of several signaling pathways can be compensated by other events downstream of mGluR1.

## Session 9: Genetics of melanocyte and melanoma development

Chair: Keith Hoek and Julia Newton Bishop

This session began with a presentation by **Patrik Ernfors** (Karolinska Institute, Sweden) on “Migratory pathways and cellular mechanisms of melanocyte development”. Patrik described his studies of melanocyte development from the neural crest and showed data which challenges traditional theories of melanocyte origin: proposing that melanocytes may also evolve from Schwann cell precursors. This talk was followed by one given by **Ze’ev Ronai** (Sanford-Burnham, USA) who discussed an emerging role for the transcription factor ATF2 in regulating MITF and melanoma development. This ubiquitously expressed protein is crucial for AP1 dimerization and therefore regulates cell cycle progression through the transcriptional control of several key genes. **Gloria Ribas** (Fundacion Investigacion Hospital Clinico, Spain) then presented a candidate gene study designed to identify inherited pigment genes acting as susceptibility genes. An Illumina platform in which 384 SNPs in 65 genomic regions was used to screen 1294 Spanish melanoma cases and 1194 controls. The study confirmed roles for *SLC45A2* and *TYR* in melanoma susceptibility. **Marie-Dominique Galibert** (CNRS UR6061 – IGDR, France) presented data which suggested that increased expression of TYRP1 plays a role in melanoma progression, showing that its knock-down in cell lines both precipitated cell cycle arrest and affected migration rates. Finally **Aaron Smith** (University of Queensland, Australia) talked about his work on NR4A nuclear receptors in melanocytes. NRF4 is a member of the nuclear receptor superfamily of transcriptional receptors, and Aaron showed evidence that the protein mediates normal and oncogenic signalling in melanocytes. Specifically his data support the view that NR4A gene expression is rapidly upregulated by wild type MC1R signaling and that it mediates MC1R-driven repair of UV-induced DNA damage.

## Session 10 : The sun, DNA damage and epidermal biology

Chair: Markus Böhm and Yoko Funasaka

By Markus Böhm

This session consisted of 4 invited talks. In the first talk, **Dr. David A. Gillespie** (from Beatson Institute for Cancer Research, UK) introduced checkpoint kinase 1 (chk1) as an important regulator of

experimental tumor development. Genetic ablation of *chk1* in a mouse model of chemical epidermal carcinogenesis (employing DMBA as a tumor initiator and TPA as a tumor promoter) had different effects depending on whether *chk1* deletion is homozygous or hemizygous. Complete loss of *chk1* was incompatible with epithelial tumorigenesis while partial loss of function promoted benign-malignant tumor progression.

**Dr. Markus Böhm** (University of Münster, Germany) highlighted modulatory effects of KdPT, a small tripeptide derivative of the last 3 C-terminal amino acids of alpha-MSH, on proinflammatory cytokine expression (IL-6 and IL-8) in normal human melanocytes and keratinocytes exposed to UVB irradiation. KdPT was previously shown not to mediate its effects via binding to MC1R. It suppressed IL-1beta-induced generation of reactive oxygen species and NF-kB signaling. Potential target structures and receptors for this small molecule, e. g. oligopeptide transporters, were discussed.

In the next talk, **Dr. Julia Newton-Bishop** (from University of Leeds, UK) reported that sunburn and intermittent sun exposure are risk factors for melanoma incidence by meta-analysis of case-control studies. She reported evidence from a new-case-control study that suggested that higher levels of regular weekend sun exposure was associated with reduced risk of melanoma in the UK and discussed the hypothesis that vitamin D resulting from that sun exposure might be protective for melanoma. Furthermore she hypothesized that sun exposure may be both causal and protective for melanoma depending on phenotype and pattern of sun exposure,

Finally, **Dr. Nicolas Mouchet** (from CNRS UMR6061, France) reported their study of whole genome microarrays in the skin of Caucasian patients with phototypes II or III five hours after irradiation by solar simulated light at 2 and 4 J/cm<sup>2</sup> *in vivo*. The results indicated a strengthening of the inflammation process and up-regulation of the JAK-STAT pathway, and parallel to the p53 pathway, the p38 stress-responsive pathway was affected. In *ex vivo* assay using a specific inhibitor of p38 (SB203580), he identified new direct p38 target genes. Their findings provided further insight into the physiological response to UV, including cell-cell interactions and cross-talk effects.

By Yoko Funasaka

**Dr. Newton-Bishop** (from University of Leeds, UK) reported that sunburn and intermittent sun exposure are risk factors for melanoma incidence by meta-analysis of case-control studies. She reported evidence from a new-case-control study that suggested that higher levels of regular weekend sun exposure was associated with reduced risk of melanoma in the UK and discussed the hypothesis that vitamin D resulting from that sun exposure might be protective for melanoma. Furthermore she hypothesized that sun exposure may be both causal and protective for melanoma depending on phenotype and pattern of sun exposure.

**Dr. Mouchet** (from CNRS UMR6061, France) reported their study of whole genome microarrays in the skin of Caucasian patients with phototypes II or III five hours after irradiation by solar simulated light at 2 and 4 J/cm<sup>2</sup> *in vivo*. The results indicated a strengthening of the inflammation process and up-regulation of the JAK-STAT pathway, and parallel to the p53 pathway, the p38 stress-responsive pathway was affected. In *ex vivo* assay using a specific inhibitor of p38 (SB203580), he identified new direct p38 target genes. Their findings provided further insight into the physiological response to UV, including cell-cell interactions and cross-talk effects.

## **Workshop A – Pigment patterns – their formation and evolution**

Chair: Greg Barsh and Nick Mundy

Not available

## Workshop B - Function and formation of the melanosome

Chair: José Carlos Garcia-Borrón, Marco d'Ischia

The symposium featured two invited lectures (delivered by M. d'Ischia, J.N. Rodriguez-Lopez) and 3 short communications (presented by K. Beaumont, F. Giordano, and A. Napolitano). It was attended by several interested participants with stimulating discussions.

The protective role of epidermal melanocytes against ultraviolet (UV) radiation and oxidative stress is believed to depend, among other factors, on functionally active MC1R receptors, competence for eumelanin synthesis and efficient melanosome transfer to keratinocytes. However, a central issue concerns the actual biological role and function of dopachrome tautomerase (Dct or Tyrp2), the enzyme responsible for the synthesis of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) in the eumelanin pathway. In the opening lecture by Marco d'Ischia and coworkers new chemical data were reported showing that both 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and its main circulating metabolite, 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MICA) are potent H-atom donors, ferric ion reducing agents and OH radical scavengers, suggesting that these indoles may serve protective antioxidant functions distal to the site of eumelanin synthesis. These results provide a convincing background to propose that the actual significance of Dct is related to the dual role of DHICA as important determinant of eumelanin protective action and as diffusible antioxidant and chemical messenger, partly in the form of 6H5MICA.

Human melanoma is characterized by its resistance to treatment by most chemotherapeutic agents, including antifolates. The group led by **Jose Neptuno Rodriguez-Lopez** at the University of Murcia is actively involved in the development of a second generation of low-toxicity antifolate drugs for the treatment of melanoma. Prof. Rodriguez-Lopez presented evidence showing that the ester-bonded gallate catechins isolated from green tea, epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG), are potent inhibitors of DHFR activity at concentrations found in the serum and tissues of green tea drinkers. However, the excellent anticancer properties of tea catechins are significantly limited by their poor bioavailability, which is related to both their low stability and their permeability characteristics. A synthetic 3,4,5-trimethoxybenzoyl analogue of ECG (TMECG) exhibited high and quite specific antiproliferative activity against malignant melanoma by acting as a prodrug selectively activated by tyrosinase. Upon activation, TMECG generated a stable quinone methide that strongly and irreversibly inhibited DHFR, and triggered apoptosis of melanoma cells. Dr Rodriguez-Lopez also presented promising results obtained in a melanoma animal model.

**Kimberley A. Beaumont**, from the Institute for Molecular Bioscience, University of Queensland, presented a talk on the regulation of melanosome trafficking by Rab GTPases. The role of several members of this family such as Rab27a in the regulation of melanosome movement is well established and highlighted by the pigmentation phenotype of mutant mice. However, the role of Rab GTPases in other steps in melanosome maturation, remains obscure. Dr Beaumont presented interesting new data on Rab17 which localizes to recycling endosomes and melanosomes, and whose expression is regulated by MITF. Analysis of melanosome number, morphology, maturation and subcellular location in melanoma cells treated with Rab17 siRNA suggest a role of Rab17 and the recycling endosome in melanosome release. It will be interesting to see if Rab17 also participates in melanosome transfer to keratinocytes.

**Francesca Giordano**, from Graça Raposo's lab at the Institut Curie in Paris, reported new data on the intracellular sorting of the OA1 protein. This is a unique G protein coupled receptor, most intriguing in that it is located within the melanosomes and lysosomes, with the putative ligand binding site oriented to the lumen of the organelle. The details of OA1 trafficking to these target organelles are mostly unknown, and therefore information on this topic is welcome. According to the data presented by Dr Giordano, OA1 can be ubiquitinated and its sorting and degradation requires functional Endosomal Sorting Complex Responsible for Transport (ESCRT) components that may recognize the ubiquitin-modified OA1 receptor. The mature of the putative ubiquitin E3 ligase responsible for OA1

ubiquitination, as well as the details of the interaction of ubiquitinated OA1 and ESCRT remain to be determined, but in any case the talk opened a new perspective for the study of protein trafficking to the melanosome.

Investigation of the main structural units of pheomelanins is of critical importance for an understanding of the basic photophysical and photochemical properties of these pigments. **Alessandra Napolitano** and her group reported the results of new biomimetic studies and oxidative degradation experiments showing for the first time that human red hair pheomelanin contains dihydroisoquinoline subunits linked to a benzothiazole ring. Model studies on isoquinoline-containing dimers isolated by biomimetic oxidation of 5-S-cysteinyldopa revealed absorption, chemical and spectral features closely resembling those of the pheomelanin polymer. These results fill an important gap in our knowledge of pheomelanin structure and provide a new basis to look at the mechanisms of UVA-induced phototoxicity and photocarcinogenesis in red haired individuals.

## **Workshop C - Vitiligo**

Chair: Mauro Picardo and Alain Taïeb

A. Taieb reviewed last year advances in the field and presented in more details the possible scenario of vitiligo with an initiation phase (silent) a clinical phase with an acceleration phase (inflammatory), and a sequellar phase, with several cycles throughout a patient's life. This scenario is based on the recent breakthroughs in the understanding of other chronic inflammatory skin diseases such as atopic dermatitis. Animals models especially new mouse models reproducing the autoimmune/inflammatory phase of vitiligo are giving clues for possible interventions in humans especially anti interferon gamma strategies. However, RCT looking at immunosuppressant effects in histologically inflammatory vitiligo should first be implemented, and methotrexate would be a good candidate.

M Picardo reviewed the latest findings in oxidative stress and lipid modifications in melanocytes grown in vitiligo patients, emphasizing an increased cholesterol level. A role of cholesterol on G protein receptors modulating ROS production is hypothesized. Recent literature confirms that antioxidant enzymes are upregulated in vitiligo melanocytes and that this increased basal level may limit responses to further stresses. The transcription factor nrf2 important to control antioxidant enzyme levels is modulated by alpha MSH signalling. The activation of PPAR gamma receptors by alpha MSH may also prove to be an interesting target for melanocyte function, since prostaglandins are ligands of these receptors.

D Parsad showed recent findings of cultured melanocytes from the border of active lesions showing aspects compatible with senescence, leading to less adhesion to substrates. Hydroxycholesterol is implicated in senescence. A recent study on LXR-alpha indicates increased levels in perilesional skin together with decreased gelatinase levels.

Y Gauthier showed recent data using the guinea pig model to induce marginal repigmentation with EDTA or 5 Fluorouracil, which seem to work by increasing intercellular spaces between keratinocytes within interfollicular epidermis, which allows a better migration of melanocytes. The effect of needling (creating a tunnel in the basal layer of the epidermis with a needle and secondary insufflation) was also presented. This presentation stimulated a larger discussion, especially on antioxidant role of EDTA and downregulation of cadherins and integrins.

S Moretti reported on a study comparing two groups of nonsegmental vitiligo patients, one with biologic evidence of serum autoantibodies and another without, indicating that the Koebner phenomenon would be associated to the non autoimmune group. The concept of autoimmune vitiligo was largely discussed and the opportunity to put this issue on the global issues consensus conference for the next IPCC was envisaged.

V Eleftheriadiou reviewed her work done at the centre for evidence based dermatology in Nottingham to set the priorities for clinical research in vitiligo. The survey indicated 660 treatments uncertainties submitted by 460 participants. A final Prioritisation Workshop ranked first a RCT in nonsegmental vitiligo with an immunosuppressant.

## HISTORY OF PIGMENT CELL RESEARCH IN EUROPE

IVth and Vth European Workshop on Melanin Pigmentation (EWMP)

By Jan Borovansky & Patrick A. Riley

*„Farewell, Edinburgh, and a´ your glittering wealth; Your Bernard´s Well, your Carlton hill, where every breeze is health; An´ spite o´ a´ your fresh sea-gales should any chance to dee, It´s no for want o´ recipe, the doctor, or the fee.“ (Edinburgh by Caroline Oliphant (1766))*

The IVth EWMP took place in Edinburgh on September 21-23, 1982. It was organized by Professor J.A.A. Hunter. It consisted of 37 lectures and 22 posters divided into 6 sessions:

1. Structure and function of the melanocyte (chaired by P. Fritsch and J.P. Ortonne)
2. Genetics of pigmentation (chaired by H. Kacser)
3. Hormonal control of melanogenesis (chaired by A.J.Thody)
4. Biology of malignant melanoma (chaired by J. Duchon and S.S. Bleehen)
5. Discussion of all posters (chaired by J.A.A. Hunter and P.A. Riley)
6. Treatment of malignant melanoma (chaired by N. Cascinelli and J.F. Smyth)

In recollection it was a windy meeting. The express train from London was delayed by several hours due to high winds, and in order to consult a street map it was necessary to seek shelter in a phone box. The workshop was held in the University of Edinburgh's Department of Dermatology which was situated in the newly reconstructed Hospital. Most of the building was empty and, after sunset, gave a strong impression of a bleak and lonely house. Only the lights on the 3rd floor radiated warmth and gave impression to passing pilgrims that something important was happening. And indeed, the workshop continued the customary tradition of first class science and friendly festive interaction. During the Discussion of the posters the lights failed and the entire session was conducted in the pitch dark with an eerie wind howling behind the windows which deepened a special romantic mood.



*Fig. 1: Prof. J.A. Hunter (on the left) having just accepted the task of organising of the IVth EWMP reacted by immediately lighting a cigar (next to him are J.P. Ortonne and J. Duchon). The picture was taken in the cellar of a winery school in Melnik, Czechoslovakia, in September 1981).*

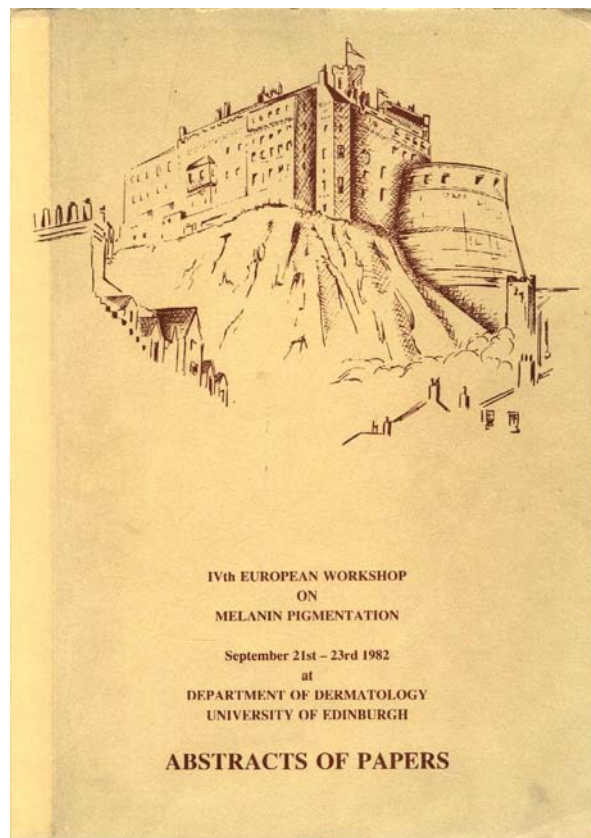


Fig.2: Book of Abstracts , IVth EWMP, 1982.

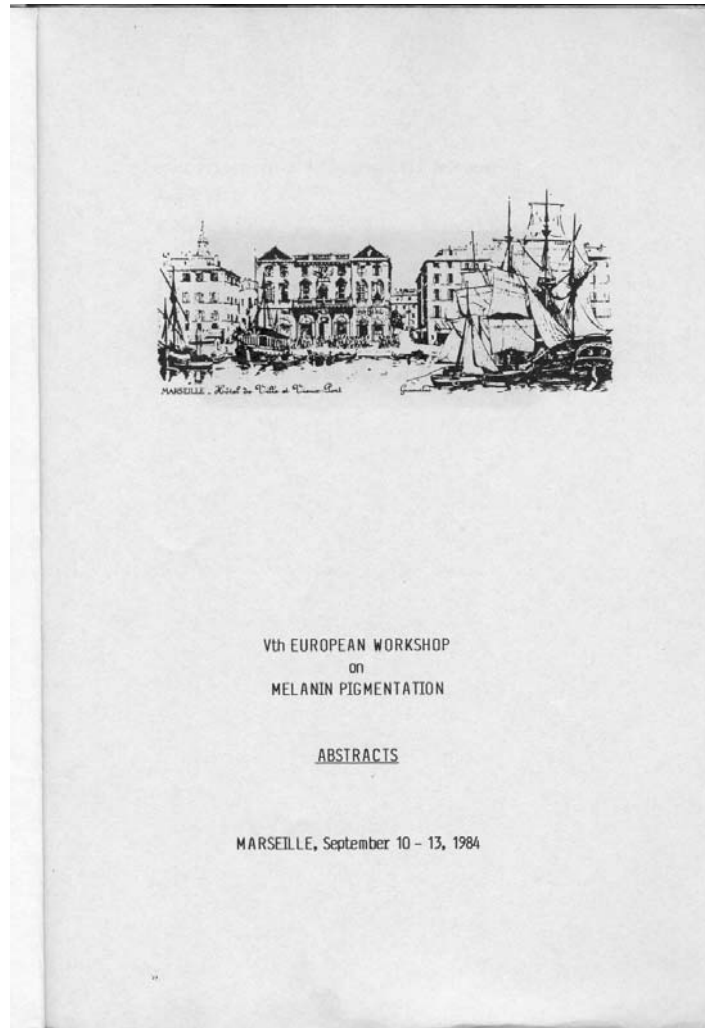
*"Virtue, my dear, is an abstract idea, varying in its manifestations with the surroundings. Virtue in Provence, in Constantinople, in London, and in Paris bears a different fruit, but is none the less virtue."* (Honoré De Balzac (1799-1850) Letter to Louise de Chaulieu).

**The V<sup>th</sup> EWMP** took place in Marseille on September 10-13, 1984. It was organized by Professor Ch. Aubert and Dr. Françoise Rougé. It consisted of 52 lectures and 31 posters divided into 7 charming brilliant sessions:

1. Pigment cell biology (chaired by W.C. Quevedo and S.S. Bleehen)
2. Enzymatic and hormonal control of melanogenesis. (chaired by R.A. King and J.A. Lozano)
3. UV light and pigmentation (chaired by B. Jacquet and F. Serri)
4. Melanin structure and biosynthesis (chaired by H. Rorsman and J. Duchon)
5. Recent advances in the biophysical studies of melanin (chaired by T. Sarna and A. Tosti)
6. Cytotoxic mechanisms in relation to pigment cells (chaired by P.A. Riley and S.Pavel)
7. Melanoma. (chaired by F.J. Lejeune and S. Sparrow).

V<sup>th</sup> EWMP was held in the Hotel Sofitel with a beautiful view over the Old Port. The meeting was opened by the Mayor of Marseille at the town hall and for many young participants it was their first experience of pastis. Provence was sunlit during the whole meeting and many participants failed to adhere to the general rule of melanoma prevention and, especially during the boat trip to Les Calanques, overlooked the application of sun protection. Fortunately, a vineyard and a chalet nearby offered some shade. Prof. Hans Rorsman proved to be the best wine conaisseur among the participants. Prof. Aubert presented each participant with a carved wooden plaque showing traditional Marseillaise fishing boats and bearing his/her name. Provence has continually attracted artists and painters and Prof. C.

Aubert belongs among them – see his still life drawn using a special technique – the dopa reaction.



*Fig.3:* Book of abstracts of the 5th EWMP.



*Fig.4:* On the boat: Dr. Ito and A. Wittbjer



*Fig.5: Dopa flowers by C. Aubert.*





## 1. Chemistry of Melanins and other Pigments

(Pr A. Napolitano)

A number of papers have appeared focused on the design and properties of melanin-based materials. Therefore these will be listed under a separate heading from now on. Among these, polydopamine films which have been studied as functional coatings for a variety of surfaces. Improved properties for these films in terms of transparency and ion permeability have been obtained by alternating deposition of dopamine-melanin particles with poly(diallyldimethylammonium chloride) (Bernsman et al ChemPhys Chem). Impedance spectra and zeta potential titration curves have also been recorded to get an insight into the electrical properties and surface chemistry of such films (Ball V Colloids and Surfaces) Polydopamine membranes were fabricated by dipping Stainless Steel substrates into alkaline dopamine solutions, fully characterized (by scanning electron microscope (SEM), energy dispersive X-ray spectroscopy (EDS), surface reflection Fourier transform IR spectrum (SR-FTIR), UV-visible spectrum) and the corrosion behaviour examined (Yu et al J. Molecular Structure). Full characterization of nanocomposite materials prepared by laponite promoted oxidation of L-Dopa has been reported (Jaber et al J. Chem Phys Lett). Hybrid materials have also been obtained from electrochemical polymerized polydopamine and single walled carbon nanotubes. Wrapping of the nanotubes with polymers resulted in a decrease of the impedance of such composite electrode and an increase of the rate of electron transfer from the electrolyte to the electrode ( Ben-Valid et al, Carbon).

Another field receiving increasing interest is the exploitation of black pigments from plant sources which are now currently referred to as plant melanins. Extraction technologies have been set up for extraction of melanin from soy sauce waste (ren et al ) Auricularia auricula fruit bodies (Zou et al Innovative Food Science & Emerging Technologies) and black waxy corn (Ma et al Faming Zhuanli Shenqing).

As usual, a variety of new melanogenesis inhibitory substances or plant extracts have been reported, among which of particular interest sulfurated derivatives of resorcinol with depigmenting, antioxidant and antibacterial properties (Poigny S Fr. demande).

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## 2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

The Aryl hydrocarbon receptor (AhR) is a member of the family of basic-helix-loop-helix transcription factors. AhR is a transcription factor that is normally inactive; upon ligand binding AhR translocates to the nucleus, dimerizes with ARNT, enabling the complex to bind to DNA recognition sequences, and eventually initiates gene transcription. Since there are several recent evidences demonstrating that AhR activation affects cell proliferation, differentiation, and apoptosis, an increasing number of studies focus on AhR-dependent modulation of cell and organ-specific functions. Two distinct papers (Jux et al., Luecke et al.) described the involvement of aryl hydrocarbon receptor in skin tanning. The paper from Jux et al., demonstrated that AhR plays a role in pigment formation by regulating the expression of genes coding for enzymes of the melanogenic pathway. UV light, the major physiological stimulus for melanogenesis, activates the aryl hydrocarbon receptor via a specific bioactive signal molecule that is formed in response to light. The study, from Luecke et al., using AhR-deficient mice showed a significantly weaker tanning than wild-type mice. Interestingly, in these mice, tyrosinase activity in the epidermis was lower as well. Tanning responses and tyrosinase activity, however, were normal in keratinocyte-specific conditional AhR knockout mice, indicating that release of melanogenic keratinocyte factors is unaffected by the UVB-AhR signaling pathway and that the diminished tanning response in AhR<sub>-/-</sub> mice is confined to the level of melanocytes. Accordingly, the number of dihydroxyphenylalanin-positive melanocytes increased significantly less on UVB irradiation in AhR<sub>-/-</sub> mice than in wild-type mice. This difference in melanocyte number was associated with a significantly reduced expression of stem cell factor-1 and c-kit in melanocytes of AhR<sub>-/-</sub> mice. In conclusion, In conclusion both studies proposed a previously unknown role of the environmental signal sensor AhR links solar UVB radiation to skin pigmentation.

Botton et al., demonstrated that the thiazolidinedione ciglitazone inhibited, independently of PPAR $\gamma$  activation, melanoma cell growth. Interestingly, ciglitazone effects are mediated through the regulation of secreted factors. Q-PCR screening of several genes involved in melanoma biology reveals that ciglitazone inhibits expression of the CXCL1 chemokine gene. CXCL1 is overexpressed in melanoma and contributes to tumorigenicity. Ciglitazone induces a diminution of CXCL1 level in different human melanoma cell lines. This effect is mediated by the downregulation of microphthalmia-associated transcription factor, MITF, the master gene in melanocyte differentiation and involved in melanoma development. Further, recombinant CXCL1 protein is sufficient to abrogate thiazolidinedione effects such as apoptosis induction, whereas extinction of the CXCL1 pathway mimics phenotypic changes observed in response to ciglitazone. Finally, inhibition of human melanoma tumor development in nude mice treated with ciglitazone is associated with a strong decrease in MITF and CXCL1 levels. The study proposed an anti-melanoma approach involving an inhibition of the MITF/CXCL1 axis.

Herraiz et al., described a new functional link between the stem cell factor receptor and Melanocortin 1 receptor (MC1R), a Gs protein-coupled receptor expressed in melanocytes. Upon stimulation by  $\alpha$ MSH, MC1R triggers the cAMP and ERK1/ERK2 MAPK pathways. In mouse melanocytes, ERK activation by  $\alpha$ MSH binding to Mc1r depends on cAMP, and melanocytes are considered a paradigm for cAMP-dependent ERK activation. However, human MC1R variants associated with red hair, fair skin [red hair color (RHC) phenotype], and increased skin cancer risk display reduced cAMP signaling but activate ERKs as efficiently as wild type in heterologous cells, suggesting independent signaling to ERKs and cAMP in human melanocytes. MC1R signaling activated the ERK pathway in normal human melanocytes and melanoma cells expressing physiological levels of endogenous RHC variants. ERK activation is comparable for wild-type and mutant MC1R and is independent on cAMP because it is neither triggered by stimulation of cAMP synthesis with forskolin nor blocked by the adenylyl cyclase inhibitor 2',5'-dideoxyadenosine. Pharmacological interference, small interfering RNA studies, expression profiles, and functional reconstitution experiments showed that  $\alpha$ MSH-induced ERK activation resulted from Src tyrosine kinase-mediated transactivation of the stem cell factor receptor, a receptor tyrosine kinase essential for proliferation, differentiation, and survival of melanocyte precursors, thus demonstrating a functional link between the stem cell factor receptor and MC1R.

The next goal for the translational research will arise from the regenerative approach. Accordingly, relevant impact should be assigned to the definition and characterization of the stem compartment. Usually, for the melanocyte lineage the hair follicle is considered the stem reservoir. Recently, Ling et al published clear indications on the dermal stem melanocytes providing their phenotypic and functional features. The paper opens new perspectives on vitiligo pathogenesis and consequent molecular-oriented approaches. Further new insights are provided by the genomewide study of Quan et al on the possible vitiligo gene association within MHC region. He identified a possible association with IBD.

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### 3. MSH, MCH, other hormones, differentiation

(Pr M. Böhm)

#### What's hot and new?

##### **A negative feedback loop for $\alpha$ -MSH-MC1R-mediated signaling identified in melanocytes.**

The intracellular amount of the ubiquitous signal transduction messenger cAMP, following activation of Gs-stimulating G protein-coupled receptors, is known to be controlled by phosphodiesterases. (PDEs). These enzymes orchestrate the magnitude, duration and subcellular localization of cAMP. Since cAMP is a crucial mediator within the  $\alpha$ -MSH-MC1R-MITF pathway that regulates melanocytes differentiation, melanogenesis, proliferation as well as the dermal tanning response. In a recent research communicated by Khaled et al. (Genes Dev. 2010; 24: 2276-81) PDE4D3 was identified as a direct target of the MSH-cAMP-MITF pathway. Using gene ablation of this enzyme, the authors showed that PDE4D3 expression is MITF-dependent. By reporter-promoter assays as well as chromatin immunoprecipitation assays it could further delineated that MITF directly acts at PDE4D3 promoter to induce expression of its gene. Most interestingly, PDE4 inhibition synergized with forskolin to induce skin pigmentation in redhead/fair-skinned mice. In summary, the identified negative homeostatic of PDE4D3 pathway highlights a potent mechanism controlling melanocyte differentiation that may be amenable to pharmacologic manipulation for skin cancer prevention.

##### **Endogenous expression of chimeric MC1R-TUBB3 isoforms in human melanoma cells.**

Alternative splicing is a well known mechanism how higher eukaryotes increase their repertoire of proteins derived from a limited number of genes. In a recent paper by Dalziel et al. (Nucleic Acids Res. 2010, Epub ahead of print) an interesting additional mechanism, i. e. splicing between adjacent genes, was investigated with regard to the MC1R gene. Using HEK293 cells transfected with various MC1R minigenes, the MC1R gene was first identified to contain an unusual and highly complex poly(A) site (PAS) with rigid positional and structural arrangements of four individuals sequence components. Such structural arrangements were not identified within the MC4R gene. Interestingly, MC1R transcripts are not cleaved at the PAS and can create chimeric MC1R-TUBB3 transcripts due to the immediate downstream neighbour locus tubulin- $\beta$ -III (TUBB3). Interestingly, these transcripts produced two distinct protein isoforms localizing to the plasma membrane and the endoplasmic reticulum. Melanoma cells expressing functional MC1R responded to  $\alpha$ -MSH or activation of the stress response kinase p38-MAPK with a shift in expression from MC1R in favour of chimeric MC1R-TUBB3 isoforms. The authors propose that expression of such chimeric MC1R proteins may also equip normal human melanocytes with cellular phenotypes required as part of the pigmentation response.

##### **Signaling cross-talks between MC1R and cKIT in normal human melanocytes.**

In previous reports it was shown that in mouse melanocytes (melanoma cells) stimulation by  $\alpha$ -MSH via cAMP activates the ERK1/ERK2 MAPK pathway. However, earlier work has suggested that this pathway may be different in normal human melanocytes. In a recent report by Herraiz et al. (Mol Endocrinol. 2010, Epub ahead of print) the impact of  $\alpha$ -MSH and artificial cAMP inducers were examined on activation of ERK1/2 and the mast cell / stem cell factor cKIT was examined in normal human melanocytes expressing wild-type MC1R and MC1R variants associated with red hair and fair skin. Surprisingly, cells expressing MC1R variants were found to activate ERKs as efficiently as wild type in heterologous cells suggesting independent signaling to ERKs and cAMP in human melanocytes. In accordance with earlier work (e. g. Böhm et al., Cell Growth Diff. 1995; 6: 291-302) ERK1/2 activation was independent on cAMP because it was neither triggered by forskolin nor blocked by the adenylyl cyclase inhibitor 2',5'-dideoxyadenosine. Interestingly, pharmacological interference, small interfering RNA studies, expression profiles, and functional reconstitution experiments revealed that  $\alpha$ -MSH-induced ERK activation resulted from Src tyrosine kinase-mediated transactivation of cKIT. The described transactivation phenomenon of the cKIT-ERK pathway in melanocytes expressing natural MC1R mutations appears to be unique and may point towards unexpected functional connection between the GPCR- and RTK-mediated signalling.

## 4. Photobiology

(Dr N. Smit)

The “indoor tanning business” once more is the topic of discussing in a number of papers that appeared in the recent literature (3, 7, 10, 12, 20, 34 and 37). Hery et al describe estimated annual percent changes (EAPCs) in the melanoma incidence in Iceland. EAPCs in men and women on the trunk have increased since 1954 and especially in women (aged less than 50) a sharp epidemic increase was observed after 1995. Use of sunbeds in Iceland expanded rapidly after 1985 and a connection therefore seems plausible. Cust et al analysed data from the Australian Melanoma Family Study and the association with sunbed use was especially strong for melanoma diagnosed at early age and with more frequent use. Lazovich et al studied a population of melanoma patients in Minnesota and found adjusted odds ratio for UVB-enhanced and primarily UVA-emitting devices of 2.86 and 4.44, respectively. Veierod et al discuss the beneficial effects of sun exposure for the production of vitamin D. They indicate that the mean irradiance from solariums is higher than that from the summer sun in Oslo and they recommend that restriction of solarium use is maintained. Grant and Schuitemaker describe that serum levels of 25-hydroxyvitamin D for optimal health would be 100-150 nmol/L. To increase the production to this level would only pose minimal increased risks of melanoma or skin cancer. Fisher (10) does not think that the skin vitamin D synthesis is a justifiable defense of indoor tanning and that strict regulation of this industry may offer one of the best ways of cancer prevention. Martinez-Levasseur et al studied sunburn and photoprotection in mammalian wildlife by photographic and histological surveys of different whale species and conclude that darker pigmentation is advantageous for the whales as in humans. Miyamura et al studied the benefits of facultative pigmentation. They found that unlike the UVB induced tanning, the pigmentation resulting from UVA treatment essentially provided no photoprotection. In this respect the findings of Mahmoud et al could be of interest since they describe pigment induced by visible light that also has different qualitative and quantitative properties than the pigment produced by UVA1 light in skin type IV-VI individuals. No pigmentation was induced in skin type II. The melanin type and content therefore will be important for the prevention of UV induced DNA damage. UVA lamps are known to generate reactive oxygen species. The paper by Jenkins et al describes a role for p16 as a regulator of oxidative stress. Melanocytes were found more susceptible to oxidative stress than fibroblasts and keratinocytes which might explain why the loss of p16 predisposes especially the melanocytes to cancerogenesis. Furthermore the paper by Wang et al in the PNAS describes the role of melanin in the production of ROS in melanocytes and the subsequent generation of oxidative DNA damage lesions. UVA induces much higher amount of such lesions in melanocytes than in fibroblasts probably due to the presence of melanin and the melanocytes showed a reduced repair capacity. The negative effects of (UVA)solaria, the role of the p16 tumor suppressor as a regulator of oxidative stress and the role of melanin mediating ROS production suggests an important role for the anti-oxidant defence of the pigment cells. In this light melanoma prevention may be achieved by antioxidants and dietary nutrients as proposed in the papers by Jensen et al and Sapkota et al.

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## 5. Neuromelanins

(Pr M. d'Ischia)

The biological roles of neuromelanin in relation to neuronal degeneration and the interaction of the pigment with metal ions are important themes in research on Parkinson's disease. Tousi et al. (2010) addressed the effects of neuromelanin on astroglial cells, an issue that has remained so far little explored, and showed that the pigment inhibits TNF $\alpha$ -induced expression of interferon  $\gamma$ -inducible protein-10 (CXCL10). Although the actual relevance of these results to the in vivo situation remains to be demonstrated, the new data point to different roles of neuromelanin in the brain, being largely cytoprotective within dopaminergic neurons, stimulating on microglia when released from damaged neurons and inhibitory on inflammatory signaling in astroglial cells. Porter et al. (2010) investigated the mechanism of coordination of dopamine and 5,6-dihydroxyindole, a putative building block of neuromelanin, with ferric ions in the presence of various ligands. Data showed that both compounds can form strong complexes that account for the notorious iron binding properties of neuromelanin.

Finally Chwiej (2010) reported use of X-ray fluorescence microscopy for two-dimensional elemental analysis of substantia nigra tissue and confirmed the statistically significant differences in elemental composition of Parkinsonian cells versus control nerve cells.

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Abstract : The role of iron in the pathogenesis of the Parkinson's disease (PD) is a current subject of research in Neurochem., since an abnormal increase in iron is reported in the substantia nigra (SN) of Parkinsonian patients. A severe loss of the cells contg. dopamine in the SN in the PD has also drawn attention towards the function of a brown-black pigment called neuromelanin, which accumulates predominantly in these dopaminergic neurons. The neuromelanin has an ability to chelate metal ions, which, in free state, may cause considerable damage to cells by reacting with their lipid-rich membranes. However, it could also potentiate free radical prodn. if it releases the bound metal ions. The highly sensitive and non-destructive micro-PIXE method suits best to quantify and map the trace elements in the SN. The accuracy in charge measurement for such microanal. studies is of utmost importance for quant. anal. Since a Faraday cup is usually placed behind the thin biol. sample to measure the charge, the primary and the secondary electrons, knocked out from the sample by traversing ion beam, hamper an exact charge detn. Hence, a new non-interceptive technique was developed for precise charge measurement and for continuous monitoring of beam current.
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**The use of cluster and discriminant analysis in the investigations of the role of trace metals in the pathogenesis of Parkinson's disease.** Journal of Trace Elements in Medicine and Biology (2010), 24(2), 78-88.  
Abstract : X-ray fluorescence microscopy was applied for two-dimensional elemental anal. of substantia nigra (SN) tissue. The samples representing Parkinson's disease (PD) and control cases were examd. at HASYLAB beamline L and at ESRF beamline ID22. Two-dimensional mapping of P, S, Cl, K, Ca, Fe, Cu, Zn, Se and Br was done with the spatial resoln. of 15 and 5  $\mu$ m. The masses per unit area of elements in neuromelanin reach nerve cells of SN were detd. The elemental data were processed using two multivariate techniques, namely cluster and discriminant anal. The statistical methods were used for data redn., both unsupervised and supervised classification as well as for the creation of a model that would simplify case identification based on the elemental anal. of SN tissue. The results of cluster anal. confirmed the statistical significance of the differences in elemental compn. of PD and control SN nerve cells. Based on the results of discriminant anal., the elements (P, Cl, Fe, Cu and Zn) that played the greatest role in the process of differentiation between neurons from examd. groups were detd.
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Abstract : DT-Diaphorase has been proposed to play a neuroprotective role in dopaminergic neurons by preventing aminochrome neurotoxicity. There are several studies supporting this idea, but in all studies, we used dicoumarol, an inhibitor of DT-diaphorase. We have designed and developed two siRNA to silence the expression of DT-diaphorase to study its role in aminochrome metab. We transduced RCSN-3 cells with retroviral particles contg. a pRetroSuper plasmid coding a siRNA for DT-diaphorase. The cells selected in the presence of puromycin generated a stable cell line RCSN-3Nq6 and RCSN-3Nq7 with low expression of DT-diaphorase (27% and 33% of wild type, resp.). A significant cell death was obsd. in RCSN-3 cells expressing siRNA Nq6 and Nq7 for DT-diaphorase when were incubated with 100  $\mu$ M aminochrome during 48 (4- and 3.5-fold, resp.;  $P < 0.01$ ). These results support the protective role of DT-diaphorase against aminochrome neurotoxicity in dopaminergic neurons contg. neuromelanin and show that Nq6 and Nq7 siRNA are very useful tools to study the role of DT-diaphorase in aminochrome metab.

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Abstract : Coordination of the neurotransmitter dopamine (DA) and the metal-binding component of neuromelanin 5,6-dihydroxyindole (DHI) with ferric iron has been studied in aq. soln. in the presence of ancillary ligands contg. amine nitrogen and carboxylate oxygen donor sites. With nitrilotriacetic acid (nta) and ethylenediamine diacetic acid (edda) coligands, coordination of the catecholate ligands DA and DHI is obsd. to be complete at physiol. pH. The resulting complexes of DA have the characteristic two-component electronic spectrum obsd. characteristically for L4Fe(Cat) complexes. The spectrum obtained with DHI consists of a single broad absorption in the visible region. Both DA and DHI are able to coordinate with Fe<sup>3+</sup> in the presence of edta, displacing carboxylate oxygen donors at pH values just above physiol. pH. These results demonstrate the strong affinity of DA and DHI for Fe<sup>3+</sup>, pointing to in vivo complex formation in neuronal mixts. at physiol. pH.
  
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**Neuromelanin inhibits CXCL10 expression in human astroglial cells.** *Neuroscience Letters* (2010), 486(1), 47-50.  
Abstract : Increasing evidence indicates neuroinflammation is instrumental in the pathogenesis of Parkinson's disease (PD). In PD, there is selective degeneration of neuromelanin (NM)-contg. dopamine neurons. Neuromelanin is predominantly cytoprotective within dopaminergic neurons, whereas, NM released from damaged neurons activates microglia. However, the effects of NM on astroglial cells remain largely unknown. Astroglia are essential to neuronal homeostasis and responsive to injury, in part, through secretion of chemokines, including interferon  $\gamma$  inducible protein-10 (CXCL10). Thus, we used an in vitro approach to identify the effects of NM on TNF $\alpha$ -induced CXCL10 expression in human astroglial cells. TNF $\alpha$ -induced CXCL10 expression was inhibited in NM exposed cells. Addnl., TNF $\alpha$ -induced NF- $\kappa$ B activation was inhibited by NM. Given that CXCL10 expression is NF- $\kappa$ B-dependent in human astroglial cells, these findings suggest that NM may inhibit CXCL10 expression, in part, through an NF- $\kappa$ B-dependent mechanism. While the in vivo consequences of NM mediated effects on astroglial CXCL10 expression remain to be fully elucidated, insights obtained in this study further our understanding of the effects of NM on inflammatory signaling in human astroglial cells.

## 6. Genetics, molecular and developmental biology

(Dr F. Beermann)

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**The fibroblast-derived paracrine factor neuregulin-1 has a novel role in regulating the constitutive color and melanocyte function in human skin.** J Cell Sci 123: 3102-3111, 2010.  
Abstract: Interactions between melanocytes and neighboring cells in the skin are important in regulating skin color in humans. We recently demonstrated that the less pigmented and thicker skin on the palms and soles is regulated by underlying fibroblasts in those areas, specifically via a secreted factor (DKK1) that modulates Wnt signaling. In this study, we tested the hypothesis that dermal fibroblasts regulate the constitutive skin color of individuals ranging from very light to very dark. We used microarray analysis to compare gene expression patterns in fibroblasts derived from lighter skin types compared to darker skin types, with a focus on secreted proteins. We identified a number of genes that differ dramatically in expression and, among the expressed proteins, neuregulin-1, which is secreted by fibroblasts derived from dark skin, effectively increases the pigmentation of melanocytes in tissue culture and in an artificial skin model and regulates their growth, suggesting that it is one of the major factors determining human skin color.
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Shortened abstract: Pigment cells of the zebrafish, *Danio rerio*, offer an exceptionally tractable system for studying the genetic and cellular bases of cell fate decisions. Cell lineage analyses using the photoconvertible marker, EosFP, revealed that both melanophores and iridophores develop from a *mitfa*<sup>+</sup> precursor. Taken together, our data reveal a *Foxd3/mitfa* transcriptional switch that governs whether a bi-potent pigment precursor will attain either an iridophore or a melanophore fate.
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Abstract: Keratinocytes contribute to melanocyte transformation by affecting their microenvironment, in part through the secretion of paracrine factors. Here we report a loss of expression of nuclear receptor RXRalpha in epidermal keratinocytes during human melanoma progression. In the absence of keratinocytic RXRalpha, in combination with mutant Cdk4, cutaneous melanoma was generated that metastasized to lymph nodes in a bigenic mouse model. Expression of several keratinocyte-derived mitogenic growth factors (Et-1, Hgf, Scf, alpha-MSH and Fgf 2 ) was elevated in skin of bigenic mice, whereas Fas, E-cadherin and Pten, implicated in apoptosis, cellular invasion and melanomagenesis, respectively, were downregulated within the microdissected melanocytic tumors. We demonstrated that RXRalpha is recruited on the proximal promoter of both Et-1 and Hgf, possibly directly regulating their transcription in keratinocytes. These studies demonstrate the contribution of keratinocytic paracrine signaling during the cellular transformation and malignant conversion of melanocytes.
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**Control of melanocyte differentiation by a MITF-PDE4D3 homeostatic circuit.** *Genes Dev* 24: 2276-2281, 2010.  
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**Clonal analyses reveal roles of organ founding stem cells, melanocyte stem cells and melanoblasts in establishment, growth and regeneration of the adult zebrafish fin.** Development 137: 3931-3939, 2010  
Abstract: In vertebrates, the adult form emerges from the embryo by mobilization of precursors or adult stem cells. What different cell types these precursors give rise to, how many precursors establish the tissue or organ, and how they divide to establish and maintain the adult form remain largely unknown. We use the pigment pattern of the adult zebrafish fin, with a variety of clonal and lineage analyses, to address these issues. Early embryonic labeling with lineage-marker-bearing transposons shows that all classes of fin melanocytes (ontogenetic, regeneration and kit-independent melanocytes) and xanthophores arise from the same melanocyte-producing founding stem cells (mFSCs), whereas iridophores arise from distinct precursors. Additionally, these experiments show that, on average, six and nine mFSCs colonize the caudal and anal fin primordia, and daughters of different mFSCs always intercalate to form the adult pattern. Labeled clones are arrayed along the proximal-distal axis of the fin, and melanocyte time-of-differentiation lineage assays show that although most of the pigment pattern growth is at the distal edge of the fin, significant growth also occurs proximally. This suggests that leading edge melanocyte stem cells (MSCs) divide both asymmetrically to generate new melanocytes, and symmetrically to expand the MSCs and leave quiescent MSCs in their wake. Clonal labeling in adult stages confirms this and reveals different contributions of MSCs and transient melanoblasts during growth. These analyses build a comprehensive picture for how MSCs are established and grow to form the pigment stripes of the adult zebrafish fins.
  
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**Sequence polymorphisms of MC1R gene and their association with depression and antidepressant response.** Psychiatr Genet 2010 (Epub, ahead of print).
  
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**Pineal-specific agouti protein regulates teleost background adaptation.** Proc Natl Acad Sci U S A 2010 (Epub, ahead of print).  
Abstract: Background adaptation is used by teleosts as one of a variety of camouflage mechanisms for avoidance of predation. Background adaptation is known to involve light sensing by the retina and subsequent regulation of melanophore dispersion or contraction in melanocytes, mediated by alpha-melanocyte-stimulating hormone and melanin-concentrating hormone, respectively. Here, we demonstrate that an agouti gene unique to teleosts, agrp2, is specifically expressed in the pineal and is required for up-regulation of hypothalamic pmch and pmchl mRNA and melanosome contraction in dermal melanocytes in response to a white background. floating head, a mutant with defective pineal development, exhibits defective up-regulation of mch mRNAs by white background, whereas nrc, a blind mutant, exhibits a normal response. These studies identify a role for the pineal in background adaptation in teleosts, a unique physiological function for the agouti family of proteins, and define a neuroendocrine axis by which environmental background regulates pigmentation.



## 7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borrón)

- Chaki M, Sengupta M, Mondal M, Bhattacharya A, Mallick S, Bhadra R, Indian Genome Variation Consortium, Ray K. **Molecular and Functional Studies of Tyrosinase Variants Among Indian Oculocutaneous Albinism Type 1 Patients.** *J Invest Dermatol.* 2010 Sep 23.
- Dalziel M, Kolesnichenko M, das Neves RP, Iborra F, Goding C, Furger A. **{alpha}-MSH regulates intergenic splicing of MC1R and TUBB3 in human melanocytes.** *Nucleic Acids Res.* 2010 Nov 11.  
Alternative splicing enables higher eukaryotes to increase their repertoire of proteins derived from a restricted number of genes. However, the possibility that functional diversity may also be augmented by splicing between adjacent genes has been largely neglected. Here, we show that the human melanocortin 1 receptor (MC1R) gene, a critical component of the facultative skin pigmentation system, has a highly complex and inefficient poly(A) site which is instrumental in allowing intergenic splicing between this locus and its immediate downstream neighbour tubulin- $\beta$ -III (TUBB3). These transcripts, which produce two distinct protein isoforms localizing to the plasma membrane and the endoplasmic reticulum, seem to be restricted to humans as no detectable chimeric mRNA could be found in MC1R expressing mouse melanocytes. Significantly, treatment with the MC1R agonist  $\alpha$ -MSH or activation of the stress response kinase p38-MAPK, both key molecules associated with ultraviolet radiation dermal insult and subsequent skin tanning, result in a shift in expression from MC1R in favour of chimeric MC1R-TUBB3 isoforms in cultured melanocytes. We propose that these chimeric proteins serve to equip melanocytes with novel cellular phenotypes required as part of the pigmentation response.
- Garcia-Molina F, Munoz-Munoz JL, Garcia-Molina M, Garcia-Ruiz PA, Tudela J, García-Cánovas F, Rodriguez-Lopez JN. **Melanogenesis inhibition due to NADH.** *Biosci Biotechnol Biochem.* 2010;74(9):1777-87.  
The effect of NADH on melanogenesis under aerobic conditions involves three types of reaction: (a) acting as tyrosinase substrate (a competitive substrate of L-tyrosine and L-DOPA), (b) irreversible inactivation acting as a suicide substrate of tyrosinase, and (c) non-enzymatic reduction of o-dopaquinone by NADH. Under anaerobic conditions, NADH irreversibly inhibits the enzymatic forms met-tyrosinase and deoxy-tyrosinase. In this paper, we kinetically characterize this coenzyme as it acts as a tyrosinase suicide substrate and propose a kinetic mechanism to explain its oxidation by tyrosinase. In addition, the compound is characterized as an irreversible inhibitor of met-tyrosinase and deoxy-tyrosinase.
- Herraiz C, Journé F, Abdel-Malek Z, Ghanem G, Jiménez-Cervantes C, García-Borrón JC. **Signaling from the Human Melanocortin 1 Receptor to ERK1 and ERK2 Mitogen-Activated Protein Kinases Involves Transactivation of cKIT.** *Mol Endocrinol.* 2010 Nov 17.  
Melanocortin 1 receptor (MC1R), a Gs protein-coupled receptor expressed in melanocytes, is a major determinant of skin pigmentation, phototype and cancer risk. Upon stimulation by  $\alpha$ MSH, MC1R triggers the cAMP and ERK1/ERK2 MAPK pathways. In mouse melanocytes, ERK activation by  $\alpha$ MSH binding to Mc1r depends on cAMP, and melanocytes are considered a paradigm for cAMP-dependent ERK activation. However, human MC1R variants associated with red hair, fair skin [red hair color (RHC) phenotype], and increased skin cancer risk display reduced cAMP signaling but activate ERKs as efficiently as wild type in heterologous cells, suggesting independent signaling to ERKs and cAMP in human melanocytes. We show that MC1R signaling activated the ERK pathway in normal human melanocytes and melanoma cells expressing physiological levels of endogenous RHC variants. ERK activation was comparable for wild-type and mutant MC1R and was independent on cAMP because it was neither triggered by stimulation of cAMP synthesis with forskolin nor blocked by the adenylyl cyclase inhibitor 2',5'-dideoxyadenosine. Stimulation of MC1R with  $\alpha$ MSH did not lead to protein kinase C activation and ERK activation was unaffected by protein kinase C inhibitors. Conversely, pharmacological interference, small interfering RNA studies, expression profiles, and functional reconstitution experiments showed that  $\alpha$ MSH-induced ERK activation resulted from Src tyrosine kinase-mediated transactivation of the stem cell factor receptor, a receptor tyrosine kinase essential for proliferation, differentiation, and survival of melanocyte precursors, thus demonstrating a functional link between the stem cell factor receptor and MC1R. Moreover, this transactivation phenomenon is unique because it is unaffected by natural mutations impairing canonical MC1R signaling through the cAMP pathway.
- Huh S, Jung E, Lee J, Roh K, Kim JD, Lee J, Park D. **Mechanisms of melanogenesis inhibition by propafenone.** *Arch Dermatol Res.* 2010 Sep;302(7):561-5.  
Melanogenesis is a physiological process that results in the synthesis of melanin pigments, which play a crucial protective role against skin photocarcinogenesis. The present study was conducted to determine the inhibitory effects of propafenone on melanogenesis and to elucidate the molecular events involved in the inhibition of melanogenesis by propafenone. To accomplish this, several experiments were conducted using human epidermal melanocyte cells. The

melanin content and cAMP production were evaluated, and western blots for proteins involved in melanogenesis were conducted. The melanin content was significantly inhibited by propafenone in a concentration-dependent manner. To clarify the mechanism of the depigmenting property of propafenone, we examined the involvement of propafenone in cAMP signaling. In the cAMP production assay, the intracellular cAMP level was reduced by propafenone. The level of microphthalmia-associated transcription factor (MITF) protein, the upstream transcription factor of tyrosinase, was also reduced by propafenone. In addition, propafenone inhibited the expression of tyrosinase, TRP-1, and TRP-2. Taken together, the results of our study show that propafenone inhibits melanogenesis by suppressing cAMP production, which is involved in the expression of melanogenesis-related proteins and suggests that propafenone may be an effective inhibitor of hyperpigmentation.

- Ito S, Wakamatsu K.

**Human hair melanins: what we have learned and have not learned from mouse coat color pigmentation.** *Pigment Cell Melanoma Res.* 2010 Sep 14. doi: 10.1111/j.1755-1471.2010.00755.x.

Hair pigmentation is one of the most conspicuous phenotypes in humans. Melanocytes produce two distinct types of melanin pigment: brown to black, indolic eumelanin and yellow to reddish brown, sulfur-containing pheomelanin. Biochemically, the precursor tyrosine and the key enzyme tyrosinase and the tyrosinase-related proteins are involved in eumelanogenesis, while only the additional presence of cysteine is necessary for pheomelanogenesis. Other important proteins involved in melanogenesis include P protein, MATP protein,  $\alpha$ -MSH, agouti signaling protein (ASIP), MC1R (the receptor for MSH and ASIP), and SLC7A11, a cystine transporter. Many studies have examined the effects of loss-of-function mutations of those proteins on mouse coat color pigmentation. In contrast, much less is known regarding the effects of mutations of the corresponding proteins on human hair pigmentation except for MC1R polymorphisms that lead to pheomelanogenesis. This perspective will discuss what we have/have not learned from mouse coat color pigmentation, with special emphasis on the significant roles of pH and the level of cysteine in melanosomes in controlling melanogenesis. Based on these data, a hypothesis is proposed to explain the diversity of human hair pigmentation.

- Jux B, Kadow S, Luecke S, Rannug A, Krutmann J, Esser C.

**The Aryl Hydrocarbon Receptor Mediates UVB Radiation-Induced Skin Tanning.** *J Invest Dermatol.* 2010 Sep 23. Melanogenesis is the vital response to protect skin cells against UVB-induced DNA damage. Melanin is produced by melanocytes, which transfer it to surrounding keratinocytes. Recently, we have shown that the aryl hydrocarbon receptor (AhR) is part of the UVB-stress response in epidermal keratinocytes. UVB triggers AhR signaling by generating the AhR ligand 6-formylindolo(3,2-b)carbazole from tryptophan. We show here that normal murine melanocytes express functional AhR. Using standard UVB tanning protocols, AhR-deficient mice were shown to tan significantly weaker than wild-type mice; in these mice, tyrosinase activity in the epidermis was lower as well. Tanning responses and tyrosinase activity, however, were normal in keratinocyte-specific conditional AhR knockout mice, indicating that release of melanogenic keratinocyte factors is unaffected by the UVB-AhR signaling pathway and that the diminished tanning response in AhR(-/-) mice is confined to the level of melanocytes. Accordingly, the number of dihydroxyphenylalanin-positive melanocytes increased significantly less on UVB irradiation in AhR(-/-) mice than in wild-type mice. This difference in melanocyte number was associated with a significantly reduced expression of stem cell factor-1 and c-kit in melanocytes of AhR(-/-) mice. Thus, the environmental signal sensor AhR links solar UVB radiation to skin pigmentation.

- Khaled M, Levy C, Fisher DE.

**Control of melanocyte differentiation by a MITF-PDE4D3 homeostatic circuit.** *Genes Dev.* 2010 Oct 15;24(20):2276-81.

Cyclic AMP (cAMP) is a ubiquitous second messenger that regulates a variety of biological processes. The magnitude and duration of cAMP expression are regulated by both production and hydrolysis. Melanocyte-stimulating hormone (MSH) plays a crucial role in pigment cell differentiation via cAMP-regulated expression of the master transcription factor MITF. We report the identification of phosphodiesterase 4D3 as a direct target of the MSH/cAMP/MITF pathway. This creates a negative feedback loop that induces refractoriness to chronic stimulation of the cAMP pathway in melanocytes. This homeostatic pathway highlights a potent mechanism controlling melanocyte differentiation that may be amenable to pharmacologic manipulation for skin cancer prevention.

- Lee DW, Kim HJ, Choi CH, Shin JH, Kim EK.

**Development of a Protein Chip to Measure PKC $\beta$  Activity.** *Appl Biochem Biotechnol.* 2010 Sep 28.

Phosphorylation of proteins by kinases plays an important role in regulating cellular processes including melanin production in the skin cells. Protein kinase C  $\beta$  (PKC $\beta$ ) is known to be involved in phosphorylating tyrosinase, the key enzyme of melanin production, regulating the skin pigmentation process. In melanogenesis, PKC $\beta$  activates the tyrosinase by phosphorylation of its two serine residues. In this study, phosphorylation activity by PKC $\beta$  was monitored on a protein chip for the screening of depigmenting agents. As a tyrosinase mimic, 11 or 30 amino acids of the C-terminal of tyrosinase was fused with maltose-binding protein (MBP). After immobilizing the MBP-fused PKC $\beta$  substrate peptide on epoxy-treated slide surface, PKC $\beta$  reaction mix was applied over the immobilized MBP-fused PKC $\beta$  substrate peptide. Phosphorylation was detected with anti-phosphoSer/Thr antibodies, followed by fluorescence-

labeled second antibodies. Phosphorylation of MBP-30aa was observed on a protein chip, and this phosphorylation was inhibited by the PKC inhibitor (GF109203X). These results indicate the potential of PKC $\beta$  protein chip as a high-throughput screening tool in the screening of depigmenting agents.

- Luecke S, Backlund M, Jux B, Esser C, Krutmann J, Rannug A.  
**The aryl hydrocarbon receptor (AHR), a novel regulator of human melanogenesis.** *Pigment Cell Melanoma Res.* 2010 Dec;23(6):828-33.  
Skin cancer, chloracne and hyperpigmentation have been associated with the exposure to environmental contaminants such as polychlorinated biphenyls, dioxins or polycyclic aromatic hydrocarbons. These compounds are xenobiotic high-affinity ligands for the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor with important physiological roles in, for example, the control of cell proliferation and inflammation. We show here that exposure of normal human melanocytes to the most potent dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), results in activation of the AHR signaling pathway and an AHR-dependent induction of tyrosinase activity, the key enzyme of the melanogenic pathway. In accordance with the upregulation of tyrosinase enzyme activity, total melanin content was also elevated in TCDD-exposed melanocytes. Neither the induction of tyrosinase enzyme activity or of total melanin could be attributed to enhanced cell proliferation, but was rather due to the induction of tyrosinase and tyrosinase-related protein 2 gene expression. Thus, the AHR is able to modulate melanogenesis by controlling the expression of melanogenic genes.
- Panzella L, Napolitano A, D'Ischia M.  
**Is DHICA the key to dopachrome tautomerase and melanocyte functions ?** *Pigment Cell Melanoma Res.* 2010 Sep 15. doi: 10.1111/j.1755-148X.2010.00771.x.
- Sato K, Takahashi H, Toriyama M.  
**Depigmenting mechanism of NSAIDs on B16F1 melanoma cells.** *Arch Dermatol Res.* 2010 Nov 16.  
The aim of the present work was to clarify the anti-melanogenic mechanism of non-steroidal anti-inflammatory drugs (NSAIDs). Mefenamic acid, diclofenac, and nimesulide were used in this study, and these drugs inhibit melanin synthesis in B16F1 melanoma cells. To elucidate the anti-melanogenic mechanism of NSAIDs, we performed western blotting analysis for melanogenic proteins, such as tyrosinase, TRP-1, and TRP-2. All NSAIDs used in this study inhibited tyrosinase protein level. Semi-quantitative RT-PCR analysis showed that the depigmentation effect of mefenamic acid and nimesulide might be due to the inhibition of tyrosinase gene transcription. These results indicate that NSAIDs inhibit  $\alpha$ -MSH-enhanced melanin synthesis, and are candidate anti-melanogenic agents since they might be effective in hyperpigmentation disorders.
- Schallreuter KU, Salem MM, Hasse S, Rokos H.  
**The redox - biochemistry of human hair pigmentation.** *Pigment Cell Melanoma Res.* 2010 Oct 19. doi: 10.1111/j.1755-148X.2010.00794.x.  
The biochemistry of hair pigmentation is a complex field involving a plethora of protein and peptide mechanisms. The in loco factory for melanin formation is the hair follicle melanocyte but it is common knowledge that melanogenesis results from a fine tuned concerted interaction between the cells of the entire dermal papilla in the anagen hair follicle. The key enzyme is tyrosinase to initiate the active pigmentation machinery. Hence, an intricate understanding from transcription of mRNA to enzyme activity including enzyme kinetics, substrate supply, optimal pH, cAMP - signalling is a must. Moreover, the role of reactive oxygen species (ROS) on enzyme regulation and functionality needs to be taken into account. So far our knowledge on the entire hair cycle relies on the murine model of the C57BL/6 mouse. Whether this data can be translated into humans still needs to be shown. This article aims to focus on the effect of H(2) O(2) -redox homeostasis on hair follicle pigmentation via tyrosinase, its substrate supply and signal transduction as well as the role of methionine sulfoxide repair via methionine sulfoxide reductases A&B (MSRA&B).
- Sendovski M, Kanteev M, Shuster Ben-Yosef V, Adir N, Fishman A.  
**Crystallization and preliminary X-ray crystallographic analysis of a bacterial tyrosinase from Bacillus megaterium.** *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 2010 Sep 1;66(Pt 9):1101-3.  
Tyrosinases are type 3 copper enzymes that are involved in the production of melanin and have two copper ions in the active site. Here, the crystallization and primary analysis of a tyrosinase from *Bacillus megaterium* is reported. The purified protein was crystallized in the absence or presence of zinc ions and the crystals diffracted to a resolution of 2.0 Å. Crystals obtained in the presence of zinc belonged to space group P2(1)2(1)2(1), while crystals grown in the absence of zinc belonged to space group P2(1). In both space groups the asymmetric unit contained a dimer, with minor differences in the crystal density and in packing interactions.
- Sendovski M, Kanteev M, Ben-Yosef VS, Adir N, Fishman A.  
**First Structures of an Active Bacterial Tyrosinase Reveal Copper Plasticity.** *J Mol Biol.* 2010 Oct 30.  
Tyrosinase is a member of the type 3 copper enzyme family that is involved in the production of melanin in a wide range of organisms. The crystal structures of a tyrosinase from *Bacillus megaterium* were determined at a resolution of 2.0-2.3 Å. The enzyme crystallized as a dimer in the asymmetric unit and was shown to be active in crystal. The overall

monomeric structure is similar to that of the monomer of the previously determined tyrosinase from *Streptomyces castaneoglobisporus*, but it does not contain an accessory Cu-binding "caddie" protein. Two Cu(II) ions, serving as the major cofactors within the active site, are coordinated by six conserved histidine residues. However, determination of structures under different conditions shows varying occupancies and positions of the copper ions. This apparent mobility in copper binding modes indicates that there is a pathway by which copper is accumulated or lost by the enzyme. Additionally, we suggest that residues R209 and V218, situated in a second shell of residues surrounding the active site, play a role in substrate binding orientation based on their flexibility and position. The determination of a structure with the inhibitor kojic acid, the first tyrosinase structure with a bound ligand, revealed additional residues involved in the positioning of substrates in the active site. Comparison of wild-type structures with the structure of the site-specific variant R209H, which possesses a higher monophenolase/diphenolase activity ratio, lends further support to a previously suggested mechanism by which monophenolic substrates dock mainly to CuA.

- Shirasugi I, Sakakibara Y, Yamasaki M, Nishiyama K, Matsui T, Liu MC, Suiko M.

**Novel Screening Method for Potential Skin-Whitening Compounds by a Luciferase Reporter Assay.** *Biosci Biotechnol Biochem.* 2010 Nov 7.

Measurement of the melanin content by using B16 melanoma cells is generally applied to find novel skin-whitening agents. However, this measurement method using B16 melanoma cells has such disadvantages, as the time taken, its sensitivity, and troublesomeness. We therefore attempted in the present study to establish a reporter assay system by measuring the tyrosinase promoter activity to use for convenient, high-throughput screening of new melanogenesis inhibitors. We first confirmed the validity of this reporter assay system by using such known skin-whitening agents, as arbutin, sulfuraphane, and theaflavin 3,3'-digallate. We then compared the effect of 56 compounds on the tyrosinase promoter activity to test this reporter assay system. Carnosol, and rottlerin strongly inhibited the tyrosinase promoter activity. Moreover, carnosol and rottlerin decreased melanin synthesis and tyrosinase expression in a dose-dependent manner when using B16 melanoma cells. These results indicate this new luciferase reported assay system to be an effective and convenient method for screening potential skin-whitening compounds.

- Tobin DJ.

**The Cell Biology of Human Hair follicle Pigmentation.** *Pigment Cell Melanoma Res.* 2010 Nov 10. doi: 10.1111/j.1755-148X.2010.00803.x.

Although we have made significant progress in understanding the regulation of the UVR-exposed epidermal-melanin unit, we know relatively little about how human hair follicle pigmentation is regulated. Progress has been hampered by gaps in our knowledge of the hair follicle's cycle controls, to which hair pigmentation appears tightly coupled. However, pigment cell researchers may have overly focused on the follicular melanocytes of the nocturnal and UVR-shy mouse as a proxy for human epidermal melanocytes. Here, I emphasise the epidermis-follicular melanocyte pluralism of human skin, as research models for vitiligo, alopecia areata and melanoma, personal care/cosmetics innovation. Further motivation could be in finding answers to; why hair follicle and epidermal pigmentary units remain broadly distinct? Why melanomas tend to originate from epidermal rather than follicular melanocytes? Why multiple follicular melanocyte sub-populations exist? Why follicular melanocytes are more sensitive to aging influences? In this perspective I attempt to raise the status of the human hair follicle melanocyte, and highlight some species-specific issues involved which the general reader of the pigmentation literature (with its substantial mouse-based data) may not fully appreciate

## 8. Melanosomes

(Pr J. Borovansky)

Reviews. Review covering tyrosinase and tyrosinase-related proteins, P-protein, MATP protein, Agouti signal protein, SLC7A11, a cystine transporter, as well as their mutations in relation to the mouse coat colour pigmentation was written by Ito & Wakamatsu with a wish to learn more about the pH and the level of cysteine in melanosomes in future. A review by Park et al. discusses melanogenesis in relation to mechanisms of deliberately-induced hypopigmentation along the following three lines: 1) interventions prior to melanin synthesis = interfering with tyrosinase transcription and/or glycosylation; 2) interventions during melanin synthesis = tyrosinase inhibition, increased tyrosinase degradation, modification of tyrosinase and TRP proteins; 3) inhibition of melanosome formation, interference with melanosome maturation, inhibition of melanosome transfer. Scherer & Kumar summarized briefly the pigmentation pathway, melanocyte function, stages of melanosome formation and in more detail the association of variants in pigmentation related genes with the risk of basal cell carcinoma, squamous cell carcinoma and cutaneous melanoma.

Melanosomal proteins. GPNMB (glycoprotein nonmetastatic melanoma protein b) is a melanosomal glycoprotein with a high homology to melanosomal structural protein Pmel17. Haraszti et al. compared melanosomes from congenic strains of the C57BL/6J mice mutant for *Gpnmb* to strain-matched controls using the standard transmission EM and synchrotron-based X-ray absorption near-edge structure analysis (XANES). Whereas the EM did not detect any ultrastructural changes in melanosomes lacking functional GPNMB, XANES uncovered multiple spectral phenotypes, which means that the GPNMB influences the chemical composition of melanosomes.

Melanin and melanosome decomposition. In model experiments using spin-coated films of synthetic eumelanin, Sharma demonstrated that melanin photodegradation is significantly accelerated by ambient humidity (cf. Melanin photodegradation in melanosomes was also shown to be dependent on the presence of water –Elleder & Borovansky-Histochem. J. 33(5):273-281, 2001). According to the Gloger's rule the extent of eumelanin pigmentation increases with the humidity of the habitat. It is inferred that a higher level of melanogenesis could be an evolutionary response to the fact that ambient humidity accelerates melanin photodegradation. An article of fundamental importance by Kempf et al. showed that a methylation induced decomposition of melanosomes as evidenced by a variety of analytical, imaging and spectroscopic techniques. The methylation resulted in solubility of melanosomes which reemphasizes the earlier observations of an increased excretion of the isomeric 5-methoxy-6-hydroxy- and 5-hydroxy-6-methoxy-indole-2-carboxylic acids in the urine of patients with advanced melanoma (see Duchoň & Matouš- Clin Chim Acta 16(3):397-402, 1967). Laser treatment using low fluence should be regarded as an effective method for the treatment of melasma through subcellular selective photothermolysis. An ultrastructural study of Mun et al using TEM revealed that laser treatment caused selective photothermolysis of Stage IV melanosomes, whereas the melanin-free Stage I and II melanosomes remained intact.

Melanosome transport. It has been known that the keratinocyte growth factor KGF/FGF7 promotes the melanosome uptake through activation of its receptor tyrosine kinase FGFR2b/KGFR. Based on the experiments with KGFR depletion by siRNA and its overexpression by transfection of the wild type KGFR, Beleudi et al demonstrated a direct effect of the receptor on phagocytosis and melanosome transfer. KGFR ligands triggered phagocytosis and melanosome transfer in differentiated keratinocytes. A receptor kinase activity and signalling were required for these effects.

Melanosome ultrastructure. Interest of paleontology in melanosomes (see ESPCR Bull 2010, No.1) has continued (Clarke et al.): Analyses of a fossil of a giant penguin *Inkayacu paracasensis* revealed that the dimensions of fossilized color-imparting melanosomes were similar to those of non-penguin avian taxa (and that the feathering may have been predominantly gray or reddish brown). In contrast, the dark black colour of extant penguin feathers is generated by large ellipsoidal melanosomes previously unknown in birds. The nanostructure of penguin feathers was thus modified after earlier macrostructural modifications of the feather shape linked to aquatic flight. Structural proteins, especially Pmel17 have been considered important for the deposition of melanin in melanosomes. Jastrzebska et al described that self-assembled DOPA-melanin in fibril-like structures built from nanoaggregates of planar melanin oligomers formed upon the drop drying of the synthetic melanin colloidal suspensions. Formation of self-assembled molecular structures is dominated by two interactions, those between the substrate and adsorbate and those between the adsorbates themselves. I believe that, as for melanosomes, the interaction between structural protein(s) and the synthesizing melanin plays a primary role in the architecture of melanosomes but the detailed arrangement of melanin constituents may depend on the quality of the surface on which melanin is deposited. Ultrastructural studies of Wu et al revealed a large reduction in the number of mature melanosomes from progressive macular hypomelanosis (PMH) lesions. There were many membrane-bound groups in PMH lesions which contained a number of smaller type II-IV melanosomes, which were distributed in clusters. No degradation of melanosomes was present in the lysosomal compartments of the PMH lesions.

Melanosomes in other contexts. Merkel cells are attached to neighbouring keratinocytes by desmosomes and contain melanosomes similar to keratinocytes. They are excitable cells in close contact with sensory nerve endings but their function is unclear. Irmak has suggested that melanosomes in Merkel cells may be involved in mammalian

magnetoreception. According to his theory, melanosome as a biological magnetite is connected by cytoskeletal filaments to mechanically gated ion channels embedded in the Merkel cell membrane. The movement of melanosome with the changing electromagnetic field may open ion channels, directly producing a receptor potential that can be transmitted to brain via sensory neurons. Dental pulp stem cells are shown by Paino et al, for the first time, to differentiate spontaneously into mature melanocytes containing melanosomes up to their terminal stage of maturation.

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## 9. Melanoma experimental, cell culture

(Dr R. Morandini)

Translational research based on cell lines is highlighted by a recent paper (Selvan *et al*): “Establishment of stable cell lines for personalized melanoma cell vaccine”.

Designing a suitable protocol to increase the chances of success appears, in this regard, very important, specially if these cells are used as tools for immunotherapy with tumor-specific lymphocytes.

Pandofilno *et al.* made an interesting comparison of three culture media for the establishment of melanoma cell lines based on the rate of success to establish cell cultures and proliferation status: 2 FCS-free media (RPMI with human serum (HS) and serum-free X-vivo 15 (X15-Medium) versus the original FCS medium. They found that the most effective medium for establishing melanoma cell lines were respectively HS-Medium, X15-Medium and FCS-Medium.

To improve the inter-lab reproducibility of the results based on cell lines, Eberle *et al.* initiated a “Multicentre study on standardisation of melanoma cell culture”.

Dieckman *et al.* developed an original cell culture method to obtain normal human melanocytes from plucked anagen hair follicles but under 5% oxygen only, in order to reduce oxidative stress.

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Contact: Web: <http://chromeageasia2011.com/>

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Contact: Web: <http://www.eado2011.com/>

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Contact: Web: [www.esdr.org](http://www.esdr.org)

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Contact: [Pr Alain TAÏEB](#)

Web: [www.ipcc2011.org](http://www.ipcc2011.org)

#### 2011 20th European Academy of Dermatology and Venereology Congress (EADV)

October 20-24, Lisbon, Portugal

Contact: Web: <http://eadvlisbon2011.org/event/eadv-2011/>

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Contact: [Dr Bernhard WEHRLE-HALLER](#)

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