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CONTENT

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

Meeting Report

THE FEBS 2009 CONGRESS
Prague 4-9 july 2009

by Professor Patrick Riley

The ESPCR was well represented at the recent **34th Congress of the Federation of European Biochemical Societies (FEBS)** which was held in Prague on July 4 – 9, 2009. The meeting attracted 2066 scientists from 68 countries. The programme consisted of 7 Plenary Lectures, 8 Symposia divided into 44 sessions, and 17 special sessions, totalling 204 lectures. In addition there were 1464 poster presentations. The programme included a Symposium Session devoted to ***The Biochemistry of Melanins and Melanosomes*** **-** the first time this topic has been addressed at a FEBS Congress.

It is no accident that this subject received attention in Prague, a centre of learning which has been at the forefront of melanogenesis research since the work of Professor Jiri Duchon and his successor in the Department of Biochemistry at the 1st Faculty of Medicine, Charles University, Professor Jan Borovansky. Jan Borovansky was a member of the local Organising Committee of the FEBS Congress and was able to persuade the Programme Committee and the supervising FEBS Executive Committee to include the Symposium.

The Symposium, which was held on July 5th, was co-chaired by Jan Borovansky and Patrick Riley (both former ESPCR Council members), and included talks by Tadeusz Sarna (former Council member), José Garcia-Borron (former ESPCR President), and Stan Pavel (former President and Secretary). Also included were presentations by Christopher Ramsden, and by Véronique Delmas (representing the laboratory of Lionel LaRue, the recently elected ESPCR President).

Speakers and co-authors at the FEBS Symposium on *The Biochemistry of Melanins and Melanosomes* (L to R):

Veronique Delmas, Christopher Ramsden, Tadeusz Sarna, Edward Land, Stan Pavel, José Garcia-Borron, Patrick Riley, and the local organizer Jan Borovansky.

The talks covered a wide ambit of topics as is evident from the Symposium programme:

T. Sarna (Jagellonian University, Krakow): Chemical and biophysical properties of eumelanin and pheomelanin.

J. Garcia-Borron (University of Murcia, Murcia): Signalling from human melanocortin 1 receptor variants associated with red hair and skin cancer.

P. Riley (Totteridge Institute for Advanced Studies, London): Physiological and pathological functions of melanosomes and their exploitation.

S. Pavel (Leiden University Medical Center, Leiden): Mutator phenotypes in pigment cells and mechanism(s) of their DNA damage.

V. Delmas (Institut Curie, Paris): Too much or too little beta catenin represses melanoblast proliferation.

C. Ramsden (Keele University, Keele): Suicide-inactivation of tyrosinase by catecholic substrates.

The Abstracts of the presentations are published in the ***FEBS Journal*, vol. 276, suppl. 1, 29-30, 2009.**

The Symposium on Melanins and Melanosomes was mentioned in the *FEBS Congress News (Issue No.2*) as being "an outstanding success, and probably responsible for prolonging the Wimbledon Final."

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Pr A. Napolitano)

Of particular interest with regard to melanin structure and properties is the joint paper by Norris' and Sarna's groups on the photoexcited states of melanin in porcine retinal pigment epithelial (RPE) cells investigated by time-resolved electron paramagnetic resonance (Wang et al. *J Phys Chem B*) . To confirm previous observations suggesting excited triplet states formation at low concentrations in synthetic eumelanin and RPE pigments higher time and field resolution were employed which allowed to obtain evidence for two distinct radical pair signals one of enhanced absorption/emission at early times and one mostly emissive at later times. The emissive character of the longer lived feature confirmed participation of an excited triplet precursor. Although this represents a minor process with respect to light energy dissipation into heat, the finding is of particular importance as it questions the purely singlet chemistry view of melanin phototoxicity. A review of the properties of melanins as revealed by advanced spectroscopic and imaging techniques, theoretical calculations, and methods of condensed-matter physics is offered in the perspective of their possible exploitation in the design of high tech materials (d'Ischia et al. *Angew Chem Intl Ed Engl*) Analysis of red hair pigments and model synthetic pheomelanins after photoexposure revealed a much different photostability of pigment components derived from 5-S-cysteinyldopa and 2-S-cysteinyldopa isomers (Greco et al *PCMR*) .
The electrical and optical properties of melanin thin films have been further investigated confirming the semiconductor-like character and a hysteretic behavior linked to an irreversible process of water mol. desorption from the melanin film (Abbas et al *Eur Phys J E: Soft Mat Biol Phys*)
As usual an impressive number of reports on the properties of compounds of natural origin or plant extracts on melanogenic pathway has appeared. New structural classes of compounds presented as melanogenesis inhibitors include curcuminoids and phlorotannins from algae.
The way hydrogen peroxide is generated during melanogensis by chemical reactions coupled to the enzymatic formation of o-quinones by tyrosinase acting on monophenols and o-diphenols and by the auto-oxidation of the o-diphenols and other intermediates was well clarified by the enzymology group at Murcia university (*Biochim Biophys Acta* 2009)
A derivatization procedure based on ethyl chloroformate allowed analysis of aminohydrophenylalanines isomers and benzothiazine /benzothiazole compounds obtained by reductive degradation of synthetic pheomelanin and melanin from the urine of a melanoma patient by gas chromatographic-mass spectrometric techniques ensuring high sensitivity levels and identification of new products by molecular weight/fragmentation pattern information (Nezirevic Dernroth et al *J. Cromatogr A*).

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2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

Stem cell systems maintain the homeostasis of tissues, which are constantly subjected to genotoxic stress. Somatic stem cell depletion due to accumulation of DNA damage has been implicated in the decline of tissue renewal capacity and the appearance of aging-related phenotypes. Hair graying is one of the most obvious sign of aging and it is caused by the incomplete maintenance of melanocyte stem cells (MSCs) with age. **Inomata *et al.*** reported that irreparable DNA damage abrogates renewal of MSCs in mice, leading to hair graying. They showed that the damaged/stressed stem cells are removed from the stem cell poll by triggering their differentiation and eventual elimination of the differentiated mature cells. Furthermore they described that deficiency of Ataxia-teleangiectasia mutated sensitizes MSCs to ectopic differentiation, suggesting that the existence of a "stemness checkpoint" to maintain the stem cell quality and to prevent hair graying.

Although it is generally accepted that the expression of keratins is restricted to epithelial cells, several studies have shown their "ectopic" or "aberrant" expression also in nonepithelial cells. Particularly, it has been reported that melanoma cells can express keratins both *in vitro* and *in vivo* and that normal human epidermal melanocytes express K16 *in situ*. **Ramot *et al.*** re-investigated the expression of K16 and K6 in normal human epidermal melanocytes in cryosections of normal human scalp skin using high-sensitivity immunostaining methods and several different monospecific primary antibodies against keratin. Their study shows that human epidermal or hair follicle melanocytes lack both K16 and K6 expression while residing in their natural habitat, supporting the old paradigm that normal human melanocytes do not express keratins in situ. Therefore, the Authors propose that, among normal cutaneous cell populations in situ, melanocytes can be distinguished for the lack of keratins immunoreactivity.

Recent data suggest that the activation of Notch signaling pathway may be implicated in the progression of melanoma. **Pinnix *et al.*** studied the effects of Notch activation on human primary melanocytes. They first assessed the activation status of the Notch pathway in a panel of melanoma cell lines and patient lesions and showed an overexpression of active Notch in malignant cells and not in melanocytes. To evaluate the role of Notch activation in melanocyte transformation and melanoma development, they used a constitutively active truncated Notch transgene construct (N^{IC}) and observed that N^{IC}-infected melanocytes displayed increased growth rate, adhesion and invasive capacity, supporting a role for Notch activity in the development of a malignant phenotype. Microarray analyses revealed that MCAM, a cell adhesion molecule up-regulated during the development and progression of melanoma, was induced in response to Notch activation. In addition, N^{IC}-infected melanocytes displayed an increased survival in limiting media conditions and anchorage-independent growth. Taken together, these data suggest that Notch signaling is involved in promoting a transformed phenotype in human melanocytes, highlight the role of its activation in melanoma and suggest that target therapy for Notch activity may be a strategy against melanoma.

VanBrocklin *et al.* screened for secondary genetic events involved in the progression of melanoma in vivo using the *Cdkn2a*-deficient mouse melanocyte cell line D6-MEL injected into nude mouse. Cell lines were established from tumours visibile in mice after a long latency and a portion of the tumour was analyzed by histological examination. Increased expression of active MET, the receptor tyrosine kinase for the hepatocyte growth factor, which is known to be involved in melanoma progression, was detected in the tumour cells. The increased Met levels resulted from gene amplification and RNA interference targeting Met in the tumor cells significantly reduced the tumor growth *in vivo*. MET represents a therapeutic target for many tumors including metastatic melanoma and the results of this work further demonstrate that targeting Met by RNAi in cells characterized by amplified Met in vivo results in a significant delay in the growth of the tumor.

The human melanocortin I receptor (MCIR) is a G protein-coupled receptor (GPCR) expressed in melanocytes which determines the amount and type of melanin, the skin phototype, the sensitivity to UV radiation and the risk of skin cancer. The *MCIR* gene is highly polymorphic and several variant alleles are associated with red hair and fair skin (the RHC phenotype). **Sanchez-Laorden *et al.*** studied the trafficking of the three highly penetrant RHC variants, R151C, R160W and D294H. The Authors demonstrate that forward trafficking was normal for D294H, but impaired for both R151C and R160W, which showed intracellular retention, preferentially found in the endoplasmic reticulum (ER) for R151C, whereas R160W resulted to be trapped in a post-ER compartment, likely the *cis*-Golgi. This is the first report of a mutant GPCR retention within this compartment. In addition, analyzing other mutants, the Authors suggest that phosphorylation of residue T157 is required for the proper forward movement of MCIR.

Skin antioxidant network includes interceptive antioxidants which could destroy ROS but also specific repair enzymes, such as methionine sulphoxide reductase (MSR), to reverse damage done to macromolecules. These two mechanisms work together to maintain a delicate redox balance which has been disturbed in the depigmenting disorder vitiligo. **Zhou *et al.*** investigated whether the decreased expression of MSRA is one of the reasons why melanocytes are especially vulnerable to oxidative stress in vitiligo. They downregulated MSRA expression in melanocytes by using the short interfering RNA (siRNA)-targeted gene silencing method. They found that the decreased expression of MSRA renders melanocytes more susceptible to H_2O_2-induced cell death. Furthermore a remarkable decline in cell viability was reported in MSRA-silenced melanocytes even without exogenous oxidative stress, suggesting that MSRA is key for normal cell survival. All together these data suggest that MSRA is crucial for melanocytes to fight against oxidative stress in vitiligo and it appears to have therapeutic potential for the treatment of vitiligo. **Elgoweini *et al.*** evaluated the efficacy of narrowband ultraviolet B phototherapy (NB-UVB) plus oral supplement of vitamin E in the treatment of vitiligo. Twenty-four patients with stable

vitiligo were recruited and divided in a group treated with NB-UVB plus vitamin E (400 IU once/d started 2 weeks before NB-UVB) (group A) and a group treated with NB-UVB as monotherapy (group B). After 6 months of treatment, an improvement in the repigmentation of the lesions was observed in 72% of patients treated with NB-UVB plus vitamin E and in 55% of patients treated only with NB-UVB. In addition, group A achieved 50% repigmentation with a mean number of treatments significantly less than for group B, experienced less mild erythema and had a significant reduction in plasma malondialdehyde (MDA; product of lipid peroxidation) compared to group B, whereas the increase in plasma glutathione (GSH) was not significant. In summary, although these data are obtained from a small number of patients, the Authors suggest that oral vitamin E, as an antioxidant, may be a valuable adjuvant therapy, preventing lipid peroxidation in melanocyte cellular membrane and improving the efficacy of NB-UVB.

The colour of the skin is controlled by the pigmentary system, i.e. melanocytes and melanin, and is primarly determined by the quantity, type and distribution of melanin within the skin. The type of melanin as well as its distribution within melanosomes is not a random event but it is generally regulated by a number of genes such as MC1R, TYR, OCA2, SLC24A5, MATP and ASIP. **Yamaguchi and Hearing** reviewed current understanding of physiological factors that regulate skin pigmentation, focusing on melanosome biogenesis, transport and transfer, melanogenic regulators in melanocytes, and factors derived from keratinocytes, fibroblasts, endothelial cells, hormones, inflammatory cells, and nerves. Abnormal changes of skin colour are observed in a vast number of diverse disorders with different underlying mechanisms. Melanotic disorders are broadly divided into hypermelanotic (due to excess melanin but normal melanocytic population)and hypermelanocytic (due to normal melanin and increased melanocytic proliferation) as well as hypomelanocitic/amelanotic and hypomelanocytic/amelanocytic which are due to melanin deficiency and reduction or absence of melanocyte number respectively. **Dessinioti and co-workers** summarized the basic concepts of melanocyte biology and discuss how molecular defects in melanocyte development and function can result in the development of hypopigmentary hereditary skin diseases. **Furuya *et al.*** studied the mechanism of induction of pigmented spots in hairless mice chronically exposed to UVB (99mj/cm^2, 3 times/week, 8 weeks), analyzing the modifications in the numbers of differentiated melanocytes containing melanin pigments (MM) and melanocyte/melanoblasts positively stained for tyrosinase-related protein (TRP)-1, TRP-2 and the tyrosine kinase receptor c-kit. TRP-2 and c-kit are markers of early melanoblasts. Tyrosinase, TRP-1 and TRP-2 are markers of differentiated melanocytes. The number of TRP-1 positive cells and that of MM greately increased after the beginning of UVB irradiation, decreased after its cessation and then gradually augmented with the development of the pigmented spots. On the contrary, cells positive for c-kit were not detected after two exposures of UVB and then started to increase during UVB irradiation and continued to augment after the cessation of irradiation. Cell positive for TRP-2 did not change after two UVB irradiation and then gradually augmented after repeated irradiation. These results demonstrate that during repeated UVB irradiation, c-kit positive cells increase first, then TRP-2, TRP-1 cells and finally melanin, as it occurs during the normal differentiation process. Taken together, these data suggest that chronic UVB irradiation leads to differentiation and proliferation of melanoblasts, followed by an increase of differentiated melanocytes, resulting in the onset of pigmented spots.

Melasma represents an acquired hypermelanosis characterized by simmetry of hyperpigmentation and by a distribution related to trigeminal nerves, suggesting a possible neural involvement in the pathogenetic mechanisms underlying the pigmentation. **Bak *et al.*** analyzed the expression of neuropeptides, neurotrophins and their receptors in lesional and non lesional facial skin of six Korean women by confocal microscopic examination. The results demonstrated a higher expression of nerve growth factor receptor (NGFR) and neutral endopeptidase (NEP) in lesional compared to non lesional skin. Based on these results, the Authors suggest that neuroactive molecules, including NGF, released from peripheral nerves may affect the microenvironment around melanocytes via NGFR and that augmented levels of NEP is important in regulating melanogenesis. Another key factor in melanogensis is the pH of melanosomes. **Cheli *et al***. showed that cAMP increased pH of melanosomes and regulates the expression of several vacuolar ATPases and ion transporters, which might be important for the control of melanosome pH. H89, a pharmacological inhibitor of protein kinase A (PKA), prevents the alkalinization of melanosomes induced by cAMP-elevating agents and completely blocks melanin synthesis independent of PKA inhibition and regulation of tyrosinase expression. These data support the hypothesis that changes of melanosome pH play a pivotal role in the regulation of melanogenesis and identify cAMP and α-MSH as key modulators of this process. Moreover the inhibition of melanin synthesis evoked by H89 suggest that compounds able to decrease melanosome pH can be considered as potential innovative treatment of hyperpigmentation diseases such as melasma and lentigo senilis. The biosafety of hydroquinone and its derivatives as skin whitening agent remains controversial. the molecular mechanism for the cytotoxicity of hydroquinone to melanocytes is not known, but may be related to: (1) its rapid spontaneous oxidation, (2) its rapid metabolic oxidation catalyzed by tyrosinase , and/or (3) the hydrophobic electron donating substitute in the *para*-position of phenol to confer high cellular permeability and high susceptibility to autooxidation. **Hu and co-workers** investigated the effects of hydroquinone, arbutin, and deoxyarbutin (d-arb) on melanogenesis and antioxidation using cultured melan-a melanocytes in the presence or absence of ultraviolet A (UVA)-induced oxidative stress and determined whether d-arb enables to be an alterative to hydroquinone and arbutin for skin whitening use. They reported that cytotoxicity of hydroquinone and arbutin was increased after UVA, whereas d-arb suppressed ROS generation. All three compounds had a similar inhibition on tyrosinase activity in dose-dependent manners with two- to three-fold decreases over the untreated control. No changes in expression of tyrosinase protein in the cells treated with arbutin or hydroquinone were detected in Western blotting assay, but significant down-regulation of tyrosinase expression was found in cells treated with d-arb. Their data indicate that d-arb is a candidate to serve as a skin whitening ingredient with the merits of potent tyrosinase inhibition, effective skin penetration, less cytotoxicity and even antioxidation to some extent. To identify novel depigmenting agent several new approaches have been proposed. **Abu Ubei *et al.*** screened an internal oligopeptide library

for inhibitory activity against mushroom and human tyrosinase and the absence of melanocyte toxicity. The peptide dose- and time-response curves showed that P3 and P4 were 6- and 17-fold more potent inhibitors of mushroom tyrosinase than HQ. The effects of P3 and P4 have been further evaluated and characterized. Kinetic studies showed that P3 and P4 are competitive inhibitors of mushroom tyrosinase. Treatment of melanocytes with P3 or P4 for 7 days led to a significant reduction in melanin content, without interference on cell proliferation and cytotoxic effects. As both oligopeptides showed substantially improved potency against tyrosinase with minimal melanocyte toxicity, the authors suggest that clinical use of these agents may obviate the need for hydroquinone, which derives its primary skin-lightening capability through its cytotoxic activity. Tyrosinase (TYR) has been the major target of pharmacological approaches for the control of skin pigmentation. **An and co-workers** examined an alternative molecular approach using TYR-small interfering RNA (siRNA) to control melanogenesis in the human melanocytes. Their study demonstrates the selective inhibitory effect of TYR-siRNA on TYR gene expression and melanin synthesis in human melanocytes, suggesting that molecular approaches using siRNA targeted to the enzymes of melanogenic pathway may provide a novel strategy for the control of cell pigmentation. However side effects of this approach, such as lower cell survival, especially under UV radiation, should be carefully considered for its use as hypo-pigmenting agent.

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3. MSH, MCH, other hormones, differentiation

(Pr M. Böhm)

Melanosomal pH – a novel target of α-MSH in pigment cells

The pH of melanosomes plays a crucial role in regulation of melanin synthesis. However, only recently Cheli *et al.* (J. Biol. Chem. 2009; 284: 18699-18706) have provided first evidence that α-MSH is capable of regulating pH of melanosomes, and molecules involved in melanosomal-cytoplasmic ion exchange. Using B16F10 murine melanoma cells, the authors show alkalization of pH of melanosomes by α-MSH and an artificial cAMP inducer. The cAMP pathway also regulated the expression of vacuolar ATPases (e. g. ATP6V0-B and others) and ionic transporters (e. g. several members of the SLC genes). Treatment of cells with H89 (a commonly used PKA pathway inhibitor) prevented the cAMP-induced melanosomal alkalization and melanin synthesis without affecting Tyrosinase expression. Interestingly, neither PKA nor other kinases inhibited by H89 were found to be involved in inhibition of melanogenesis.

Proteoglycans, melanoma cells and α-MSH – a new connection

The syndecans are a family of transmembrane cell surface heparan sulfate proteoglycans that regulate the adhesion-dependent signal transduction of many cell types. In a recent paper by Lee *et al.* (J. Biol. Chem. 2009, E-Pub) the authors demonstrate that expression of syndecan-2 is elevated in tissue samples from nevi and cutaneous melanomas but not in melanocytes of normal skin. Syndecan-2 overexpression was also observed in several human melanoma cell lines. Importantly, overexpression of syndecan-2 increased migration of A375 human melanoma cells as demonstrated in transwell migration assays. Moreover B16 melanoma cells transfected with syndecan-2, moreover, exhibited increased invasion in an in vitro wound healing assay while the opposite was observed in cells treated with syndecan-2 siRNA. Interestingly, α-MSH decreased the mRNA and cell surface expression of syndecan-2 expression and melanoma cell migration, and furthermore decreased B16 melanoma cell migration and invasion.

Thymosin, NF-κB and melanoma cell invasion – impact of α-MSH and cAMP-inducing agents

Thymosin beta4 (Tbeta4) is a major actin-sequestering protein that is involved in cell growth, survival, motility, and metastasis of certain tumors. In a study by Kim *et al.* (Exp. Cell Res. 2009, E-Pub) the authors investigated the effects of α-MSH and artificial cAMP-induced agents on Tbeta4 expression and the metastatic potential of melanoma cells. α-MSH and IBMX suppressed Tbeta4 expression and regulated epithelial-mesenchymal transition-associated genes through the suppression of NF-***κ***B activation in B16F10 melanoma cells. The in vitro invasiveness and anchorage-independent growth of B16F10 in a semi-solid agar of these cells were also inhibited by α-MSH and IBMX. In addition, in animal experiments, the metastatic potential of α-MSH- or IBMX-treated B16F10 melanoma cells was decreased compared to untreated control cells.

Melanoma cell targeting by α-MSH peptides – a further step towards clinical application ?

Melanoma cell targeting by MC1R-binding peptides conjugated to cytostatic drugs or radiochemicals has been a goal for years. In a recent study Yang *et al. (*Bioconjugate Chem., 2009, E-Pub) evaluated a Arg-Gly-Asp-conjugated α-MSH hybrid peptide for potential melanoma therapy. The authors coupled the RGD motif {cyclic(Arg-Gly-Asp-dTyr-Asp)} was coupled to [$Cys^{3,4,10,}$ $_D$Phe^7, Arg^{11}α-MSH_{3-13} {(Arg^{11})CCMSH} to generate a RGD-Lys-(Arg^{11})CCMSH hybrid peptide. After confirming MC1R binding affinity of the peptide in B16/F1 melanoma cells, internalization and efflux, melanoma targeting and pharmacokinetic properties and single photon emission computed tomography/CT (SPECT/CT) imaging of ^{99m}Tc-RGD-Lys-(Arg^{11})CCMSH were determined in B16/F1 melanoma cells and melanoma-bearing C57 mice. In addition, clonogenic cytotoxic effects of RGD-Lys-(Arg^{11}))CCMSH were determined in melanoma cells in vitro. Importantly, ^{99m}Tc-RGD-Lys-(Arg^{11})CCMSH showed rapid internalization and extended retention in B16/F1 cells. The cellular uptake of the peptide was MC1 receptor-mediated. ^{99m}Tc-RGD-Lys-(Arg^{11})CCMSH exhibited high tumor uptake (14.83 +/- 2.94% ID/g 2 h post-injection) and prolonged tumor retention (7.59 +/- 2.04% ID/g 24 h post-injection) in B16/F1 melanoma-bearing mice. Non-target organ uptakes were generally low except for the kidneys. Flank melanoma tumors could be clearly imaged by small animal SPECT/CT using ^{99m}Tc-RGD-Lys-(Arg^{11})CCMSH as an imaging probe 2 h post-injection. Single treatment (3 h incubation) with 100 nM of RGD-Lys-(Arg^{11})CCMSH significantly ($p < 0.05$) decreased the clonogenic survival of B16/F1 cells by 65% compared to the untreated control cells. These data indicate a favorable melanoma targeting property of ^{99m}Tc-RGD-Lys-(Arg^{11})CCMSH and remarkable cytotoxic effect of RGD-Lys-(Arg^{11})CCMSH in B16/F1 cells warranting the further evaluation of ^{188}Re-labeled α-MSH hybrid peptides as novel therapeutic peptides for melanoma treatment.

4. Photobiology

(Dr N. Smit)

In June 2009 the 15th International Congress on Photobiology (ICP) was held in Dusseldorf, Germany. Many important aspects of photobiology were presented at the occasion (see for program; http://www.iuf.uni-duesseldorf.de/ICP2009/index.html). Next to sessions on "Melanocyte Photobiology" and "UV and Malignant Melanoma" special attention was given to the role of UVA in mutagenesis and in signal transduction. Next to UVA increasing interest can be observed for the influences of infrared (IR) radiation. An excellent overview of Photoimmunology was given by contributions of leading speakers in the field and the special (Finsen Medal) Lecture held by Margaret Kripke. Another highlight for me at this meeting was the new work presented by Edward de Fabo. Two pathways to melanoma have been identified in their mouse melanoma model system, a UVB-dependent but pigment independent pathway and a UVA-pigment dependent pathway. The latter is proposed to act via photo-oxidation of melanin or its precursors. The work on conversion of UV light and cell signaling via the aryl hydrocarbon receptor may also be of importance for melanocyte photobiology.
The cellular response to UVA in relation to photocarcinogenesis is the topic of the review by Ridley et al. As was obvious during the ICP meeting this topic is considered a major challenge for the photobiology field in the 21st century. The visible light is responsible for 50% of the skin oxidative burden, as reported by Zastrow et al. This may be one of the examples of the increased interest in IR radiation and its influence on skin.
Marrot and colleages presented some work at the ICP on (photo)oxidative stress in melanocyte and the role of the transcription factor Nrf2 that controls phase 2 antioxidant enzymes such as HO-1, NQO1 and GCL. Kokot et al now published a paper on the role of MSH on gene expression of Nrf2 and some of the Nrf2-dependent enzymes in both keratinocytes and melanocytes. Results suggest a regulatory role for alpha-MSH and related melanocortin peptides in antioxidative cytoprotection.
In the review by Greinert the status of new biomarkers for skin cancer, especially those that emerge from gene expression profiling is described. Microarray studies on changes during the transition of "normal to cancer cells" or induced by UV has resulted in conflicting data but some of the studies provide some possible candidates for new biomarkers that should be tested for their functionality in future studies.
In the paper by Laurin some known MM susceptibility genes were resequenced. Next to mutations in CDKN2A and MC1R, rare variants in tyrosinase were observed that may be regarded as important risk factors for skin cancer. Transplant patients are another group showing highly increased risk of obtaining skin cancer. In a group of 398 transplant recipients Urwin et al have developed a predictive index (PI) allocating patients in three significantly different groups for development of NMSC.
Filip et al provide an overview of natural agents and their protection against UV exposed damage and mechanism of action. Various other studies appeared that also investigated the (photo)protective effects by natural agents (Afaq, de la Coba, Cai, Jeon, Kolapposwamy and Zhang et al). Next to these studies the paper by van der Pols et al demonstrates that high concentrations of the serum antioxidant selenium are associated with decreased BCC and SCC tumor incidence in an Australian community. Heffernan et al describe in the JID 129 the mechanism that may be involved in the protective role of caffeine against UV induced skin cancer. In a comment by Kerzendorfer our daily coffee is considered a therapeutic option, a reassuring thought to me.

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5. Neuromelanins

(Pr M. d'Ischia)

The role of iron in neuromelanin synthesis and its biological significance in relation to Parkinson's disease is the subject of a critical review by Snyder and Connor (2009). Although literature on this topic is huge and spans over several decades, experimental data and views as to how iron accumulation impacts on degenerative mechanisms of pigmented dopaminergic neurones are still increasing. Here, the authors strive to provide an integrated view of the subject in which analytical aspects of iron determination are critically reviewed together with more biologically-oriented implications related to the etiopathogenesis of neuronal degeneration.

The close association of neuromelanin with lipids has long been known, but studies by Zecca, Simon and their associates have focused renewed attention to this topic following characterization of dolichol derivatives and related isoprenoid lipids from the human pigment. Now, in their recent paper (Ward et al., 2009) they go on to show that these lipids are present both in their native and oxidized forms, and their relative distribution patterns vary from one region of the brain to the other. These results may suggest that dolichol derivatives play a role as antioxidants capable of mitigating the effects of lipid peroxidation, and may therefore serve as biomarkers of aging. Further studies of neuromelanin-associated lipids may help gain a better understanding of the structural architecture and functional role of the pigment in its lipid matrix.

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  Abstract**:** Background: Iron status is higher in the substantia nigra than in other brain regions but can fluctuate as function of diet and genetics and disease. Of particular note is the compartmentalization of the iron-enrichment in this region; the pars reticulata contains higher levels of stainable iron as compared to the pars compacta. The latter area is where the dopaminergic neurons reside. How this compartmentalization impacts the interpretation of data that iron contributes to cell death as in Parkinson's disease or iron deficiency contributes to altered dopaminergic activity is unknown. Nonetheless, that iron can influence neuronal cell death and dopamine function is clear. Methods: The mechanisms by which iron may be managed in the substantia nigra, particularly in the neuromelanin cells where minimal levels of ferritin the iron storage protein have been detected are addressed. The current approaches to detect iron in the substantia nigra are also reviewed. In addn., the potential mechanisms by which iron enrichment may occur in the substantia nigra are explored. General Significance: This review attempts to provide a crit. evaluation of the many avenues of exploration into the role of iron in one of the most iron-enriched and clin. investigated areas of the brain, the substantia nigra.

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  Abstract**:** Neuromelanin (NM) isolated from 7 regions of the human brain was found to contain series of natural and oxidized isoprenoid lipids. Specifically, dolichols (dol) and dolichoic acids (dol-CA) with 14-22 and 14-21 isoprene units were identified. Stds. of nor-dolichol and nor-dolichoic acid were used to det. the relative amts. of dol and dol-CA compared to the total lipids present in NM for each region. The cerebellum, putamen, globus pallidus, and premotor cortex contained similar amts. of dol, comprising approx. 8-9.5% of the total lipid wt. Interestingly, the corpus callosum contained substantially lower quantities of both dol and dol-CA compared to the other regions, <4% dol relative to the total lipid wt. Oxidized and reduced dolichol-related species were identified and detd. to be region-dependent.

6. Genetics, molecular and developmental biology

(Dr F. Beermann)

Selected highlights:

*1. **Genetically engineered (transgenic) melanoma models***: many melanoma models have been described over the past 2 decades, most of them reflecting the human disease to a certain degree. However, all of them carry the genetic lesion (T-antigen, Ha-Ras^{V12G}, N-Ras^{Q61K}, HGF) from birth (see for review Larue and Beermann, Pigment Cell Res. 20, 485-497). Two papers from the groups of Richard Marais, Martin McMahon and Marcus Bosenberg (by Dhomen et al, Cancer Cell and Dankort et al., Nature Genetics) have now overcome this issue, using sophisticated Cre/loxP targeting strategy to allow replacement of an endogenous and wildtype B-Raf by a mutant one (B-Raf^{V600E}) upon inducible Cre-expression in adult mice. In both models, melanocyte proliferation was achieved, and melanoma were forming either spontaneously or upon additional inactivation of the Pten tumor suppressor. The interested reader is recommended to have a look at the "News and Views" by Glenn Merlino in the recent PCMR issue (PCMR 2009, 22, 246-247).

*2. **It is commonly accepted that melanocyte stem cells*** are residing in the bulge of the hair follicle. Buiding up on previous work, the group of Emi Nishimura has now convincingly shown in a recent Cell paper (Inomata et al., 2009) that natural and oxidative stress-mediated hair graying will affect melanocyte stem cells not by inducing apoptosis but rather by a premature differentiation of such stem cells in the bulge region, where they can be observed as fully pigmented cells (so called EPMs - ectopically pigmented melanocytes). A "News and Views" article has been written by Choi and Artandi (Cell Stem Cell 5, July 2009) and by Shinichi Nishikawa in PCMR (PCMR 2009, accepted DOI: 10.1111/j.1755-148X.2009.00605.x)

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FOXD3 regulates the lineage switch between neural crest-derived glial cells and pigment cells by repressing MITF through a non-canonical mechanism. Development 136: 1849-1858, 2009.
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melanoblasts. Moreover, FOXD3 represses melanogenesis. Here we show that avian MITF expression begins very early during melanoblast migration and that loss of MITF in melanoblasts causes them to transdifferentiate to a glial phenotype. Ectopic expression of FOXD3 represses MITF in cultured neural crest cells and in B16-F10 melanoma cells. We also show that FOXD3 does not bind directly to the MITF promoter, but instead interacts with the transcriptional activator PAX3 to prevent the binding of PAX3 to the MITF promoter. Overexpression of PAX3 is sufficient to rescue MITF expression from FOXD3-mediated repression. We conclude that FOXD3 controls the lineage choice between neural/glial and pigment cells by repressing MITF during the early phase of neural crest migration.
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See also Fujimura et al., 2009.

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See News and Views by Sarode and Beermann, PCMR 22, 380-381.

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7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borron)

- Abdel-Malek ZA, Ruwe A, Kavanagh-Starner R, Kadekaro AL, Swope V, Haskell-Luevano, C, Koikov L, Knittel JJ.
alpha-MSH tripeptide analogs activate the melanocortin 1 receptor and reduce UV-induced DNA damage in human melanocytes. Pigment Cell Melanoma Res. 2009 Jun 23. [Epub ahead of print]
Summary: One skin cancer prevention strategy that we are developing is based on synthesizing and testing melanocortin analogs that reduce and repair DNA damage resulting from exposure to solar ultraviolet (UV) radiation, in addition to stimulating pigmentation. Previously, we reported the effects of tetrapeptide analogs of alpha-melanocortin (alpha-MSH) that were more potent and stable tan the physiological alpha-MSH, and mimicked its photoprotective effects against UV-induced DNA damage in human melanocytes. Here, we report on a panel of tripeptide analogs consisting of a modified alpha-MSH core His(6)-d-Phe(7)-Arg(8), which contained different N-capping groups, C-terminal modifications, or arginine mimics. The most potent tripeptides in activating cAMP formation and tyrosinase of human melanocytes were three analogs with C-terminal modifications. The most effective C-terminal tripeptide mimicked alpha-MSH in reducing hydrogen peroxide generation and enhancing nucleotide excision repair following UV irradiation. The effects of these three analogs required functional MC1R, as they were absent in human melanocytes that expressed non-functional receptor. These results demonstrate activation of the MC1R by tripeptide melanocortin analogs. Designing small analogs for topical delivery should prove practical and efficacious for skin cancer prevention.

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Short-Sequence Oligopeptides with Inhibitory Activity against Mushroom and Human Tyrosinase.
J Invest Dermatol. 2009 May 14. [Epub ahead of print]
Cutaneous hyperpigmentation is a common disorder due to excess melanin production by the enzyme tyrosinase. The gold standard for treatment is hydroquinone (HQ), which reduces pigmentation through its toxicity to melanocytes rather than via tyrosinase inhibition. We screened an internal library for oligopeptides that inhibited both mushroom and human tyrosinase but showed no cytotoxicity to human melanocytes. We identified two highly active inhibitory sequences, P3 and P4, of 8- and 10-amino-acid-length, respectively. Mushroom tyrosinase inhibition was dose-dependent with IC(50) (half-maximal inhibitory concentration) values of 123 and 40 muM, respectively, compared with 680 muM for HQ. Other oligopeptides showed weaker or no inhibitory activity. Kinetic studies showed that P3 and P4 are competitive inhibitors of mushroom tyrosinase. At 100 muM, P3 and P4 inhibited human tyrosinase by 25-35%. This inhibition partially depended on whether L-dopa or L-tyrosine was the substrate, suggesting that tyrosinase may contain contains two distinct catalytic sites. Treatment of melanocytes with 100 muM P3 or P4 for 7 days led to a 27 or 43% reduction in melanin content. This inhibition was independent of cell proliferation and cytotoxic effects. Our data suggest that peptide-mediated inhibition of melanogenesis is due to reduction in tyrosinase activity.

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Inhibition of melanogenesis by tyrosinase siRNA in human melanocytes. BMB Rep. 42(3):178-83, 2009.
Tyrosinase (TYR) plays a critical role in cellular melanogenesis and, thus, has been the major target of pharmacological approaches for the control of skin pigmentation. This study examined an alternative molecular approach using TYR-small interfering RNA (siRNA) to control melanogenesis in the human melanocytes. Both the mRNA and protein levels of TYR were significantly lowered by TYR-siRNA treatment, whereas TYR-related protein 1 and TYR-related protein 2 displayed no such changes. TYR-siRNA treatment inhibited the cellular melanin synthesis from the externally supplied TYR substrate L-tyrosine. TYR-siRNA also suppressed melanin synthesis and decreased the viability of cells exposed to ultraviolet radiation, supporting a critical role of melanin in protection against ultraviolet radiation. These results suggest that molecular approaches using siRNA targeted to the enzymes of melanogenic pathway may provide a novel strategy for the control of cell pigmentation.

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Tyrosinase is a multifunctional, glycosylated, and copper-containing oxidase, which catalyzes the first two steps in mammalian melanogenesis and is responsable for enzymatic browning reactions in damaged fruits during post-harvest handling and processing. Neither hyperpigmentation in human skin nor enzymatic browning in fruits are desirable. These phenomena have encouraged researchers to seek new potent tyrosinase inhibitors for use in foods and cosmetics. This article surveys tyrosinase inhibitors newly discovered from natural and synthetic sources. The inhibitory strength is compared with that of a standard inhibitor, kojic acid, and their inhibitory mechanisms are discussed.

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{alpha}MSH and Cyclic AMP Elevating Agents Control Melanosome pH through a Protein Kinase A-independent Mechanism. J Biol Chem. 284(28):18699-706, 2009. Epub 2009 Apr 22.
Melanins are synthesized in melanocytes within specialized organelles called melanosomes. Numerous studies have shown that the pH of melanosome plays a key role in the regulation of melanin synthesis. However, until now, acute

regulation of melanosome pH by a physiological stimulus has never been demonstrated. In the present study, we show that the activation of the cAMP pathway by alphaMSH or forskolin leads to an alkalinization of melanosomes and a concomitant regulation of vacuolar ATPases and ion transporters of the solute carrier family. The solute carrier family members include SLC45A2, which is mutated in oculocutaneous albinism type IV, SLC24A4 and SLC24A5, proteins implicated in the control of eye, hair, and skin pigmentation, and the P protein, encoded by the oculocutaneous albinism type II locus. Interestingly, H89, a pharmacological inhibitor of protein kinase A (PKA), prevents the cAMP-induced pigmentation and induces acidification of melanosomes. The drastic depigmenting effect of H89 is not due to an inhibition of tyrosinase expression. Indeed, H89 blocks the induction of melanogenesis induced by LY294002, a potent inhibitor of the PI 3-kinase pathway, without any effect on tyrosinase expression. Furthermore, PKA is not involved in the inhibition of pigmentation promoted by H89 because LY294002 induces pigmentation independently of PKA. Also, other PKA inhibitors do not affect pigmentation. Taken together, our results strengthen the support for a key role of melanosome pH in the regulation of melanin synthesis and, for the first time, demonstrate that melanosome pH is regulated by cAMP and alphaMSH. Notably, these are both mediators of the response to solar UV radiation, the main physiological stimulus of skin pigmentation.

- De Lucia M, Panzella L, Pezzella A, Napolitano A, d'Ischia M.
Plant catechols and their S-glutathionyl conjugates as antinitrosating agents: expedient synthesis and remarkable potency of 5-S-glutathionylpiceatannol. Chem Res Toxicol. 21(12):2407-13, 2008.
With a view to elucidating the structural requisites for effective antinitrosating properties in plant polyphenolics and their metabolites, we have undertaken a comparative investigation of the nitrite scavenging effects of representative catechol derivatives of dietary relevance in the 2,3-diaminonaphthalene (DAN) nitrosation and tyrosine nitration assays. Compounds tested included caffeic acid (1), chlorogenic acid (2), piceatannol (3), hydroxytyrosol (4), and the corresponding S-glutathionyl conjugates 5-8, which were prepared using either tyrosinase (5 and 6) or a novel, o-iodoxybenzoic acid (IBX)-based oxygenation/ conjugation methodology (7b and 8). In the DAN nitrosation assay at pH 4.0, the rank order of inhibitory activities was found to be 5-S-glutathionylpiceatannol (7b) > 3 > 1 > 2 > 2-S-glutathionylcaffeic acid (5) > 2-S-glutathionylchlorogenic acid (6) > 4 approximately 5-S-glutathionylhydroxytyrosol (8). Quite unexpectedly, in the tyrosine nitration assay in 0.5 M HCl, 2 was the most efficient inhibitor followed by 1 > 4 > 3 > 7b approximately 5 > 8

- Devi S, Kedlaya R, Maddodi N, Bhat KM, Weber CS, Valdivia HH, Setaluri V.
Calcium Homeostasis in Human Melanocytes: Role of Transient Receptor Potential Melastatin 1 (TRPM1) and its Regulation by Ultraviolet Light. Am J Physiol Cell Physiol. 2009 Jul 8. [Epub ahead of print]
Transient Receptor Potential, Melastatin (TRPM) is a subfamily of ion channels that are involved in sensing taste, ambient temperature, low pH, osmolarity and chemical ligands. Melastatin 1 /TRPM1, the founding member, was originally identified as melanoma metastasis suppressor based on its expression in normal pigment cells in the skin and the eye but not in aggressive, metastasis-competent melanomas. The role of TRPM1 and its regulation in normal melanocytes and in melanoma progression is not understood. Here, we studied the relationship of TRPM1 expression to growth and differentiation of human epidermal melanocytes. TRPM1 expression and intracellular Ca(2+) levels are significantly lower in rapidly proliferating melanocytes compared to the slow growing, differentiated melanocytes. We show that lentiviral shRNA- mediated knockdown of TRPM1 results in reduced intracellular Ca(2+) and decreased Ca(2+) uptake suggesting a role for TRPM1 in Ca(2+) homeostasis in melanocytes. TRPM1 knockdown also resulted in a decrease in tyrosinase activity and intracellular melanin pigment. Expression of the tumor suppressor p53 by transfection or induction of endogenous p53 by ultraviolet B(UVB) radiation caused repression of TRPM1 expression accompanied by decrease in mobilization of intracellular Ca(2+) and uptake of extracelular Ca(2+). These data suggest a role for TRPM1-mediated Ca(2+) homeostasis, which is also regulated by UVB, in melanogenesis.

- Gheibi N, Saboury AA, Rajaei F, Pahlevan AA.
Dual effects of aliphatic carboxylic acids on cresolase and catecholase reactions of mushroom tyrosinase. J Enzyme Inhib Med Chem. 2009 Mar 19. [Epub ahead of print]
Catecholase and cresolase activities of mushroom tyrosinase (MT) were studied in presence of some n-alkyl carboxylic acid derivatives. Catecholase activity of MT achieved its optimal activity in presence of 1.0, 1.25, 2.0, 2.2 and 3.2 mM of pyruvic acid, acrylic acid, propanoic acid, 2-oxo-butanoic acid, and 2-oxo-octanoic acid, respectively. Contrarily, the cresolase activity of MT was inhibited by all type of the above acids. Propanoic acid caused an uncompetitive mode of inhibition (K(i)=0.14 mM), however, the pyruvic, acrylic, 2-oxo-butanoic and 2-oxo-octanoic acids showed a competitive manner of inhibition with the inhibition constants (K(i)) of 0.36, 0.6, 3.6 and 4.5 mM, respectively. So, it seems that, there is a physical difference in the docking of mono- and o-diphenols to the tyrosinase active site. This difference could be an essential determinant for the course of the catalytic cycle. Monophenols are proposed tobind only the oxyform of the tyrosinase. It is likely that the binding of acids occurs through their carboxylate group with one copper ion of the binuclear site. Thus, they could completely block the cresolase reaction, by preventing monophenol binding to the enzyme. From an allosteric point of view, n-alkyl acids may be involved in activation of MT catecholase reactions.

- Jiang Z, Xu J, Long M, Tu Z, Yang G, He G.

2, 3, 5, 4'-tetrahydroxystilbene-2-O-beta-d-glucoside (THSG) induces melanogenesis in B16 cells by MAP kinase activation and tyrosinase upregulation. Life Sci. 2009 Jun 13. [Epub ahead of print]
AIMS: The 2, 3, 5, 4'-tetrahydroxystilbene-2-O-beta-d-glucoside (THSG), a water-soluble active component extracted from dried tuber root of Polygonum multiflorum, has been found to induce pigmentation in B16 cells, but the details of the underlying mechanism remain unknown. The present study was conducted to investigate the mechanism of stimulatory effect of THSG on melanogenesis using B16F1 melanoma cells. MAIN METHODS: Several experiments were performed in B16F1 melanoma cells. We studied melanin content, tyrosinase activity, cell viability, and performed reverse transcription polymerase chain reaction and Western blots for proteins involved in melanogenesis. KEY FINDINGS: THSG increased the melanin content and tyrosinase activity in a concentration-dependent manner and treatment with 10 microg/ml THSG enhanced the expression of tyrosinase time-dependently in B16 cells. We then investigated whether THSG influences the expression of microphthalmia-associated transcription factor (MITF), which is required for tyrosinase expression. THSG was found to induce sustained MITF up-regulation and cAMP response element (CRE) binding protein (CREB) activation, suggesting that THSG-mediated MITF activation may be cAMP dependent. Furthermore, Western blot analysis revealed that THSG elevated the level of phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) significantly at 1-6 h; a p38 MAPK inhibitor, SB203580, almost completely attenuated the THSG-mediated up-regulation of melanin synthesis and induction of MITF and tyrosinase expression. SIGNIFICANCE: THSG exerts its stimulatory effect on melanogenesis by MAP kinase activation and MITF-induction of tyrosinase.

- Laughlin KM, Luo D, Liu C, Shaw G, Warrington KH Jr, Law BK, Harrison JK.
Hematopoietic- and neurologic-expressed sequence 1 (Hn1) depletion in B16.F10 melanoma cells promotes a differentiated phenotype that includes increased melanogenesis and cell cycle arrest. Differentiation. 78(1):35-44, 2009. Epub 2009 May 7.
The Hematopoietic- and neurologic-expressed sequence 1 (Hn1) gene encodes a small protein that is highly conserved among species. Hn1 expression is upregulated in regenerating neural tissues, including the axotomized adult rodent facial motor nerve and dedifferentiating retinal pigment epithelial cells of the Japanese newt. It is also expressed in numerous tissues during embryonic development as well as in regions of the adult brain that exhibit high plasticity. Hn1 has also been reported as a marker for human ovarian carcinoma and it is expressed in high-grade human gliomas. This study was directed toward understanding the function of Hn1 in a murine melanoma cell line. Hn1 mRNA and protein were identified in B16.F10 cells and in tumors formed from these cells. Inhibition of Hn1 protein expression with siRNA increased melanogenesis. Hn1-depleted cells expressed higher levels of the melanogenic proteins tyrosinase and Trp2 and an increased interaction between actin and Rab27a. The in vitro cell growth rate of Hn1-depleted cells was significantly reduced due to G1/S cell cycle arrest. This was consistent with a reduction in the phosphorylation of retinoblastoma protein as well as lower levels of p27 and increased expression of p21. Decreased expression of c-Met, the receptor for hepatocyte growth factor, was also detectedin the Hn1-depleted cells, however HGF-dependent stimulation of phosphorylated-ERK was unaffected. Hn1 depletion also led to increased basal levels of phosphorylated p38 MAPK, while basal ERK phosphorylation was reduced. Moreover, Hn1-depleted cells had reduced expression of transcription factors MITF and USF-1, and increased expression of TFE3. These data, coupled with reports on Hn1 expression in regeneration and development, suggest that Hn1 functions as a suppressor of differentiation in cells undergoing repair or proliferation.

- Manini P, Napolitano A, Westerhof W, Riley PA, d'Ischia M.
A Reactive ortho-Quinone Generated by Tyrosinase-Catalyzed Oxidation of the Skin Depigmenting Agent Monobenzone: Self-Coupling and Thiol-Conjugation Reactions and Possible Implications for Melanocyte Toxicity. Chem Res Toxicol. 2009 Jul 17. [Epub ahead of print]
Monobenzone (hydroquinone monobenzylether, 1) is a potent skin depigmenting agent that causes irreversible loss of epidermal melanocytes by way of a tyrosinase-dependent mechanism so far little understood. Herein, we show that 1 can be oxidized by mushroom tyrosinase to an unstable o-quinone (1-quinone) that has been characterized by comparison of its properties with those of a synthetic sample obtained by o-iodoxybenzoic acid-mediated oxidation of 1. Preparative scale oxidation of 1 with tyrosinase and catalytic l-DOPA, followed by reductive workup and acetylation, led to the isolation of two main products that were identified as the acetylated catechol derivative 4 and an unusual biphenyl-type dimer of 4, acetylated 5, arising evidently by coupling of 4 with 1-quinone. In the presence of l-cysteine or N-acetyl-l-cysteine, formation of 4 and 5 was inhibited, and the reaction led instead to monoadducts (6 or 9) and diadducts (7 and 8). A similar behavior was observed when the tyrosinase-promoted oxidation of 1 was carried out in the presence of sulfhydryl-containing peptides, such as reduced glutathione, or proteins, such as bovine serum albumin (BSA), as inferred by detection of adduct 9 by high pressure liquid chromatography-electrochemical detection (HPLC-ED) after acid hydrolysis. The generation and reaction chemistry of 1-quinone described in this article may bear relevance to the etiopathogenetic mechanisms of monobenzone-induced leukoderma as well as to the recently proposed haptenation hypothesis of vitiligo, a disabling pigmentary disorder characterized by irreversible melanocyte loss.

- Munoz-Munoz JL, García-Molina F, Varón R, Tudela J, García-Cánovas F, Rodríguez-López JN.

Generation of hydrogen peroxide in the melanin biosynthesis pathway. Biochim Biophys Acta. 1794(7):1017-29, 2009. Epub 2009 Apr 15.
The generation of H(2)O(2) in the melanin biosynthesis pathway is of great importance because of its great cytotoxic capacity. However, there is controversy concerning the way in which H(2)O(2) is generated in this pathway. In this work we demonstrate that it is generated in a series of chemical reactions coupled to the enzymatic formation of o-quinones by tyrosinase acting on monophenols and o-diphenols and during the auto-oxidation of the o-diphenols and other intermediates in the pathway. The use of the enzymes such as catalase, superoxide dismutase and peroxidase helps reveal the H(2)O(2) generated. Based on the results obtained, we propose a scheme of enzymatic and non-enzymatic reactions that lead to the biosynthesis of melanins, which explains the formation of H(2)O(2).

- Nikodinovic-Runic J, Martin LB, Babu R, Blau W, O'Connor KE.
Characterization of melanin-overproducing transposon mutants of Pseudomonas putida F6. FEMS Microbiol Lett. 2009 Jun 30. [Epub ahead of print]
Abstract: Two melanin-overproducing Pseudomonas putida F6 mutants were generated using transposon (Tn5) mutagenesis. Mutants were disrupted in a transcriptional regulator (TR) and a homogentisate 1,2-dioxygenase (HDO) gene. Colonies of mutant F6-TR overproduced a black pigment on solid medium. The same mutant (F6-TR) had a 3.7-fold higher tyrosinase activity compared with the wild-type strain when induced with ferulic acid. However in tyrosine uptake assays whole cells of the mutant strain F6-TR consumed eight times less tyrosine compared with the wild-type strain. Mutant F6-HDO produced a diffusible red pigment into the growth medium. Pigment production by mutant F6-HDO is sixfold higher than the wild-type strain. The biomass yield of mutant F6-HDO grown on tyrosine as the sole source of carbon and energy was 1.2-fold lower than the wild-type strain. While the growth of the wild-type strain was completely inhibited by 5 min of exposure to UV light (254 nm) both mutant strains showed survival rates >30%. Mutant F6-HDO was able to tolerate higher concentrations of hydrogen peroxide (H(2)O(2)) exhibiting 1.5 times smaller zones of inhibition at 10 mM H(2)O(2) compared with mutant F6-TR and the wild-type strain. The pigments produced by all strains were purified and confirmed to be melanins.

- Op't Holt BT, Vance MA, Mirica LM, Heppner DE, Stack TD, Solomon EI.
Reaction coordinate of a functional model of tyrosinase: spectroscopic and computational characterization. J Am Chem Soc. 131(18):6421-38, 2009.
The mu-eta(2):eta(2)-peroxodicopper(II) complex synthesized by reacting the Cu(I) complex of the bis-diamine ligand N,N'-di-tert-butyl-ethylenediamine (DBED) with O(2) is a functional and spectroscopic model of the coupled binuclear copper protein tyrosinase. This complex reacts with 2,4-di-tert-butylphenolate at low temperature to produce a mixture of the catechol and quinone products, which proceeds through three intermediates (A-C) that have been characterized. A, stabilized at 153 K, is characterized as a phenolate-bonded bis-mu-oxo dicopper(III) species, which proceeds at 193 K to B, presumably a catecholate-bridged coupled bis-copper(II) species via an electrophilic aromatic substitution mechanism wherein aromatic ring distortion is the rate-limiting step. Isotopic labeling shows that the oxygen inserted into the aromatic substrate during hydroxylation derives from dioxygen, and a late-stage ortho-H(+) transfer to an exogenous base is associated with C-O bond formation. Addition of a proton to B produces C, determined from resonance Raman spectra to be a Cu(II)-semiquinone complex. The formation of C (the oxidation of catecholate and reduction to Cu(I)) is governed by the protonation state of the distal bridging oxygen ligand of B. Parallels and contrasts are drawn between the spectroscopically and computationally supported mechanism of the DBED system, presented here, and the experimentally derived mechanism of the coupled binuclear copper protein tyrosinase.

- Park SH, Kim DS, Lee HK, Kwon SB, Lee S, Ryoo IJ, Kim WG, Yoo ID, Park KC.
Long-term suppression of tyrosinase by terrein via tyrosinase degradation and its decreased expression. Exp Dermatol. 18(6):562-6, 2009.
Previously, we reported that a fungal metabolite, terrein, decreases melanin synthesis via downregulation of microphthalmia-associated transcription factor (MITF). In the present study, we further investigated the long-term hypopigmenting action of terrein in a spontaneously immortalized mouse melanocyte cell line, Mel-Ab. Treatment with terrein at a concentration of 50 mum strongly decreased melanogenesis in a time-dependent manner. Interestingly, the decreased tyrosinase protein levels lasted for at least 7 days, even though the MITF protein levels were restored after 3 days of treatment. In accordance with the results of Western blot analyses, the tyrosinase mRNA levels were found to be continuously decreased for at least 7 days, even though recovery of the MITF mRNA levels began after 3 days of terrein treatment. Therefore, we evaluated tyrosinase downregulation to determine if it is caused by proteasomal degradation. We found that the reduction in tyrosinase levels that was induced by terrein was clearly recovered by MG-132, a proteasome inhibitor. Moreover, ubiquitination of tyrosinase increased following treatment with terrein in the presence of MG-132. Taken together, these results suggest that terrein decreases melanogenesis through ubiquitin-dependent proteasomal degradation as well as via decreased expression of its mRNA.

- Schallreuter KU, Hasse S, Rokos H, Chavan B, Shalbaf M, Spencer JD, Wood JM.
Cholesterol regulates melanogenesis in human epidermal melanocytes and melanoma cells. Exp Dermatol. 2009 Mar 7. [Epub ahead of print]

Cholesterol is important for membrane stability and is the key substrate for the synthesis of steroid hormones and vitamin D. Furthermore, it is a major component of the lipid barrier in the stratum corneum of the human epidermis. Considering that steroid hormone synthesis is taking place in epidermal melanocytes, we tested whether downstream oestrogen receptor/cAMP signalling via MITF/tyrosine hydroxylase/tyrosinase/pigmentation could be possibly modulated by cholesterol. For this purpose, we utilized human primary melanocyte cell cultures and human melanoma cells with different pigmentation capacity applying immunofluorescence, RT-PCR, Western blotting and determination of melanin content. Our in situ and in vitro results demonstrated that melanocytes can synthesize cholesterol via HMG-CoA reductase and transport cholesterol via LDL/Apo-B100/LDLR. Moreover, we show that cholesterol increases melanogenesis in these cells and in human melanoma cells of intermediate pigmentation (FM55) in a time- and dose-dependent manner. Cellular cholesterol levels in melanoma cells with different pigmentation patterns, epidermal melanocytes and keratinocytes do not differ except in the amelanotic (FM3) melanoma cell line. This result is in agreement with decreasing cholesterol content versus increasing pigmentation in melanosomes. Cholesterol induces cAMP in a biphasic manner i.e. after 30 min and later after 6 and 24 h, meanwhile protein expression of oestrogen receptor beta, CREB, MITF, tyrosine hydroxylase and tyrosinase is induced after 72 h. Taken together, we show that human epidermal melanocytes have the capacity of cholesterol signalling via LDL/Apo-B100/LDL receptor and that cholesterol under in vitro conditions increases melanogenesis.

- Sturm RA.
Molecular genetics of human pigmentation diversity. Hum Mol Genet. 18(R1):R9-17, 2009.
The genetic basis underlying normal variation in the pigmentary traits of skin, hair and eye colour has been the subject of intense research directed at understanding the diversity seen both between and within human populations. A combination of approaches have been used including comparative genomics of candidate genes and the identification of regions of the human genome under positive selection, together with genome-wide and specific allele association studies. Independent selection for different pigmentation gene sets has been found between Asian, European and African populations. Several genome-wide association studies for pigmentation have now been conducted and identified single nucleotide polymorphism (SNP) markers in known, TYR, TYRP1, OCA2, SLC45A2, SLC24A5, MC1R, ASIP, KITLG and previously unknown SLC24A4, IRF4, TPCN2, candidate genes. The contribution of SNP polymorphisms present in populations from South Asia have been tested and alleles found at TYR, SLC45A2 and SLC24A5 can largely account for differences between those of darkest and lightest skin reflectance using a simple additive model. Skin and hair colour associations in Europeans are found within a range of pigmentation gene alleles, whereas blue-brown eye colour can be explained by a single SNP proposed to regulate OCA2 expression. Functional testing of variant alleles has begun to connect phenotype correlations with biological differences. Variant MC1R alleles show direct correlations between the biochemical signalling properties of the encoded receptor and the red-hair fair skin pigmentation phenotype. Direct testing of a range of clonal melanocyte cultures derived from donor skin tissue characterized for three causal SNPs within SLC45A2, SLC24A5 and OCA2 has assessed their impact on melanin content and tyrosinase enzyme activity. From a culmination of genetic and functional studies, it is apparent that a number of genes impacting melanosome biogenesis or th e melanin biosynthetic pathway are candidates to explain the diversity seen inhuman pigmentation.

- Sutay Kocabas D, Pearson AR, Phillips SE, Bakir U, Ogel ZB, McPherson MJ, Trinh CH.
Crystallization and preliminary X-ray analysis of a bifunctional catalase-phenol oxidase from Scytalidium thermophilum. Acta Crystallogr Sect F Struct Biol Cryst Commun. 65(Pt 5):486-8, 2009.
Epub 2009 Apr 24.
Catalase-phenol oxidase from Scytalidium thermophilum is a bifunctional enzyme: its major activity is the catalase-mediated decomposition of hydrogen peroxide, but it also catalyzes phenol oxidation. To understand the structural basis of this dual functionality, the enzyme, which has been shown to be a tetramer in solution, has been purified by anion-exchange and gel-filtration chromatography and has been crystallized using the hanging-drop vapour-diffusion technique. Streak-seeding was used to obtain larger crystals suitable for X-ray analysis. Diffraction data were collected to 2.8 A resolution at the Daresbury Synchrotron Radiation Source. The crystals belonged to space group P2(1) and contained one tetramer per asymmetric unit.

- Tamura K, Ohbayashi N, Maruta Y, Kanno E, Itoh T, Fukuda M. **Varp is a novel Rab32/38-binding protein that regulates Tyrp1 trafficking in melanocytes.** Mol Biol Cell. 20(12):2900-8, 2009. Epub 2009 Apr 29.
Two small GTPase Rabs, Rab32 and Rab38, have recently been proposed to regulate trafficking of melanogenic enzymes to melanosomes in mammalian epidermal melanocytes; however, the exact molecular mechanism of Rab32/38-mediated transport of melanogenic enzymes has never been clarified, because no Rab32/38-specific effector has ever been identified. In this study, we screened for a Rab32/38-specific effector by a yeast two-hybrid assay using a guanosine triphosphate (GTP)-locked Rab32/38 as bait and found that VPS9-ankyrin-repeat protein (Varp)/Ankrd27, characterized previously as a guanine nucleotide Exchange factor (GEF) for Rab21, functions as a specific Rab32/38-binding protein in mouse melanocyte cell line melan-a. Deletion analysis showed that the first ankyrin-repeat (ANKR1) domain functions as a GTP-dependent Rab32/38-binding domain, but that the N-terminal VPS9 domain (i.e., Rab21-GEF domain) does not. Small interfering RNA-mediated knockdown of endogenous Varp in melan-a cells caused a dramatic reduction in Tyrp1 (tyrosinase-related protein 1) signals from melanosomes but did not cause any

reduction in Pmel17 signals. Furthermore, expression of the ANKR1 domain in melan-a cells also caused a dramatic reduction of Tyrp1 signals, whereas the VPS9 domain had no effect. Based on these findings, we propose that Varp functions as the Rab32/38 effector that controls trafficking of Tyrp1 in melanocytes.

- Truschel ST, Simoes S, Gangi Setty SR, Harper DC, Tenza D, Thomas PC, Herman KE, Sackett SD, Cowan DC, Theos AC, Raposo G, Marks MS.
  ESCRT-I Function is Required for Tyrp1 Transport from Early Endosomes to the Melanosome Limiting Membrane. Traffic. 2009 Jun 9. [Epub ahead of print]
  Melanosomes are lysosome-related organelles that coexist with lysosomes within melanocytes. The pathways by which melanosomal proteins are diverted from endocytic organelles toward melanosomes are incompletely defined. In melanocytes from mouse models of Hermansky-Pudlak syndrome that lack BLOC-1, melanosomal proteins such as tyrosinase-related protein 1 (Tyrp1) accumulate in early endosomes. Whether this accumulation represents an anomalous pathway or an arrested normal intermediate in melanosome protein trafficking is not clear.Here, we show that early endosomes are requisite intermediates in the trafficking of Tyrp1 from the Golgi to late stage melanosomes in normal melanocytic cells. Kinetic analyses show that very little newly synthesized Tyrp1 traverses the cell surface and that internalized Tyrp1 is inefficiently sorted to melanosomes. Nevertheless, nearly all Tyrp1 traverse early endosomes since it becomes trapped within enlarged, modified endosomes upon overexpression of Hrs. Although Tyrp1 localization is not affected by Hrs depletion, depletion of the ESCRT-I component, Tsg101, or inhibition of ESCRT function by dominant-negative approaches results in a dramatic redistribution of Tyrp1 to aberrant endosomal membranes that are largely distinct from those harboring traditional ESCRT-dependent, ubiquitylated cargoes such as MART-1. The lysosomal protein content of some of these membranes and the lack of Tyrp1 recycling to the plasma membrane in Tsg101-depleted cells suggests that ESCRT-I functions downstream of BLOC-1. Our data delineate a novel pathway for Tyrp1 trafficking and illustrate a requirement for ESCRT-I function in controlling protein sorting from vacuolar endosomes to the limiting membrane of a lysosome-related organelle.

- Wakamatsu K, Ohtara K, Ito S.
  Chemical analysis of late stages of pheomelanogenesis: conversion of dihydrobenzothiazine to a benzothiazole structure. Pigment Cell Melanoma Res. 22(4):474-86, 2009. Epub 2009 Jun 11.
  Pheomelanogenesis is a complex pathway that starts with the oxidation of tyrosine (or DOPA, 3,4-dihydroxyphenylalanine) by tyrosinase in the presence of cysteine, which results in the production of 5-S-cysteinyldopa and its isomers. Beyond that step, relatively little has been clarified except for a possible intermediate produced, dihydro-1,4-benzothiazine-3-carboxylic acid (DHBTCA). We therefore carried out a detailed study on the course of pheomelanogenesis using DOPA and cysteine and the physiological enzyme tyrosinase. To elucidate the later stages of pheomelanogenesis, chemical degradative methods of reductive hydrolysis with hydroiodic acid and alkaline peroxide oxidation were applied. The results show that: (1) DHBTCA accumulates after the disappearance of the cysteinyldopa isomers, (2) DHBTCA is then oxidized by a redox exchange with dopaquinone to form ortho-quinonimine, which leads to the production of pheomelanin with a benzothiazine moiety, and (3) the benzothiazine moiety gradually degrades to form a benzothiazole moiety. This latter process is consistent with the much higher ratio of benzothiazole-derived units in human red hair than in mouse yellow hair. These findings may be relevant to the (photo)toxic effects of pheomelanin.

- Wang ZQ, Si L, Tang Q, Lin D, Fu Z, Zhang J, Cui B, Zhu Y, Kong X, Deng M, Xia Y, Xu H, Le W, Hu L, Kong X.
  Gain-of-function mutation of KIT ligand on melanin synthesis causes familial progressive hyperpigmentation. Am J Hum Genet. 84(5):672-7, 2009. Epub 2009 Apr 16.
  Familial progressive hyperpigmentation (FPH) is an autosomal-dominantly inherited disorder characterized by hyperpigmented patches in the skin, present in early infancy and increasing in size and number with age. The genetic basis for FPH remains unknown. In this study, a six-generation Chinese family with FPH was subjected to a genome-wide scan for linkage analysis. Two-point linkage analysis mapped the locus for FPH at chromosome 12q21.31-q23.1, with a maximum two-point LOD score of 4.35 (Ø = 0.00) at D12S81. Haplotype analysis confined the locus within an interval of 9.09 cM, flanked by the markers D12S1667 and D12S2081. Mutation profiling of positional candidate genes detected a heterozygous transversion (c. 107A-->G) in exon 2 of the KIT ligand (KITLG) gene, predicted to result in the substitution of a serine residue for an asparagine residue at codón 36 (p.N-->S). This mutant "G" allele cosegregated perfectly with affected, but not with unaffected, members of the FPH family. Function analysis of the soluble form of sKITLG revealed that mutant sKITLGN36S increased the content of the melanin by 109% compared with the wild-type sKITLG in human A375 melanoma cells. Consistent with this result, the tyrosinase activity was significantly increased by mutant sKITLGN36S compared to wild-type control. To our knowledge, these data provided the first genetic evidence that the FPH disease is caused by the KITLGN36S mutation, which has a gain-of-function effect on the melanin synthesis and opens a new avenue for exploration of the genetic mechanism of FPH.

- Yamaguchi Y, Hearing VJ.
  Physiological factors that regulate skin pigmentation. Biofactors. 35(2):193-9, 2009.
  More than 150 genes have been identified that affect skin color either directly or indirectly, and we review current understanding of physiological factors that regulate skin pigmentation. We focus on melanosome biogenesis, transport

and transfer, melanogenic regulators in melanocytes, and factors derived from keratinocytes, fibroblasts, endothelial cells, hormones, inflammatory cells, and nerves. Enzymatic components of melanosomes include tyrosinase, tyrosinase-related protein 1, and dopachrome tautomerase, which depend on the functions of OA1, P, MATP, ATP7A, and BLOC-1 to synthesize eumelanins and pheomelanins. The main structural component of melanosomes is Pmel17/gp100/Silv, whose sorting involves adaptor protein 1A (AP1A), AP1B, AP2, and spectrin, as well as a chaperone-like component, MART-1. During their maturation, melanosomes move from the perinuclear area toward the plasma membrane. Microtubules, dynein, kinesin, actin filaments, Rab27a, melanophilin, myosin Va, and Slp2-a are involved in melanosome transport. Foxn1 and p53 up-regulate skin pigmentation via bFGF and POMC derivatives including alpha-MSH and ACTH, respectively. Other critical factors that affect skin pigmentation include MC1R, CREB, ASP, MITF, PAX3, SOX9/10, LEF-1/TCF, PAR-2, DKK1, SCF, HGF, GM-CSF, endothelin-1, prostaglandins, leukotrienes, thromboxanes, neurotrophins, and neuropeptides. UV radiation up-regulates most factors that increase melanogenesis. Further studies will elucidate the currently unknown functions of many other pigment genes/proteins.

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Geometric and electronic structure differences between the type 3 copper sites of the multicopper oxidases and hemocyanin/tyrosinase. Proc Natl Acad Sci U S A. 106(16):6585-90, 2009. Epub 2009 Apr 3.
The coupled binuclear "type 3" Cu sites are found in hemocyanin (Hc), tyrosinase (Tyr), and the multicopper oxidases (MCOs), such as laccase (Lc), and play vital roles in O(2) respiration. Although all type 3 Cu sites share the same ground state features, those of Hc/Tyr have very different ligand-binding properties relative to those of the MCOs. In particular, the type 3 Cu site in the MCOs (Lc(T3)) is a part of the trinuclear Cu cluster, and if the third (i.e., type 2) Cu is removed, the Lc(T3) site does not react with O(2). Density functional theory calculations indicate that O(2) binding in Hc is approximately 9 kcal mol(-1) more favorable than for Lc(T3). The difference is mostly found in the total energy difference of the deoxy states (approximately 7 kcal mol(-1)), where the stabilization of deoxy Lc(T3) derives from its long equilibrium Cu-Cu distance of approximately 5.5-6.5 A, relative to approximately 4.2 A in deoxy Hc/Tyr. The O(2) binding in Hc is driven by the electrostatic destabilization of the deoxy Hc site, in which the two Cu(I) centers are kept close together by the protein for facile 2-electron reduction of O(2). Alternatively, the lack of O(2) reactivity in Lc(T3) reflects the flexibility of the active site, capable of minimizing the electrostatic repulsion of the 2 Cu(I)s. Thus, the O(2) reactivity of the MCOs is intrinsic to the trinuclear Cu cluster, leading to different O(2) intermediates as required by its function of irreversible reduction of O(2) to H(2)O.

8. Melanosomes

(Pr J. Borovansky)

Reviews. Review by *Yamaguchi and Hearing* focused on melanosome biogenesis, transport and transfer, melanogenic regulators in melanocytes, and factors derived from keratinocytes, fibroblasts, endothelial cells, hormones, inflammatory cells and nerves. The text is accompanied by beautiful graphic colour illustrations.
Ortonne and Bissett devoted their review to hyperpigmentary disorders (postinflammatory hyperpigmentation, solar lentigos, and melasma) reflecting pigmentation control targets and effective agents as described in recent literature. Melanosomes are mentioned in various contexts. Mechanisms behind the colour change including the role of the melanosome size, speed and transport molecules -dynein, myosin , kinesin were compared between fish, frogs and mammals by *Aspengren et al. Dell´ Angelica* summarized ideas about the specific roles of the adaptor protein (AP)-3 complex in the protein trafficking and organelle biogenesis within the endosomal-lysosomal system that emerged over the last ten years.
Mills and Patterson introduce the concept of the vertebrate pigment pattern. Whereas a vast majority of studies have so far focused on just one type of pigment –melanin synthesized by one type of pigment cells – melanocytes in melanosomes, the current review emphasizes the existence of many types of pigment and pigment cells in nature. Together they generate an extraordinary array of pigment patterns.

Pigment cells and organelles in the nanometer scale. Understanding the hierarchical organization of molecules and organelles within the interior of the cell is a challenge of cell biology. *Heymann et al.* obtained 3D imaging of the MNT-1 cell architecture by means of ion-abrasion scanning EM, i.e. a technique which combines iterative removal of material from the surface of a bulk specimen using focused ion-beam milling with SEM. The 3D images demonstrated a spatial relationships between various organelles such as mitochondria and membranes of ER and the distribution of melanosomes.

Melanosomal constituents and antigens. Melan-A/MART-1, a glycoprotein associated with the melanosome, is a well-known melanoma antigen recognized by autologous cytotoxic cells. *Song et al* constructed a fluorescent cellular reporter system for screening chemicals augmenting the expression of melanoma antigens such as Melan-A/MART-1. A study evaluating the association betwen genetic variants in the pigmentation and pigmentary phenotypes and skin cancer risk was performed by *Nan et al*. As for the melanosome, some proteins, such as TYR, TYRP1, P-protein, SLC24A5 and MATP, which are involved in the induction of the melanization of pheomelanosome or eumelanosome, are presented in Fig.1.

Melanosomes and free radicals. To determine whether sub-lethal oxidative stress to the RPE by the visible light treatment affects the translocation of organelles, notably phagosomes and melanosomes, *Burke and Zareba* studied ARPE-19 cells that contained phagocytized isolated porcine melanosomes. The cultures were blue light-treated to generate reactive oxygen intermediates. The photic stress impaired the movement and positioning of RPE organelles, which could have widespread consequences for maintaining a functionally efficient subcellular organization. Melanosomes protect pigmented cells against oxidative stress induced by light, e.g. RPE cells, but it has not been known whether aging modulates their photoprotective function. Model experiments of *Zadlo et al.* demonstrated that natural melanin was only a moderately efficient photoprotective pigment, which upon photoaging might lose its antioxidant efficiency and even become a photosensitizer.

Melanosome trafficking and transport. To give a broader picture of the molecular regulators of melanogenesis, *Ganesan et al* used a genome-wide siRNA functional genomic approach to identify 92 novel regulators of the melanin production in pigmented MNT-1 cells. The screening approach identified several genes that impact on melanosome trafficking/sorting of melanosome protein cargo. A close relationship between melanogenesis and autophagy was demonstrated. In Xenopus melanophores, aggregation and dispersion of pigment granules are regulated by the second messenger cAMP through the protein kinase A (PKA) signalling pathway. The PKA is bound to pigment granules where it forms complexes with molecular motors involved in pigment transport. *Semenova et al* report that PKA anchoring protein moesin is bound to pigment granules. See also *Burke and Zareba.*

Melanosomes under pathologic situations. Two cases of amelanotic melanoma of oesophagus with immunohistochemical and genetic (*KIT* and *PDGFR*) analyses were performed by *Terada*. In both the cases tumour cells were positive for melanosome (HMB45), S100, p53 protein, KIT and PDGFR A. A case report of dyschromatosis was presented by *Yusuf et al*.. Although the precise etiology of this disorder is not yet known, the clinicopathological findings implicate an inherent abnormality of the melanosomes or melanin processing. *Lazova et al* show that malignant melanoma cells display high levels of autophagy, a cytoplasmic process of the protein and organelle digestion that provides an energy source in times of nutrient deprivation therapies. The autophagosomes were filled with heavily melanized melanosome-like structures and debris. The melanosomes seemed to be partially digested and might have represented only residual, undigested melanin

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9. Melanoma experimental, cell culture

(Dr R. Morandini)

Developing 3D Cell Culture model is more and more a key features to better understand cell interaction and between cells and their environment. A 3D model may serve as a good intermediate between in vitro and in vivo systems, reducing the use of laboratory animals for experiments.
Marrero and Heller have developed a new and original 3D model ("*An ideal cell culture model is one where a 3D construct is capable of providing cells the ability to network with one another in an unrestricted manner and independent of other natural stimuli.*").
Melanoma progression has been studied using a lot of 3D methods, such as the liquid overlay method, matrigel/algi matrix, cytodex/plastic beads scaffold and Extracel sponges. Experimentation using these methods has proven to be successful, promoting cellular aggregates of melanoma ranging in size between 50 and 500 μm in diameter. Although these techniques provide useful information on a microscale level, this new model is able to produce tumor sizes close to 1 cm.
Briefly, the 3D model consists of growing keratinocytes into spheroids using bioreactors. Keratinocytes cells mimic epithelia found in mammals, to act as scaffolding that supports cellular growth of tumor cells such as melanoma. Keratinocytes can promote cellular development because they are known to synthesize their own basement membrane (BM) for structural stability similar to the BM sitting on the extracellular matrix. The bioreactor system known as the High Aspect Ratio Vessel (HARVs) was used to provide a microgravity environment. The aggregates are floating in the medium. After formation of the aggregates the melanoma cells were injected inside the spheroids.

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E. Melanoma Experimental

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ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2009 15th Annual Meeting Pan American Society for Pigment Cell Research
The Pigmentary System: Securing a Place under the Sun
September 4-7, UTHSC, Memphis, TN
Contact: Dr. Andrzej Slominski
E-mail : aslominski@utmem.edu

2009 39th Annual ESDR Meeting
September 9-12, Budapest, Hungary
Contact: Website: www.esdr.org

2009 XVth Meeting of the ESPCR
September 20-23, Münster, Germany
Contact: Pr Markus Böhm
E-mail: bohmm@uni-muenster.de
Web: www.espcr.org

2009 18th EADV Congress
October 7-11, Berlin, Germany

2009 6th World Conference on SKIN AGEING
October 29-3O, Malta
Contact: Website: www.malta-skinageing.com

2009 International Melanoma Congress (Society for Melanoma Research)
November, 1-4, Boston, Massachusetts
Contact: Website: www.melanomacongress09.com/ www.societymelanomaresearch.org/

2010 XVIth Meeting of the ESPCR
September, Bath, UK
Contact: Dr Robert Kelsh

2010 40th Annual ESDR Meeting
September 8-11, Helsinski, Finland

2010 16th Annual Meeting Pan American Society for Pigment Cell Research
Vancover, Canada
Contact: Dr. Youwen Zhou

2011 41st Annual ESDR Meeting

September 7-10 Barcelona, Spain

Contact: Website: www.esdr.org

2011 XXIth IPCC

September, 21-24, Bordeaux, France

Contact: Pr Alain Taïeb

2012 XVIIth Meeting of the ESPCR

September, Geneva, Switzerland

Contact: Dr Bernhard Wehrle-Haller