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LETTER TO ESPCR MEMBERS

SHIGEKI SHIBAHARA, M.D., Ph.D.
President, IFPCS

Dear Friends, Members of ESPCR

It is my great honor and pleasure to write a presidential letter for the ESPCR Bulletin. As the new President of the IFPCS, I would like to begin my greetings by expressing profound thanks to the outgoing Officers of the IFPCS: Zalfa Abdel-Malek (Ex-President) and Jose Carlos Garcia-Borron (Ex-Secretary-Treasurer), with every appreciation of all their hard work for the Federation over the past three years. I would also like to welcome our new IFPCS officers, Mauro Picardo, ESPCR (Vice-President), Caroline Le Poole, PASPCR (Treasurer), and Prasad Kumarasinghe, ASPCR (Secretary). Because of heavy work of the Secretary-Treasurer, as evident from the great performance and enormous efforts of Jose Carlos Garcia-Borron, we established the Secretary and the Treasurer as separate positions at the IFPCS Council meeting held in Sapporo during the 20th IPCC. Thus, each of four regional societies is now able to contribute equally to the management of the IFPCS.

The IFPCS already enjoyed the "Change" in 2008: the launch of Pigment Cell & Melanoma Research (PCMR) and the merge of 20th International Pigment Cell Conference (IPCC) and 5th International Melanoma Research Congress (IMRC). The historical conjoined meeting was organized by Prof. Kowichi Jimbow, Sapporo Medical University, and ended successfully.

Pigment Cell & Melanoma Research (PCMR)
The IFPCS has supported and promoted the Pigment Cell Research/PCMR, which under the outstanding editorship of Colin Goding (official term: 2004-2009) has steadily increased its scientific quality, output and Impact Factor (4.288 in 2007). However, due to the term of five years, the next Editor-in-Chief was selected as Dr. Ze'ev Ronai from Society for Melanoma Research (SMR) at the IFPCS Council meeting held in Sapporo. At the same time, Dr. Jose Carlos Garcia-Borron was elected as the Executive editor from IFPCS. The Executive editors are nominated by the respective Societies or federations affiliated with PCMR (namely, IFPCS and SMR) and approved by the Editor-in-Chief. Unfortunately, however, Dr. Jose Carlos Garcia-Borron recently resigned as the Executive editor because of the delicate reasons concerning the editorial concept. We respect his decision and appreciate his past great contributions to the achievement of PCR/PCMR. Consequently, we have elected Dr. Heinz Arnheiter (PASPCR) as a new Executive editor for PCMR. To ensure the smooth transition, a new editorial team will be involved in publication of the Nov/Dec 2009 issue. I am confident that PCMR will advance further under the leadership of Dr. Ze'ev Ronai, with both Executive editors from IFPCS (Dr. Heinz Arnheiter) and SMR (Dr. Glenn Merlino).
On the other matter, IFPCS has decided to introduce the mandatory online subscription to PCMR with a special price in 2009. You can get more information through the IFPCS Web site, shown below.

New IFPCS WEB Site
First of all, we are grateful to Dr. Bill Oetting, who has been managing the IFPCS website for many years. He kindly transferred various types of information to Dr. Lluis Montoliu (ESPCR). Thanks to

enormous efforts of Lluis, the IFPCS has a new independent Web site since July 2008. Please access to the IFPCS WEB Site (http://www.ifpcs.org). You will realize the excellent work achieved by Lluis.

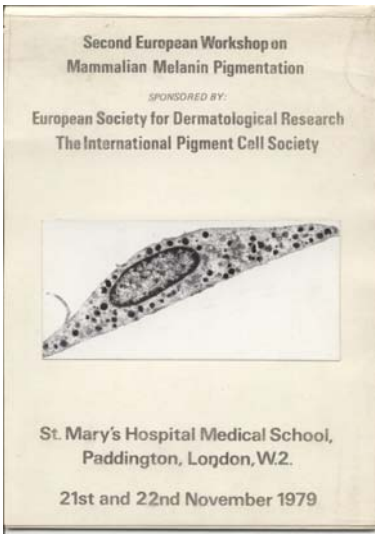
Next IFPCS Council Meeting
The last 3 council meetings were held in Barcelona in 2006, Singapore 2007 and Japan 2008. According to the rotation, the next Council Meeting will be held in Memphis, Tenn. during the 15th Annual Meeting of PASPCR on 4-7 September 2009, organized by Dr. Andrzej Slominski and other committee members.

Next IPCC Meeting
Dr. Alain Taieb will organize the next IPCC in Bordeaux, France in 2011. The main theme is 'Skin and Other Pigment Cells: Bridging Clinical Medicine and Science'. As a matter of course, members of the SMR will be welcome to participate in the 2011 IPCC, although the next IPCC will not be an official joint meeting with the SMR. I look forward to seeing many of you in the next IPCC.

I wish all of you a peaceful and productive year 2009.

Best wishes,
Shigeki Shibahara, M.D., Ph.D.
President, IFPCS

THE HISTORY OF PIGMENT CELL RESEARCH ACTIVITIES IN EUROPE
Part 2
By Prof. Jan Borovanský

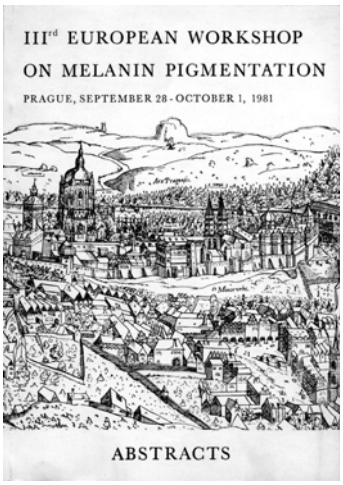


A year minus two days after the first European Workshop on Melanin Pigmentation (EWMP) in Lyon (see ESPCR Res. Bulletin No.62) the 2nd EWMP took place at St Mary´s Hospital Medical School in London, U.K. on November 21-22, 1979 and was organized by Prof. Aodan S. Breathnach and his secretary Linda Blay. In my recollections it was a typical cosy University meeting held in a small lecture theatre with the Workshop Dinner in the Medical School refectory. The University mood penetrated all the participants, not only due to the dominant appearance of Prof. Breathnach but probably also because everyone was aware of the beginning of the penicillin story in the historic precincts of St.Mary´s Hospital. The University mood was reflected also by abundant open discussions after each of the 32 lectures. The programme was divided into six sessions:

1. Melanocyte: Biology and Functional Morphology (chaired by M.Prunieras)
2. Biochemistry and Melanogenesis (chaired by G. Prota)
3. Control of Pigmentation (chaired by H. Rorsman)
4. Biology of Melanoma (chaired by J.A.A. Hunter)
5. Immunology of Melanoma (chaired by Rona Mackie)
6. Chemotherapy of Melanoma (chaired by P.A. Riley)

The Czech participants (i.e. Prof. Duchon and I) enjoyed this meeting enormously,each delivering two lectures. We also entered into lasting friendship with Fritz Anders, Dieter Schachtschabel (a remarkable man who needs only 2 hours of sleep a day) and John Hunter.
Moreover, for me the occasion was notable because my son was born whilst I was on the return flight from London to Prague. Unfortunately, in 1979 I did not possess any flash equipment and, therefore, my photodocumentation of the meeting was rather limited.

The 3rd European Workshop on Melanin Pigmentation was organized by Associate Prof. J. Duchon and myself at the Congress Facility of the Physician´s House in Prague, Czechoslovakia on September 28 – October 1, 1981.



The Prague Workshop introduced plenty of novelties: a) It was the first EWMP organized behind the Iron Curtain, which enabled scientists from Eastern Europe to come in large numbers, especially from Poland, East Germany and the Soviet Union (including Prof. Kurbanov from Turkmenia). Two Italian male participants found difficulty in concentrating on the lectures, being preoccupied by a Russian blonde beauty. Mutual cooperation between laboratories from various parts of Europe was initiated at this meeting and has proliferated up to the present time. b) For the first time American scientists took part in the EWMP, namely the President of the International Pigment Cell Society, Prof. T.B. Fitzpatrick (attracted by J. Duchon to taste a typical Czech goose), Jean Burnett and Frank Meyskens. c) For the first time there was a Japanese contribution presented at a EWMP by Dr Kowichi Jimbow who, even

after several days spent in Prague did not change his mind that the best beer in the world was Sapporo beer. d) For the first time there was a series of biophysical contributions delivered mostly by scientists from the Jagiellonian University in Krakow (Prof. S. Lukiewicz, Tadeusz Sarna and their coworkers). e) An unexpectedly high number of Abstracts forced us to introduce posters in the format of the EWMP and to divide the contributions into 66 lectures and 45 posters.

The Sections were as follows:

1. Ultrastructure and cell biology of pigment cells (chaired by K.Jimbow, I.Rosdahl, J.Svejda)
2. Biophysical, biochemical and physiological properties of melanins, melanosomes and melanocytes. Due to a high number of contributions the section was run in 3 consecutive sessions chaired by a) S. Lukiewicz, G. Prota; b) T.B. Fitzpatrick, C. Voulot, J. Duchon; c) P.A. Riley, D.O. Schachtschabel, J. Borovanský.
3. Melanoma: Basic properties. (Again in 3 sessions chaired by: a) J.A.A. Hunter, F. Vosmík; b) J.A. Lozano, H. Rorsman, B. Matous; c) K. Kurbanov, F. Meyskens, J. Sula
4. Immunologic properties of melanoma (J. Doré, H. Duchková)
5. Treatment of melanoma (M. Vaglini, K.D. Wozniak, Z. Mechl)
6. Normal and abnormasl pigmentation (G.Niebauer, J.P. Ortonne, J. Konopík)

After many years I can disclose a failure by us - the Organizers. The social programme included a guided tour to Konopiste Castle, followed by a Farewell Party at a nearby Gamekeeper´s Lodge. Sunsets in October come early and it had been necessary to divide the castle visitors into three groups. When the last participants left the castle, it was completely dark outside. We lost our way and, instead of a 200 metre walk to the Gamekeeper´s Lodge, we walked for a half an hour through the forest. Fortunately, the lady in charge of the trip had had an intuition (in 1981 there were no mobile phones) and had arranged for the coaches to be parked at the end of the forest path, so that many participants thought that the nocturnal Forest Walk was an imaginative planned feature of the programme.

The Opening Ceremony of the Prague 1981 Workshop in Aula Magna of the Charles University. From the left: F.Anders, G.Prota, K.Kurbanov, J.Riman (*president of the Czechoslovak Academy of Sciences)*, J.P. Ortonne, Thomas B. Fitzpatrick, J. Borovanský, Z.Mechl, J. Duchon, K.D.Wozniak, H.Duchková, P.A. Riley, J.Svejda, J.Homolka, F. Konopík, H. Rorsman, B.Matous.

J. Duchon, D.O.Schachtschabel,
F. Anders

Bengt Larson, ?,
H. Rorsman

D. Botti, ? a lady prof
from Italy

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Pr A. Napolitano)

Chemistry of melanin and related pigments

A few reports on the properties of natural melanins have appeared. The optical properties of melanin in melanocytic nevi are described in the paper by Zonios et al (*Photochem. Photobiol*) and compared with those of various skin types and skin lesions such as melanocytic nevi and melanoma previously reported by the same authors. Based on the antioxidant properties of the melanin pigment present in the muscles of the Gallus gallus Brisson chicken its potential use as a natural antioxidant in the food, cosmetic and pharmaceutical industries is suggested (Tu *et al Food Chem.*) . The investigators from the State Key Laboratory of Food Science and Technology of Nanchang University have previously extensively studied this pigment identifying it as a melanin by esr spectroscopy.

As usual a number of compounds of natural origin or obtained by synthetic procedures exhibiting a variety of structural features are proposed for melanogenesis control. Of particular interest is a patent disclosing the use of 2,2'-cyclolignans for inducing, restoring or stimulating the pigmentation of the skin, hair or hairs. (Bernard *et al* PCT Int. Appl., 2009),

A detailed investigation of melanin from melanocytes of different skin types in comparison with synthetic pigments was carried out by pyrolysis in combination with gas chromatography and mass spectrometry (Py-GC/MS) (Stepien *et al J. Am. Soc. Mass Spectrom.*). Products specific of eumelanin and pheomelanin were identified which offers a potential alternative to chemical degradation methods for the differentiation of epidermal melanin types.

On the side of melanin application in materials science a detailed characterization of self-arranged ordered and amorphous melanin films was gained by absorption spectra, spectra of photovoltage, and spectra of time-resolved photoluminescence (Davidenko *et al Molecular Crystals and Liquid Crystals*, 2008) Use of melanin containing hybrids as sunscreens having stronger UV protecting synergy effects was proposed by a Korean group (Park *et al*. *Bull Korean Chem Soc*) New org.-inorg. nanohybrid compounds were prepared by reaction of dopa with TiO_2 and were characterized by $^{1}H/^{13}C$ cross-polarization magic angle spinning (CPMAS) solid-state NMR and FT-IR spectroscopy . Preparation of thiol-capped gold nanoparticles modified with iron complexed melanin combining magnetic properties and biocompatibility together with a new strategy for their delivery was also reported. (Grumelli *et alChem Phys Chem*).

Structure, Reactivity and Properties

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2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

The review by **Aspengren and co-workers** will focus on the melanophore/melanocyte systems in fish, amphibians, and mammals. The authors describe the roles of melanin, melanophores, and melanocytes in animals, current views on how the three motor proteins dynein, kinesin, and myosin-V are involved in melanosome transport along microtubules and actin filaments, and how signal transduction pathways regulate the activities of the motors to achieve aggregation and dispersion of melanosomes. The authors also show how melanosomes are transferred to surrounding skin cells in amphibians and mammals. Comparative studies have revealed that the ability of physiological color change is lost during evolution while the importance of morphological color change, mainly via transfer of pigment to surrounding skin cells, increases. The authors conclude that in humans, pigment mainly has a role in protection against ultraviolet radiation, but also perhaps in the immune system.

Bedogni and Powell review the role of tissue oxygenation, and in particular physiologic skin hypoxia, on cell survival and senescence and how it contributes to melanocyte transformation and melanoma development. The tissue microenvironment plays a critical role in cell survival and growth and can contribute to cell transformation and tumor development. Cellular interactions with the stroma and with other cells provide key signals that control cellular arrest or division, survival or death, and entrance or exit from a quiescent state. Together, these decisions are essential for maintenance of tissue homeostasis. Tissue oxygenation is an important component of the microenvironment that can acutely alter the behaviour of a cell through the direct regulation of genes involved in cell survival, apoptosis, glucose metabolism, and angiogenesis. Loss of tissue homeostasis due to, for example, oncogene activation leads to the disruption of these signals and eventually can lead to cell transformation and tumor development.

Cutaneous melanoma deriving from the transformation of melanocytes is one of the most lethal cancers among young adults. The paper by **Botton et al.** shows that ciglitazone inhibits melanoma growth by inducing apoptosis and cell-cycle arrest, whereas normal melanocytes are resistant to ciglitazone. In melanoma cells, ciglitazone-induced apoptosis is associated with caspase activations and a loss of mitochondrial membrane potential. Induction of cell-cycle arrest by ciglitazone is associated with changes in expression of key cell-cycle regulators such as p21, cyclin D1, and pRB hypophosphorylation. Cell-cycle arrest occurs at low ciglitazone concentrations and through a PPARg-dependent pathway, whereas the induction of apoptosis is caused by higher ciglitazone concentrations and independently of PPARg. These results allow an effective molecular dissociation between proapoptotic effects and growth inhibition evoked by ciglitazone in melanoma cells. Finally, the authors show that in vivo treatment of nude mice by ciglitazone dramatically inhibits human melanoma xenograft development. The data presented suggest that ciglitazone might be a better candidate for clinical trials in melanoma treatment than the thiazolidinediones currently used in the treatment of type 2 diabetes, such as rosiglitazone, which is devoid of a proapoptotic PPARg-independent function.

Human pigmentation is a polygenic trait which may be shaped by different kinds of gene-gene interactions. Recent studies have revealed that interactive effects between HERC2 and OCA2 may be responsible for blue eye colour determination in humans. **Branicki and co-workers** performed a population study, examining important polymorphisms within the HERC2 and OCA2 genes. Furthermore, pooling these results with genotyping data for MC1R, ASIP and SLC45A2 obtained for the same population sample the authors also analysed potential genetic interactions affecting variation in eye, hair and skin colour. Results confirmed the association of HERC2rs12913832 with eye colour and showed that this SNP is also significantly associated with skin and hair colouration. It is also concluded that OCA2 rs1800407 is independently associated with eye colour. Finally, using various approaches the authors were able to show that there is an interaction between MC1R and HERC2 in determination of skin and hair colour in the studied population sample.

Chien and co-workers demonstrates that in malignant melanoma, hight levels of nuclear beta-catenin in both primary tumors and metastases correlate with reduced expression of a marker of proliferation and with improved survival, in contrast to other types of tumour such as colorectal cancer. The reduction in proliferation observed *in vivo* is evidenced in B16 murine melanoma cells and in human melanoma cell lines cultured *in vitro* with either WNT3A or small-molecule activators of beta-catenin signaling. In accord with these data, B16 melanoma cells expressing WNT3A also exhibit decreased tumor dimension and decreased metastasis popential when implanted *in vivo* into mice. Genome-wide transcriptional profiling showed that WNT3A up-regulates genes implicated in melanocyte differentiation, several of which are down-regulated with melanoma progression. These findings suggest that WNT3A can mediate transcriptional changes in melanoma cells in a manner reminiscent of the known role of Wnt/beta-catenin signaling in normal melanocyte development, thereby altering melanoma cell fate to one that may be less proliferative and potentially less aggressive. The authors conclude that their results may explain the observed loss of nuclear beta-catenin with melanoma progression in human tumors, which could reflect a dysregulation of cellular differentiation through a loss of homeostatic Wnt/beta-catenin signaling.

Melanin synthesis, is mainly dependent by tyrosinase activity. In tyrosinase-positive amelanotic melanomas this rate limiting enzyme is inactive because of acidic endo-melanosomal pH. The E5 oncogene of the Human Papillomavirus Type 16 is a small transmembrane protein with a weak transforming activity and a role during the early steps of viral infections. E5 has been shown to interact with 16 kDa subunit C of the trans-membrane Vacuolar ATPase proton pump ultimately resulting in its functional suppressions. However, the cellular effects of such an interaction are still under debate. **Di Domenico and co-workers** explored whether the HPV16 E5 oncoprotein does indeed interact with the vacuolar ATPase proton pump once expressed in intact human cells and whether this interaction has functional consequences on cell

metabolism and phenotype. The authors provide evidence that in the E5 expressing cells interaction between E5 and V-ATPase determines an increase of endo-cellular pH. The cellular alkalinisation in turn leads to the post-translational activation of tyrosinase, melanin synthesis and phenotype modulation. These effects are associated with an increased activation of tyrosine analogue anti-blastic drugs. The authors conclude that once expressed within intact human cells the HPV16-E5 oncoprotein does actually interact with the vacuolar V-ATPase proton pump and this interaction induces a number of functional effects. In amelanotic melanomas these effects can modulate the cell phenotype and can induce a higher sensitivity to tyrosine related anti-blastic drugs.

UV solar radiation is the major environmental risk factor for malignant melanoma. A great effort is currently posed on the search of new compounds able to prevent or reduce UV-mediated cell damage. Ferulic acid is a natural compound recently included in the formulation of solar protecting dermatological products. The purpose of the present work, **by Domenico et al**, was to assess whether its ethyl ester derivative, FAEE, could protect skin melanocytes from UV-induced oxidative stress and cell damage. Experiments on human melanocytes irradiated with UVB showed that FAEE treatment reduced the generation of ROS, with a net decrease of protein oxidation. FAEE treatment was accompanied by an induction of HSP70 and heme oxygenase, by a marked suppression of PARP activation and a significant suppression of apoptosis. Moreover FAEE prevented iNOS induction, thus suppressing the secondary generation of NO-derived oxidizing agents. FAEE may represent a potentially effective pharmacological approach to reduce UV radiation-induced skin damage.

The genetic background of cutaneous malignant melanoma (CMM) includes both germ line aberrations in high-penetrance genes, like CDKN2A, and allelic variation in low-penetrance genes like the melanocortin-1 receptor gene, MC1R. Red-hair colour associated MC1R alleles (RHC) have been associated with red hair, fair skin and risk of CMM. **Höiom et al.** investigated MC1R and CDKN2A variation in relation to phenotype, clinical factors and CMM risk in the Swedish population. The study cohort consisted of sporadic primary melanoma patients, familial melanoma patients and a control group. An allele-dose dependent increase in melanoma risk for carriers of variant MC1R alleles (after adjusting for phenotype), with an elevated risk among familial CMM patients, was observed. This elevated risk was found to be significantly associated with an increased frequency of dysplastic nevi (DN) among familial patients compared to sporadic patients. MC1R variation was found to be less frequent among acral lentiginous melanomas (ALM) and dependent on tumour localisation. No association was found between CDKN2A gene variants and general melanoma risk. Two new variants in the POMC gene were identified in red haired individuals without RHC alleles.

The melanocortin-1 receptor (MC1R) is a key regulator of pigmentation in mammals and some specific polymorphisms are tightly associated with an increased risk of skin cancers. Physiologically activated by alpha-melanocyte stimulating hormone (alphaMSH), MC1R function can be antagonized by a secreted factor, agouti signal protein (ASP), which is responsible for the lighter phenotypes in mammals, including humans, and is also associated with increased risk of skin cancer. It is therefore of great interest to characterize the molecular effects elicited by those MC1R ligands. **Le Pape and co-workers** determined the gene expression profiles of murine melan-a melanocytes treated with ASP or alphaMSH over a 4-day time course using genome-wide oligonucleotide microarrays. As expected, there were significant reductions in expression of numerous melanogenic proteins elicited by ASP, which correlates with its inhibition of pigmentation. ASP also unexpectedly modulated the expression of genes involved in various other cellular pathways, including glutathione synthesis and redox metabolism. Many genes up-regulated by ASP are involved in morphogenesis, cell adhesion, and extracellular matrix-receptor interactions. Concomitantly, ASP enhanced the migratory potential and the invasiveness of melanocytic cells in vitro. The results they are obtained demonstrate the role of ASP in the de-differentiation of melanocytes, identify pigment-related genes targeted by ASP and by alphaMSH, and provide insights into the pleiotropic molecular effects of MC1R signaling that may function during development and may affect skin cancer risk.

Hu and co-workers analysed the amount of Eumelanin and Pheomelanin in four immortal human uveal melanoma cell lines, by chemical degradation and microanalytical high-performance liquid chromatography and compared with those from 39 normal human uveal melanocyte cell lines. Uveal melanoma cells had a very low Eumelanin/Pheomelanin ratio, which was significantly lower than that from normal melanocytes isolated both from eyes with light-colored irides or dark-colored irides. This low ratio was caused by a low level of Eumelanin in melanoma cells, which was only 1/8 and 1/31 of that in melanocytes from eyes with light-colored irides and dark-colored irides, respectively. The Pheomelanin level in uveal melanoma cells was not statistically different from normal melanocytes from eyes with light-colored irides or dark-colored irides. The total quantity of Eumelanin and Pheomelanin in uveal melanoma cells was significantly less than that in normal melanocytes. This difference was because of the low level of Eumelanin in uveal melanoma cells. The results indicate that the changes of melanin content in uveal melanoma cells are mainly relate to the decrease of Eumelanin content. Low melanin and Eumelanin content may make melanoma cells more susceptible to mutagenic effects of ultraviolet radiation and oxidative stress, which may enhance the proliferation of melanoma cells and accelerate progression of melanoma.

The binding of alpha-melanocortin and its specific receptor, on the plasma membrane of melanin synthesising cells, plays a crucial role in melanins biosynthesis. Furthermore, loss of MC1R function is associated with an increased incidence of melanoma and non-melanoma skin cancer. The expression of the alpha-melanocortin receptor gene is highly controlled but until now, region responsible for tissue-specific activity of the gene promoter has not been identified. **Miccadei and co-workers** have cloned the genomic sequences upstream the human MC1R coding gene. A DNA fragment of 5 kilobases upstream the human MC1R encoding sequence was placed in front of a reporter gene and several deletion mutants of such fragment have been prepared. These constructs have been tested for the ability to drive the melanocyte-specific gene expression of the reporter gene using transfection experiments in melanocyte and non-melanocyte cell lines. From these experiments the authors identified a DNA fragment with the ability to drive the gene transcription in a tissue-specific way

and we used this small DNA fragment in DNA-protein interaction assays. the authors show that the 150 base pairs upstream the MC1R gene initiation codon are able to drive the melanocyte-specific gene transcription. Furthermore, they provide evidences suggesting that on such minimal melanocyte-specific gene promoter can assemble tissue-specific complexes. The authors conclude that the their results strongly imply that the transcriptional regulation of the melanocyte-specific MC1R gene requires an internal promoter located in the 150 base pairs upstream the initiation codon.

Although considered a significant psychosocial distress, little is known about the detailed mechanisms of hyperpigmentation. Recently, protein p53 has been demonstrated to promote ultraviolet B-induced skin pigmentation by stimulating the transcription of a melanogenic cytokine, pro-opiomelanocortin, in keratinocytes. Considering that p53 can be activated by various stresses, such as sun exposure, inflammation, and aging, this finding led **Murase and co-workers** to examine the involvement of p53 in cytokine receptor signaling, which might result in skin hyperpigmentation. Immunohistochemical and reverse transcription-PCR analyses revealed the increased expression and phosphorylation of p53 in the epidermis of hyperpigmented spots, accompanied by the higher expression of melanogenic cytokines, including stem cell factor, endothelin-1, and POMC. The involvement of p53 in hyperpigmentation was also indicated by the significantly higher expression of p53 transcriptional targets in the epidermis of hyperpigmented spots. Treatment of human keratinocytes and melanocytes with known p53 activators or inhibitors, including pifithrin-alpha (PFT), demonstrated significant increases or decreases, respectively, in the expression of melanogenic factors, including cytokines and their receptors. Additionally, PFT administration abolished stem cell factor-induced phosphorylation of mitogen-activated protein kinase in human melanocytes. Furthermore, when organ-cultured hyperpigmented spots, in vitro human skin substitutes, and mouse skin were treated with PFT or p53 small interfering RNA, the expression of melanogenic cytokines and their receptors was significantly decreased, as were levels of tyrosinase and melanogenesis. Taken together, these data reveal the essential role of p53 in hyperpigmentation of the skin via the regulation of paracrine-cytokine signaling, both in keratinocytes and in melanocytes.

Spry and co-workers describe the long-term effects of chronic topical forskolin treatment in this animal model. Forskolin-induced eumelanin production persisted through 3 months of daily applications, and forskolin-induced eumelanin remained protective against UV damage as assessed by minimal erythematous dose. No toxic changes were observed in the skin or overall health of animals exposed to prolonged forskolin therapy. Body weights were maintained throughout the course of topical forskolin application. Topical application of forskolin was associated with an increase in the number of melanocytes in the epidermis and thickening of the epidermis due, at least in part, to an accumulation of nucleated keratinocytes. Together, these data suggest in this animal model, short-term topical regular application of forskolin promotes eumelanin induction and that over time, topical forskolin treatment is associated with persistent melanization, epidermal cell accumulation, and skin thickening.

The eruption of nevi after an immunosuppressive condition is a phenomenon indicating that the immune system may play a major role in limiting proliferation of melanocytes. **Zattra and co-workers** analyze the role of immunosuppressive regimens on melanocyte proliferation. In particular, they discuss the eruptive nevi phenomenon, which is determined by the inability of the immune system to inhibit melanocyte proliferation. These clinical observations indicate that the immune system has a pivotal role in restraining melanocyte proliferation. However, although the role of the immune system in the development of non-melanoma skin cancer has been shown clearly in several studies involving organ transplant patients, the role of immunosuppression in melanoma genesis has not yet been established. Further investigations are required to establish the real immunogenicity of melanoma, particularly in the light of the dichotomy between the eruptive nevi phenomenon in immunosuppressed patients and the low incidence of melanoma in transplanted patients.

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3. MSH, MCH, other hormones, differentiation

(Pr M. Böhm)

POMC, alpha-MSH and MC-Rs

ß-MSH, MC-4R and epidermal cells – new insight into pigmentation and differentiation

Several previous reports already indicated that not only α- but also ß-melanocyte-stimulating hormone (MSH), especially in combination with ultraviolet (UV) radiation, can induce pigmentation. Moreover, binding sites for ß-MSH were detected in murine melanocytes and keratinocyte-derived cell lines many years ago. Regarding pigmentation, however, the melanocortin-1 receptor (MC-1R) which binds α-MSH and adrenocorticotropin with similar affinity has attained most attention. In a recent paper **(Spencer & Schallreuter, Endocrinology 2009; 150: 1250-1258)**, evidence is now provided that MC-4R immunoreactivity can be detected in human epidermis. By means of RT-PCR and Western immunoblotting, the authors further show in vitro expression of MC-4R in human epidermal melanocytes and keratinocytes, in the latter cell type, especially in differentiated cells. MC-4R expression in both human keratinocytes and melanocytes was subsequently confirmed by radiolabelled [^{125}]ß-MSH revealing the highest number of ß-MSH binding sites in differentiated keratinocytes. At the functional level, pharmacological blockade of MC-4R by a MC-4R antagonist (HS014) inhibited ß-MSH-induced melanin formation in human melanocytes. Mechanistically, ß-MSH not only increased intracellular cAMP but also protein levels of tyrosinase and Microphthalmia-associated transcription factor, two important downstream targets of the MCR-cAMP pathway orchestrating pigmentation. These data are of stimulating interest for our current concepts on pigmentation, but perhaps also for our funderstanding in keratinocyte biology.

Agouti signal protein in murine melanocytes- more than a pigmentation-inhibiting peptide

A major regulator of skin and hair pigmentation of many vertebrates is the MC-1R-dependent pathway. UVB irradiation induces cutaneous expression of proopiomelanocortin (POMC) and α-MSH expression which in turn not only induces pigmentation but also protects from UVB-induced DNA damage. It is also well established that the product of the *agouti* locus, agouti signal protein (ASP in mice, ASIP in humans) antagonizes MC-1R-mediated pigmentation as evidenced by various agouti phenotypes in mice. Recently, several lines of evidence suggest that ASIP which is widely expressed in various human tissues has a role in the development of cutaneous melanoma and non-melanoma skin cancer. Using a genome-wide oligonucleotide microarray approach, **Le Pape, Passeron, Giubellino, Valencia, Wolber and Hearing (Proc Natl Acad Sci USA 2009; 106: 1802-1807)** determined the expression profiles of murine melan-a melanocytes exposed to ASP or -MSH over a 4 day course. In accordance with its role of as a pigmentation-inhibiting peptide, ASIP affected the expression of various melanogenic genes such as tyrosinase, tyrp1 or tyrp2. Most interestingly, however, ASP also modulated the expression of other gene families including those involved in redox metabolism, morphogenesis, cell adhesion and extracellular matrix-receptor interaction. Accordingly, ASP increased cell migration and invasiveness of melanocytic cells in vitro suggesting that ASIP via such mechanisms could participate in the development of UVB-induced skin cancer.

Regulation of Nrf2 and Nrf-dependent enzymes – a novel cytoprotective facet of a-MSH

Human skin is constantly exposed to UV light which represents the most ubiquitous environmental stressor inducing DNA damage and oxidative stress. Among the key players orchestrating the cellular redox metabolism in response to oxidative stress are the so-called Nrf transcription factors. These factors regulate the expression of phase-II detoxifying enzymes like heme oxygenase (HO), a guardian of tissue damage. In a recent paper by **Kokot, Metze, Mouchet, Galibert, Schiller, Luger and Böhm (Endocrinology 2009; E-Pub, ahead of print),** the authors investigated the expression and regulation of Nrf1-3 in human skin cells in vitro, ex vivo and in situ. In particular, they examined whether α-MSH is capable of modulating Nrf2 and Nrf-dependent gene expression. Nrf1-3 mRNAs were detected in various cutaneous cell types in vitro but Nrf2 expression was most pronounced within the basal layer of the epidermis, especially in keratinocytes. Surprisingly, UVB irradiation at physiological doses *reduced* Nrf2 and Nrf-dependent gene expression in normal keratinocytes and melanocytes in vitro as well as ex vivo in skin organ cultures. α-MSH alone significantly increased Nrf2 as well as Nrf-dependent HO-1, α-glutamylcysteine-synthetase and glutathione-S-transferase Pi gene expression in both keratinocytes and melanocytes. This effect of α-MSH occurred at physiological doses, was due to transcriptional induction, mimicked by the artificial cAMP inducer forskolin, and blocked by PKA pathway inhibition. In silico promoter analysis of Nrf2 further identified several putative binding sites for activator protein 1 and cAMP response element binding protein, transcription factors typically activated by α-MSH. Importantly, α-MSH prevented or even over-compensated the UVB-induced suppression of Nrf2 and Nrf-dependent genes not only in normal keratinocytes and melanocytes in vitro but also in skin organ cultures. These findings reveal regulation of Nrf2 and Nrf-dependent genes by α-MSH. The data also highlight a new facet in the cytoprotective and antioxidative effector mechanism of α-MSH.

4. Photobiology

(Dr N. Smit)

In the Int Rev Cell Mol Biol Sara Aspengren *et al.* describe various aspects of melanosome transport in a chapter of 57 pages. Unfortunately, the role of UV in these processes is only mentioned briefly in some paragraphs of the chapter. Nevertheless, the authors provide a nice overview for those of us who are not familiar with this interesting field of pigment cell research (see also melanosomal section Pr J Borovansky).
Birlea, Costin and Norris produced a review of 33 pages in Medicinal Research Reviews, that may shed some new light on the function of vitamin D in pigmentation in general and in the repigmentation in vitiligo. In this case the combination with UV and corticosteroids for treatment of vitiligo patients and the mechanisms involved are described. In this review a role for UV in these mechanisms is discussed in more detail.
Interestingly, several vitamin D analogs are proposed that might show much better therapeutic efficacy than the conventional synthetic vitamin D compounds (e.g. 1,25(OH)2D3) used so far. The paper by Zmijewski et al describes the use of compounds that are converted by UVB and have antiproliferative properties against melanoma cells. Among the photoconversion products vitamin D like structures were identified. Some of the newly synthesized compounds were more potent inhibitors of melanoma growth than 1,25(OH)2D3.
Deacon et al describe studies addressing the problem of autologous tumor cell vaccines that may still contain viable tumor cells. Interestingly, UV(A and B) irradiation strongly improved the effects of gamma irradiation; controlling the melanoma cell proliferation.
Melanoma risk seems to be inversely correlated to smoking as was earlier reported by Freedman DM et al in 2003. On the other hand smoking has been shown to increase the risk of squamous cell carcinoma (De Hertog et al. 2001). Grant WB found a strong inverse relation between lung cancer and melanoma. Since both smoking and chronic UV irradiation result in skin aging and elastosis, the author suggests that the skin aging process is connected with reduced risk of melanoma.
In PCMR Hornyak et al describe Mitf as a regulator of apoptosis in melanocytes after UV irradiation. Wild type Mitf melanocytes were more resistant to UV than melanocytes partially deficient. As shown by comparison with albino melanocytes the difference was independent of melanin content or production indicating that Mitf itself is responsible for improved melanocyte survival after UV irradiation.
Merkel cell carcinoma (MCC) is described by Peggy B Liao. These tumors have a mortality rate higher than that of melanoma. Chronic sun exposure and immuno suppression are considered as risk factors for MCC. The epidemiological study by Lanoy et al among persons with AIDS in the US indeed show a greatly increased risk for MCC. Only modest excess risk of melanoma among the AIDS patients was found which was not related to immuno suppression.

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5. Neuromelanins

(Pr M. d'Ischia)

The present commentary is focused on some interesting papers on neuromelanin and neuronal degeneration that may open new scenarios in the field. A study by Zecca et al. (2008) sheds light on a new class of melanin-type pigments that occurs in the putamen, cortex, cerebellum, and other major regions of human brain. The most remarkable finding is that the neuromelanin-like pigments appear to originate from cysteinyldopa rather than cysteinyl-dopamine, as in the substantia nigra pigment. This is an important discovery that may open new vistas in the mechanisms of tyrosine metabolism and pigment formation in the brain. The origin from dopa and the possible involvement of enzymatic systems should be a major focus for future research, since it may reflect the occurrence of a pheomelanin-type pathway separate from catecholamine metabolism and driven by an oxidative stress-related aberrant conversion of dopa. Of interest is also a study by Bush et al. (2009) showing that neuromelanin pigments from the various brain regions show the same surface photoionization potentials, suggesting similar functional roles.

Using synchrotron x-ray microspectroscopy, Bohic et al. (2008) provide a description of the microenvironment of neuromelanin in whole neurons with age. High spatial resolution data in particular showed iron-rich microdomains colocalized with other elements within the pigment, a finding of possible relevance to the biosynthesis of the pigment. The demonstration of reduced sulfur derivatives and various forms of oxidized sulfur, including a significant increase in sulfonate, is an additional important observation which integrates and extends our current understanding of the ontogenesis and regulation of neuromelanin in human brain.

Finally, Herrero Hernandez (2009) draws attention on a possible, hitherto overlooked link between melanoma and Parkinson's disease that resides in genes that regulate pigmentation, e.g. those involved in the synthesis of dopamine and related compounds.

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  Abstract: The microchem. environment of neuromelanin (NM) in whole neurons from formalin fixed and paraffin embedded human substantia nigra sections were characterized using synchrotron chem. x-ray microscopy. Concns. of NM-assocd. elements increased in the developing brain; the highest levels of most elements were found in the mature brain but the temporal pattern of the accumulation of different elements varied. High spatial resoln. investigations, using a unique hard x-ray nanoprobe, revealed iron-rich microdomains colocalized with other elements within the pigment. These microdomains represent the first visualization of a structure regulating the metal-binding properties of NM and supporting a physiol. role for NM in the regulation of functionally important elements in pigmented neurons. The authors' results demonstrate that the local chem. environment of iron in NM is similar to that found in ferritin and points to a possible role of iron in NM biosynthesis. Intracellular speciation of sulfur contained in NM revealed the presence of reduced sulfur compds. and various forms of oxidized sulfur compds. which have not previously been reported. Further, a significant increase in sulfonate in NM in the mature brain suggests that in vivo metab. of the pigment via an as yet unidentified pathway occurs. The current data add to the understanding of the development and regulation of NM in the human brain.

- Bush WD, Garguilo J, Zucca FA, Bellei C, Nemanich RJ, Edwards GS, Zecca L, Simon JD.
  Neuromelanins isolated from different regions of the human brain exhibit a common surface photoionization threshold. Photochemistry and Photobiology 85(1): 387-390, 2009.
  Abstract: Neuromelanin (NM) isolated from the premotor cortex, cerebellum, putamen, globus pallidus, and corpus callosum of the human brain was studied by scanning probe microscopy and photoelectron emission microscopy (PEEM) and the results were compared with previously published work on NM from the substantia nigra. SEM revealed a common structure for all NMs. All NMs exhibited spherical entities of diams. of 200-400 nm, composed of smaller spherical substructures, .apprx.30 nm in diam. These features were similar to those obsd. for many melanin systems including Sepia cuttlefish, bovine eye, and human eye and hair melanosomes. Photoelectron microscopy images were collected for all NMs at specific wavelengths of UV light of 248-413 nm, using the spontaneous emission output from the Duke free electron laser. Anal. of the data established a common threshold PEEM photoionization potential for neuromelanins of 4.7 ± 0.2 eV, corresponding to an oxidn. potential of -0.3 ± 0.2 V vs the normal H electrode (NHE). These results were consistent with previously reported potentials for NM from the substantia nigra of 4.5 ± 0.2 eV (-0.1 ± 0.2 V vs NHE). All NMs exhibited a common low surface oxidn. potential, reflecting their eumelanic component and their inability to trigger redox processes with neurotoxic effect.

- Goodman G, Bercovich D.
  Melanin directly converts light for vertebrate metabolic use: Heuristic thoughts on birds, Icarus and dark human skin. Medical Hypotheses 71(2): 190-202, 2008.

Abstract: A review. Pigments serve many visually obvious animal functions (e.g. hair, skin, eyes, feathers, and scales). One is melanin, unusual in an absorption across the UV-visual spectrum which is controversial. Any polymer or macro-structure of melanin monomers is melanin. Its roles derive from complex structural and phys.-chem. properties e.g. semiconductor, stable radical, conductor, free radical scavenger, and charge-transfer. Clinicians and researchers are well acquainted with melanin in skin and ocular pathologies and now increasingly are with internal, melanized, pathol.-assocd. sites not obviously subject to light radiation (e.g. brain, cochlea). At both types of sites some findings puzzle: pos. and neg. neuromelanin effects in Parkinsons; unexpected melanocyte action in the cochlea, in deafness; melanin reduces DNA damage, but can promote melanoma; in melanotic cells, mitochondrial no. was 83% less, respiration down 30%, but development similar to normal amelanotic cells. A little known, avian anatomical conundrum may help resolve melanin paradoxes. One of many unique adaptations to flight, the pecten, strange intra-ocular organ with unresolved function(s), is much enlarged and heavily melanized in birds fighting gravity, hypoxia, thirst, and hunger during long-distance, frequently sub-zero, non-stop migration. The pecten may help cope with energy and nutrient needs under extreme conditions, by a marginal but crit., melanin-initiated conversion of light to metabolic energy, coupled to local metabolite recycling. Similarly in Central Africa, redn. in body hair and melanin increase may also have lead to photomelanometabolism which, though small scale/unit body area, in total may have enabled a sharply increased development of the energy-hungry cortex and enhanced human survival generally. Animal inability to utilize light energy directly has been traditionally assumed. Melanin and the pecten may have unexpected lessons also for human physiol. and medicine.

- Herndon E S, Hladik CL, Shang P, Burns DK, Raisanen J, White CL.
Neuroanatomic Profile of Polyglutamine Immunoreactivity inHuntington Disease Brains. Journal of Neuropathology & Experimental Neurology 68(3): 250-261, 2009.
Abstract: A pathol. hallmark of Huntington disease (HD) is the presence of intraneuronal aggregates of polyglutamine-contg. huntingtin protein fragments. Monoclonal antibody 1C2 is a com. antibody to normal human TATA-binding protein that detects long stretches of glutamine residues. Using 1C2 as a surrogate marker formutant huntingtin protein, we immunostained 19 HD cases, 10 normal controls, and 10 cases of frontotemporal degeneration with ubiquitinated inclusions as diseased controls. In the HD cases, there was consistent 1C2 immunoreactivity in the neocortex, striatum, hippocampus, lateral geniculate body, basis pontis, medullary reticular formation, and cerebellar dentate nucleus. The normal and diseased controls demonstrated 1C2 immunoreactivity only in the substantia nigra, locus coeruleus, and pituitary gland. Staining of 5 HD cases and 5 normal controls revealed a less consistent and less diagnostically useful morphol. immunoreactivity profile. These results indicate that widespread 1C2 immunoreactivity is present in diverse central nervous system areas in HD, and that in the appropriate setting, 1C2 staining can be a useful tool in the postmortem diagnosis of HD when neuromelanin-contg. neuronal populations are avoided.

- Herrero Hernandez E.
Pigmentation genes link Parkinson's disease to melanoma, opening a window on both etiologies. Medical Hypotheses 72(3): 280-284, 2009.
Summary: Melanomas occur more frequently among subjects with Parkinson's disease (PD) and a biol. explanation for this epidemiol. observation is lacking. It is also well-known that pigmentation genes play an important role in the development of melanomas. It is therefore suggested that the link between both diseases resides in genes that regulate pigmentation. Among these, those involved in the synthesis of dopamine and related compds. as melanin appear to be the most plausible candidates. While it is known that individuals with fair phototypes have an increased risk for melanoma, this hypothesis suggests that the same applies to Parkinson's disease. It is therefore postulated that the accurate anal. of the phototype could be used to identify subjects at higher risk for both diseases, possibly allowing preventative interventions (photoprotective, nutritional, occupational) and prediction of risk in childhood. Another possible implication of this hypothesis is that therapeutic strategies targeting melanogenesis could maintain or perhaps restore the physiol. concns. of neuromelanin in the substantia nigra and achieve protection against neuronal loss in subjects at risk of developing PD.

- Karlsson O, Berg C, Brittebo EB, Lindquist NG.
Retention of the cyanobacterial neurotoxin β -N-methylamino-L-alanine in melanin and neuromelanin-containing cells - a possible link between Parkinson-dementia complex and pigmentary retinopathy. Pigment Cell & Melanoma Research 22(1): 120-130, 2009.
Abstract: β-N-methylamino-L-alanine (BMAA), a neurotoxic amino acid produced by cyanobacteria, has been suggested to be involved in the etiol. of a neurodegenerative disease complex which includes Parkinson-dementia complex (PDC). In PDC,neuromelanin-contg. neurons in substantia nigra are degenerated. Many PDC patients also have an uncommon pigmentary retinopathy. The aim of this study was to investigate the distribution of 3H-BMAA in mice and frogs, with emphasis on pigment-contg. tissues. Using autoradiog, a distinct retention of 3H-BMAA was obsd. in melanin-contg. tissues such as the eye and neuromelanin-contg. neurons in frog brain. Anal.of the binding of 3H-BMAA to Sepia melanin in vitro demonstrated two apparent binding sites. In vitro-studies with synthetic melanin revealed a stronger interaction of 3H-BMAA with melanin during synthesis than the binding to preformed melanin. Long-term exposure to BMAA may lead to bioaccumulation in melanin- and neuromelanin-contg. cells causing high intracellular levels, and potentially changed melanin characteristics via incorporation of BMAA into the melanin

polymer. Interaction of BMAA with melanin may be a possible link between PDC and pigmentary retinopathy.

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  New melanic pigments in the human brain that accumulate in aging and block environmental toxic metals. Proceedings of the National Academy of Sciences of the United States of America 105(45):17567-17572, 2008.
  Abstract: Neuronal pigments of melanic type were identified in the putamen, cortex, cerebellum, and other major regions of human brain. These pigments consist of granules 30 nm in size, contained in organelles together with lipid droplets, and they accumulate in aging, reaching concns. as high as 1.5-2.6 μg/mg tissue in major brain regions. These pigments, which we term neuromelanins, contain melanic, lipid, and peptide components. The melanic component is arom. in structure, contains a stable free radical, and is synthesized from the precursor mol. cysteinyl-3,4-dihydroxyphenylalanine. This contrasts with neuromelanin of the substantia nigra, where the melanic precursor is cysteinyl-dopamine. These neuronal pigments have some structural similarities to the melanin found in skin. The precursors of lipid components of the neuromelanins are the polyunsatd. lipids present in the surrounding organelles. The synthesis of neuromelanins in the various regions of the human brain is an important protective process because the melanic component is generated through the removal of reactive/toxic quinones that would otherwise cause neurotoxicity. Furthermore, the resulting melanic component serves an addnl. protective role through its ability to chelate and accumulate metals, including environmentally toxic metals such as mercury and lead.

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  Human neuromelanin induced microglial overactivation: a novel mechanism for the progressive dopaminergic neurodegeneration. Zhongguo Shenjing Mianyixue He Shenjingbingxue Zazhi 15(4): 274-278, 1 plate, 2008.
  Abstract: The objective of the paper is to investigate the role and mechanism of microglia in dopaminergic neurodegeneration induced by human neuromelanin (HNM) from substantia nigra pars compacta (SNpc) by using multiple primary mesencephalic culture systems. In primary mesencephalic glia-reconstituted cultures, the role of microglia on HNM-elicited dopaminergic neurodegeneration was evaluated by measuring DA uptake. In microglia-enriched cultures, functional and morphol. microglial overactivation by HNM was explored by staining microglia with OX-42 antibody and detecting the prodn. of a series of pro-inflammatory and neurotoxic factors. The 5.0 μg/mL HNM-induced DA uptake were 58%, 51% and 35% of neuron enriched cultures (control) in 10%, 20% and 30% microglia-reconstituted cultures resp., which were statistically significant compared with control group; 5.0 μg/mL HNM-induced DA uptake were 97%, 92% and 93% of neuron-enriched cultures (control)in 40%, 50% and 60% astroglia reconstituted cultures resp., which weren't statistically significant compared with control group. The 5.0 μg/mL HNM increased the no. of activated microglia, which was 5.77 folds of control group, and was statistically significant; HNM changed the morphol. of microglia indicated by enlarging cell body, irregular shape such as rod and/or amoeboid-like shape and intensified staining. The 5.0 μg/mL HNM produced a large amt. of intracellular reactive oxygen species, nitrite oxide, tumor necrosis factors α, interleukin-1β and prostaglandin E2, which were all statistically significant compared with control group. HNM overactivated microglia functionally and morphol., accelerating dopaminergic neurodegeneration. Thus inhibition of microglial overactivation might be a novel target for PD therapy.

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  Human neuromelanin induced selective and progressive dopaminergic neurodegeneration. Zhongguo Shenjing Mianyixue He Shenjingbingxue Zazhi 15(4): 259-262, 312, 1 plate, 2008.
  Abstract : Objective: To investigate the role and mechanism of human neuromelanin (HNM) extd. from substantia nigra pars compacta (SNpc) on dopamine (DA) neurons. Methods: (1) Mixed neuron-glia cultures were treated with 5 μg/mL HNM for 10 d and the effect of HNM on DA neurons was evaluated by detecting the uptake capacity of DA neurons, counting tyrosine hydroxylase (TH)-pos. neuronal no., measuring neuronal dendrite length and observing morphol. changes. (2) Difference of uptake capacity between DA and γ-aminobutyric acid (GABA) neurons and no. between DA and total neurons were compared. (3) Uptake capacities of DA neurons in mixed neuron-glia, neuron-enriched and neuron-astroglia cultures were detected. Results: (1) In mixed neuron-glia cultures, 5 μg/mL HNM-induced uptake capacity of DA neurons, TH (+) neuronal no. and neuronal dendrite length were 36%, 41% and 40% of the control ($P < 0.001$, $P < 0.05$, $P < 0.001$, resp.). The cell bodies were shrunk and smaller, the cytoplasmic stainings were reduced and the neuronal dendrites were decreased, shorter, thinner, broken and even gone. (2) The difference of DA and GABA uptake elicited by 5 μg/mL HNM for 10 d was 28% ($P < 0.005$), and were 26% and 29% after treating the cells for 7 d and 10 d ($P < 0.05$, $P < 0.01$), resp. The difference of neuronal no. between DA and total neurons was 33% ($P < 0.01$), which all were statistically significant. (3) DA uptake produced by 5 μg/mL HNM were 49%, 81% and 83% of the control in mixed neuron-glia, neuron-enriched and neuron-astroglia cultures, resp., and the difference was significant between the first and second group ($P < 0.01$) and was not significant between the second and third group. Conclusions: (1) HNM induced dopaminergic neurodegeneration was functionally and morphol. selective to DA neurons. (2) HNM produced dopaminergic neurotoxicity was potentiated by microglia to some degree. (3) HNM was a pivotal endogenous mechanism for PD and might be a promising model for PD study.

6. Genetics, molecular and developmental biology

(Dr F. Beermann)

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  Molecular and evolutionary history of melanism in North American gray wolves. Science 323: 1339-1343, 2009.
  Shortened abstract: Melanism in the gray wolf, Canis lupus, is caused by a different melanocortin pathway component, the K locus, that encodes a beta-defensin protein that acts as an alternative ligand for Mc1r. We show that the melanistic K locus mutation in North American wolves derives from past hybridization with domestic dogs, has risen to high frequency in forested habitats, and exhibits a molecular signature of positive selection. The same mutation also causes melanism in the coyote, Canis latrans, and in Italian gray wolves, and hence our results demonstrate how traits selected in domesticated species can influence the morphological diversity of their wild relatives.

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  The p53 tumor suppressor causes congenital malformations in Rpl24-deficient mice and promotes their survival. Mol Cell Biol, 2009 [Epub ahead of print]
  Abstract: Hypomorphic mutation in one allele of ribosomal protein l24 gene (Rpl24) is responsible for the Belly Spot and Tail (Bst) mouse, which suffers from defects of the eye, skeleton, and coat pigmentation. It has been hypothesized that these pathological manifestations result exclusively from faulty protein synthesis. Herein, we demonstrate that up-regulation of the p53 tumor suppressor during the restricted period of embryonic development significantly contributes to the Bst phenotype. However, in the absence of p53 a large majority of Rpl24(Bst/+) embryos die. We showed that p53 promotes survival of these mice via p21-dependent mechanism. Our results imply that activation of a p53-dependent checkpoint mechanism in response to various RP deficiencies might also play a role in the pathogenesis of congenital malformations in humans.

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  The chemokine SDF-1/CXCL12 regulates the migration of melanocyte progenitors in mouse hair follicles. Differentiation 77: 395-411, 2009.
  Abstract: Mouse skin melanocytes originate from the neural crest and subsequently invade the epidermis and migrate into the hair follicles (HF) where they proliferate and differentiate. Here we demonstrate a role for the chemokine SDF-1/CXCL12 and its receptor CXCR4 in regulating the migration and positioning of melanoblasts during HF formation and cycling. CXCR4 expression by melanoblasts was upregulated during the anagen phase of the HF cycle. CXCR4-expressing cells in the HF also expressed the stem cell markers nestin and LEX, the neural crest marker SOX10 and the cell proliferation marker PCNA. SDF-1 was widely expressed along the path taken by migrating CXCR4-expressing cells in the outer root sheath (ORS), suggesting that SDF-1-mediated signaling might be required for the migration of CXCR4 cells. Skin sections from CXCR4-deficient mice, and skin explants treated with the CXCR4 antagonist AMD3100, contained melanoblasts abnormally concentrated in the epidermis, consistent with a defect in their migration. SDF-1 acted as a chemoattractant for FACS-sorted cells isolated from the anagen skin of CXCR4-EGFP transgenic mice in vitro, and AMD3100 inhibited the SDF-1-induced migratory response. Together, these data demonstrate an important role for SDF-1/CXCR4 signaling in directing the migration and positioning of melanoblasts in the HF.

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  Mice with mutations of Dock7 have generalized hypopigmentation and white-spotting but show normal neurological function. Proc Natl Acad Sci U S A 106: 2706-2711, 2009.
  Abstract: The classical recessive coat color mutation misty (m) arose spontaneously on the DBA/J background and causes generalized hypopigmentation and localized white-spotting in mice, with a lack of pigment on the belly, tail tip, and paws. Here we describe moonlight (mnlt), a second hypopigmentation and white-spotting mutation identified on the C57BL/6J background, which yields a phenotypic copy of m/m coat color traits. We demonstrate that the 2 mutations are allelic. m/m and mnlt/mnlt phenotypes both result from mutations that truncate the dedicator of cytokinesis 7 protein (DOCK7), a widely expressed Rho family guanine nucleotide exchange factor. Although Dock7 is transcribed at high levels in the developing brain and has been implicated in both axon development and myelination by in vitro studies, we find no requirement for DOCK7 in neurobehavioral function in vivo. However, DOCK7 has non-redundant role(s) related to the distribution and function of dermal and follicular melanocytes.

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Summary: Whereas c-kit deficiency leads to a loss of melanophores, an additional mutation of foxd3 or downregulation of foxd3 at least partially rescues melanophores from cell death. This suggests a kit-dependent role for foxd3 in the regulation of melanophore survival.

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Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet, 2009 [Epub ahead of print].
A novel mouse model for melanoma / abstract: Mutational activation of BRAF is the earliest and most common genetic alteration in human melanoma. To build a model of human melanoma, we generated mice with conditional melanocyte-specific expression of BRaf(V600E). Upon induction of BRaf(V600E) expression, mice developed benign melanocytic hyperplasias that failed to progress to melanoma over 15-20 months. By contrast, expression of BRaf(V600E) combined with Pten tumor suppressor gene silencing elicited development of melanoma with 100% penetrance, short latency and with metastases observed in lymph nodes and lungs. Melanoma was prevented by inhibitors of mTorc1 (rapamycin) or MEK1/2 (PD325901) but, upon cessation of drug administration, mice developed melanoma, indicating the presence of long-lived melanoma-initiating cells in this system. Notably, combined treatment with rapamycin and PD325901 led to shrinkage of established melanomas. These mice, engineered with a common genetic profile to human melanoma, provide a system to study melanoma's cardinal feature of metastasis and for preclinical evaluation of agents designed to prevent or treat metastatic disease.

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Inhibition of PAX3 by TGF-beta modulates melanocyte viability. Mol Cell 32: 554-563, 2008
Abstract: The protein encoded by paired-box homeotic gene 3 (PAX3) is a key regulator of the microphthalmia-associated transcription factor (Mitf) in the melanocyte lineage. Here, we show that PAX3 expression in skin is directly inhibited by TGF-beta/Smads. UV irradiation represses TGF-beta in keratinocytes, and the repression of TGF-beta/Smads upregulates PAX3 in melanocytes, which is associated with a UV-induced melanogenic response and consequent pigmentation. Furthermore, the TGF-beta-PAX3 signaling pathway interacts with the p53-POMC/MSH-MC1R signaling pathway, and both are crucial in melanogenesis. The activation of p53-POMC/MSH-MC1R signaling is required for the UV-induced melanogenic response because PAX3 functions in synergy with SOX10 in a cAMP-response element (CRE)-dependent manner to regulate the transcription of Mitf. This study will provide a rich foundation for further research on skin cancer prevention by enabling us to identify targeted small molecules in the signaling pathways of the UV-induced melanogenic response that are highly likely to induce naturally protective pigmentation.
See commentaries by Loeken MR (2009) Pigment Cell Melanoma Res 22: 146-147 and Moustakas A (2008) Dev Cell 15: 797-799, 2008.

7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borron)

- Ayres JS, Schneider DS.
  A signaling protease required for melanization in Drosophila affects resistance and tolerance of infections. PLoS Biol. 6(12):2764-73, 2008.
  Organisms evolve two routes to surviving infections-they can resist pathogen growth (resistance) and they can endure the pathogenesis of infection (tolerance). The sum of these two properties together defines the defensive capabilities of the host. Typically, studies of animal defenses focus on Esther understanding resistance or, to a lesser extent, tolerance mechanisms, thus providing little understanding of the relationship between these two mechanisms. We suggest there are nine possible pairwise permutations of these traits, assuming they can increase, decrease, or remain unchanged in an independent manner. Here we show that by making a single mutation in the gene encoding a protease, CG3066, active in the melanization cascade in Drosophila melanogaster, we observe the full spectrum of changes; these mutant flies show increases anddecreases in their resistance and tolerance properties when challenged with a variety of pathogens. This result implicates melanization in fighting microbial infections and shows that an immune response can affect both resistance and tolerance to infections in microbe-dependent ways. The fly is often described as having an unsophisticated and stereotypical immune response where single mutations cause simple binary changes in immunity. We report a level of complexity in the fly's immune response that has strong ecological implications. We suggest that immune responses are highly tuned by evolution, since selection for defenses that alter resistance against one pathogen may change both resistance and tolerance to other pathogens.

- Chawla S, deLong MA, Visscher MO, Wickett RR, Manga P, Boissy RE.
  Mechanism of tyrosinase inhibition by deoxyArbutin and its second-generation derivatives. Br J Dermatol.159(6):1267-74, 2008. Epub 2008 Sep 20.
  BACKGROUND: Disorders, such as age spots, melasma and hyperpigmentation at sites of actinic damage, emanate from the augmentation of an increased amount of epidermal melanin. OBJECTIVES: The ineptness of current therapies in treating these conditions, as well as high cytotoxicity, mutagenicity, poor skin penetration and low stability of skin-depigmenting formulations led us to investigate new compounds that meet the medical requirements for depigmentation agents. We have shown previously that the tyrosinase inhibitor deoxyArbutin (dA) is a more effective and less toxic skin lightener than hydroquinone (HQ). METHODS: The efficacy and reversibility of dA and its derivatives on inhibiting tyrosine hydroxylase and DOPAoxidase was assessed using standard assays. RESULTS: dA and its second-generation derivatives inhibit tyrosine hydroxylase and DOPAoxidase activities of tyrosinase dose dependently thereby inhibiting melanin synthesis in intact melanocytes, when used at concentrations that retain 95% cell viability in culture. This depigmenting effect was completely reversible when the compounds were removed. Tyrosinase inhibition was also observed in vitro when tested using human and purified mushroom tyrosinase, establishing that they are direct enzyme inhibitors. Lineweaver-Burk reciprocal plot analysis using mushroom tyrosinase illustrated that dA and its derivatives are more robust competitive inhibitors than HQ, when tyrosine is used as substrate. CONCLUSIONS: Thus, dA and its second-generation derivatives, which inhibit melanogenesis at safe concentrations by specifically acting on the tyrosinase enzyme at a post-translational level, are promising agents to ameliorate hyperpigmented lesions or lighten skin.

- Chung SW, Ha YM, Kim YJ, Song S, Lee H, Suh H, Chung HY.
  Inhibitory effects of 6-(3-hydroxyphenyl)-2-naphthol on tyrosinase activity and melanin synthesis. Arch Pharm Res. 32(2):289-94, 2009.
  As a part of an ongoing project searching for new skin-lightening agents, the inhibitory property of 6-(3-Hydroxyphenyl)-2-naphthol (HPN) on melanogenesis was investigated. The inhibitory action of HPN (IC(50)=15.2 muM) on mushroom tyrosinase was revealed. To further explore the action of HPN on melanogenesis, the inhibition of tyrosinase and melanin levels were measured in B16 melanoma cells (B16 cells). Results show that HPN inhibited tyrosinase activity and reduced melanin in B16 cells. Therefore, our data indicate HPN as a new candidate for depigmentation reagents.

- Cook AL, Chen W, Thurber AE, Smit DJ, Smith AG, Bladen TG, Brown DL, Duffy DL, Pastorino L, Bianchi-Scarra G, Leonard JH, Stow JL, Sturm RA.
  Analysis of cultured human melanocytes based on polymorphisms within the SLC45A2/MATP, SLC24A5/NCKX5, and OCA2/P loci. J Invest Dermatol. 129(2):392-405, 2009.
  Single nucleotide polymorphisms (SNPs) within the SLC45A2/MATP, SLC24A5/NCKX5, and OCA2/P genes have been associated with natural variation of pigmentation traits in human populations. Here, we describe the characterization of human primary melanocytic cells genotyped for polymorphisms within the MATP, NCKX5, or OCA2 loci. On the basis of genotype, these cultured cells reflect the phenotypes observed by others in terms of both melanin content and tyrosinase (TYR) activity when comparing skin designated as either "White" or "Black". We found a statistically significant association of MATP-374L (darker skin) with higher TYR protein abundance that was not observed for any NCKX5-111 or OCA2 rs12913832 allele. MATP-374L/L homozygous strains displayed significantly

lower MATP transcript levels compared to MATP-374F/F homozygous cells, but this did not reach statistical significance based on NCKX5 or OCA2 genotype. Similarly, we observed significantly increased levels of OCA2 mRNA in rs12913832-T (brown eye) homozygotes compared to rs12913832-C (blue eye) homozygous strains, which was not observed for MATP or NCKX5 gene transcripts. In genotype-phenotype associations performed on a collection of 226 southern European individuals using these same SNPs, we were able to show strong correlations in MATP-L374F, OCA2, and melanocortin-1 receptor with skin, eye, and hair color variation, respectively.

- Di Domenico F, Foppoli C, Blarzino C, Perluigi M, Paolini F, Morici S, Coccia R, Cini C, De Marco F.
Expression of human papilloma virus type 16 E5 protein in amelanotic melanoma cells regulates endo-cellular pH and restores tyrosinase activity. J Exp Clin Cancer Res. 28:4,2009.
BACKGROUND: Melanin synthesis, the elective trait of melanocytes, is regulated by tyrosinase activity. In tyrosinase-positive amelanotic melanomas this rate limiting enzyme is inactive because of acidic endo-melanosomal pH. The E5 oncogene of the Human Papillomavirus Type 16 is a small transmembrane protein with a weak transforming activity and a role during the early steps of viral infections. E5 has been shown to interact with 16 kDa subunit C of the trans-membrane Vacuolar ATPase proton pump ultimately resulting in its functional suppressions. However, the cellular effects of such an interaction are still under debate. With this work we intended to explore whether the HPV16 E5 oncoprotein does indeed interact with the vacuolar ATPase proton pump once expressed in intact human cells and whether this interaction has functional consequences on cell metabolism and phenotype. METHODS: The expression of the HPV16-E5 oncoproteins was induced in two Tyrosinase-positive amelanotic melanomas (the cell lines FRM and M14) by a retroviral expression construct. Modulation of the intracellular pH was measured with Acridine orange and fluorescence microscopy. Expression of tyrosinase and its activity was followed by RT-PCR, Western Blot and enzyme assay. The anchorage-independence growth and the metabolic activity of E5 expressing cells were also monitored. RESULTS: We provide evidence that in the E5 expressing cells interaction between E5 and V-ATPase determines an increase of endo-cellular pH. The cellular alkalinisation in turn leads to the post-translational activation of tyrosinase, melanin synthesis and phenotype modulation. These effects are associated with an increased activation of tyrosine analogue anti-blastic drugs. CONCLUSION: Once expressed within intact human cells the HPV16-E5 oncoprotein does actually interact with the vacuolar V-ATPase proton pump and this interaction induces a number of functional effects. In amelanotic melanomas these effects can modulate the cell phenotype and can induce a higher sensitivity to tyrosine related anti-blastic drugs.

- Gandía-Herrero F, Jiménez-Atiénzar M, Cabanes J, Escribano J, García-Carmona F.
Fluorescence detection of tyrosinase activity on dopamine-betaxanthin purified from Portulaca oleracea (common purslane) flowers. J Agric Food Chem. 57(6):2523-8, 2009.
Tyrosinase or polyphenol oxidase (EC 1.14.18.1) is one of the key enzymes for the biosynthesis of natural pigment betalains. These are an important class of water-soluble pigments, characteristic of plants belonging to the order Caryophyllales. In this work, dopamine-betaxanthin (also known as miraxanthin V) is reported as the pigment responsible for the bright coloration in yellow flowers of Portulaca oleracea (common purslane). The natural pigment is purified, and used as a substrate for the catecholase (diphenolase) activity of the enzyme tyrosinase. A new, continuous method to follow the activity is developed based on the fluorescent properties of the betaxanthin. Fluorescence of the enzyme activity derived products is reported for the first time. Relevance of the fluorescent phenomenon is discussed based on fluorescence images and the description of a physiological inner filter effect present in flowers of P. oleracea. The first description of the betalain content in flower pistils is also provided.

- Ganesan AK, Ho H, Bodemann B, Petersen S, Aruri J, Koshy S, Richardson Z, Le LQ, Krasieva T, Roth MG, Farmer P, White MA.
Genome-wide siRNA-based functional genomics of pigmentation identifies novel genes and pathways that impact melanogenesis in human cells. PLoS Genet. 4(12):e1000298, 2008. Epub 2008 Dec 5.
Melanin protects the skin and eyes from the harmful effects of UV irradiation, protects neural cells from toxic insults, and is required for sound conduction in the inner ear. Aberrant regulation of melanogenesis underlies skin disorders (melasma and vitiligo), neurologic disorders (Parkinson's disease), auditory disorders (Waardenburg's syndrome), and opthalmologic disorders (age related macular degeneration). Much of the core synthetic machinery driving melanin production has been identified; however, the spectrum of gene products participating in melanogenesis in different physiological niches is poorly understood. Functional genomics based on RNA-mediated interference (RNAi) provides the opportunity to derive unbiased comprehensive collections of pharmaceutically tractable single gene targets supporting melanin production. In this study, we have combined a high-throughput, cell-based, one-well/one-gene screening platform with a genome-wide arrayed synthetic library of chemically synthesized, small interfering RNAs to identify novel biological pathways that govern melanin biogenesis in human melanocytes. Ninety-two novel genes that support pigment production were identified with a low false discovery rate. Secondary validation and preliminary mechanistic studies identified a large panel of targets that converge on tyrosinase expression and stability. Small molecule inhibition of a family of gene products in this class was sufficient to impair chronic tyrosinase expression in pigmented melanoma cells and UV-induced tyrosinase expression in primary melanocytes. Isolation of molecular machinery known to support autophagosome biosynthesis from this screen, together with in vitro and in vivo validation, exposed a close functional relationship between melanogenesis and autophagy. In summary, these studies illustrate the

power of RNAi-based functional genomics to identify novel genes, pathways, and pharmacologic agents that impact a biological phenotype and operate outside of preconceived mechanistic relationships.

- Güell M, Luis JM, Solà M, Siegbahn PE.
  Theoretical study of the hydroxylation of phenolates by the Cu(2)O (2)(N,N'-dimethylethylenediamine) (2) (2+) complex. J Biol Inorg Chem. 14(2):229-42, 2009. Epub 2008 Oct 30.
  Tyrosinase catalyzes the ortho hydroxylation of monophenols and the subsequent oxidation of the diphenolic products to the resulting quinones. In efforts to create biomimetic copper complexes that can oxidize C-H bonds, Stack and coworkers recently reported a synthetic mu-eta(2):eta(2)-peroxodicopper(II)(DBED)(2) complex (DBED is N,N'-di-tert-butylethylenediamine), which rapidly hydroxylates phenolates. A reactive intermediate consistent with a bis-mu-oxo-dicopper(III)-phenolate complex, with the O-O bond fully cleaved, is observed experimentally. Overall, the evidence for sequential O-O bond cleavage and C-O bond formation in this synthetic complex suggests an alternative mechanism to the concerted or late-stage O-O bond scission generally accepted for the phenol hydroxylation reaction performed by tyrosinase. In this work, the reaction mechanism of this peroxodicopper(II) complex was studied with hybrid density functional methods by replacing DBED in the mu-eta(2):eta(2)-peroxodicopper(II)(DBED)(2) complex by N,N'-dimethylethylenediamine ligands to reduce the computational costs. The reaction mechanism obtained is compared with the existing proposals for the catalytic ortho hydroxylation of monophenol and the subsequent oxidation of the diphenolic product to the resulting quinone with the aim of gaining some understanding about the copper-promoted oxidation processes mediated by 2:1 Cu(I)O(2)-derived species.

- Hachiya A, Sriwiriyanont P, Kobayashi T, Nagasawa A, Yoshida H, Ohuchi A, Kitahara T, Visscher MO, Takema Y, Tsuboi R, Boissy RE.
  Stem cell factor-KIT signalling plays a pivotal role in regulating pigmentation in mammalian hair. J Pathol. 2008 Dec 1. [Epub ahead of print]
  Hair greying is one of the most distinct but least comprehended features of senescence. The signalling of stem cell factor (SCF) and its receptor KIT has been documented to regulate essential roles in the maintenance of embryonic melanocyte lineages and postnatal cutaneous melanogenesis, although little is known about its detailed mechanisms in postnatal hair pigmentation. To address this, anagen human hair follicles and C57BL/6 murine pelage were analysed in this study. Molecular biological analyses of murine follicular skin indicated a significant increase of membrane-bound SCF expression, reaching its peak 8-16 days after anagen induction in concert with the escalation of cutaneous tyrosinase activity and corresponding pigmentation. Administration of KIT-neutralizing antibody abolished MITF and tyrosinase expressions, resulting in a reversible hair depigmentation in murine regenerated hair and human hair organ culture. Quantitative RT-PCR of human hair follicles indicated that KIT expression as well as the expression of several melanogenic factors, including MITF, was significantly lower in unpigmented than in pigmented follicles. Taken together, these data revealed a pivotal role of SCF-KIT signalling in the maintenance of human hair follicle melanogenesis during the anagen cycle and its involvement in physiological ageing of the hair follicle pigmentary unit.

- Hasegawa J, Goto Y, Murata H, Takata M, Saida T, Imokawa G.
  Downregulated melanogenic paracrine cytokine linkages in hypopigmented palmoplantar skin. Pigment Cell Melanoma Res. 21(6):687-99, 2008.
  The hypo-pigmentation of human skin on the palms and the soles compared with other areas of the body has recently been reported to be due to mesenchymal-epithelial interactions via a fibroblast-derived factor, dickkopf 1, an inhibitor of the canonical Wnt signaling pathway. Recently, it has been reported that keratinocytes play a significant role in skin color determination by producing cytokines involved in melanogenesis. Thus, we hypothesized that the downregulated expression of keratinocyte- or fibroblast-derived melanogenic cytokines may also be responsible for the decreased function of palmoplantar (PP) melanocytes in addition to the suppressive effects of dickkopf 1 on melanogenic function in epidermal melanocytes. Immunohistochemistry revealed that the number of tyrosinase, S100alpha, c-KIT, endothelin B receptor (ETBR), SOX10, and microphthalmia-associated transcription factor (MITF) immuno-positive melanocytes is significantly reduced in PP epidermis. In contrast, dopa-histochemistry demonstrated no substantial reduction in melanocyte populations in PP epidermis. Real-time RT-PCR revealed that the expression of stem cell factor (SCF) and endothelin (ET)-1 mRNAs in PP skin was significantly downregulated. In parallel, immunohistochemistry revealed that SCF and ET-1 immuno-staining was markedly attenuated in PP skin. Western blotting revealed that the expression of SCF, c-KIT, and MITF-M proteins was significantly decreased in PP skin. These findings suggest the possibility that downregulation of ET-1/SCF/receptor linkages is also associated with the decreased function of melanocytes in PP skin.

- Hiyoshi M, Konishi H, Uemura H, Matsuzaki H, Tsukamoto H, Sugimoto R, Takeda H, Dakeshita S, Kitayama A, Takami H, Sawachika F, Kido H, Arisawa K.
  D-Dopachrome tautomerase is a candidate for key proteins to protect the rat liver damaged by carbon tetrachloride. Toxicology. 255(1-2):6-14, 2009.
  Carbon tetrachloride (CCl4) is known to induce liver damage. Animal experiments with CCl4 injections have revealed many findings, especially mechanisms of liver damage and liver regeneration. Recently, proteomic approaches have been introduced in various studies to evaluate the quantitative and qualitative changes in the comprehensive proteome level. The aim of this research is to elucidate the key protein for liver damage, liver protection and liver regeneration by

using proteomic techniques. 50 % (v/v) CCl4 in corn oil was administered intraperitoneally to adult male rats at a dose of 4ml/kg body weight. Approximately 24h after the injection, the liver was removed and extracted proteins were analyzed with cleavable isotope coded affinity tag (cICAT) reagents, two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS). A twelvefold increase in D-dopachrome tautomerase (DDT) was indicated. This enzyme has been reported to be involved in the biosynthesis of melanin, an antioxidant. According to the histological analysis, melanin levels were increased in un-damaged hepatocytes of CCl4-treated rats. These results suggest that the increase in DDT is a response to liver damage, accelerates melanin biosynthesis and protects the liver from oxidative stress induced by CCl4.

- Kim A, Yang Y, Lee MS, Yoo YD, Lee HG, Lim JS.
NDRG2 gene expression in B16F10 melanoma cells restrains melanogenesis via inhibition of Mitf expression. Pigment Cell Melanoma Res. 21(6):653-64, 2008.
NDRG2 (N-myc downstream-regulated gene 2) is a candidate tumor suppressor implicated in control of glioblastoma proliferation and dendritic cell differentiation. The microphthalmia-associated transcription factor (Mitf) plays a crucial role in the melanocyte lineage and in melanoma by controlling survival, differentiation, cell cycle entry and exit, and melanoma metastasis. Identifying upstream regulators of Mitf expression, therefore, remains a key issue. In this study, we aimed to assess whether the candidate tumor suppressor NDRG2 can modulate Mitf expression. Here, we show that NDRG2 acts to prevent cAMP and beta-catenin-mediated activation of the Mitf promoter, thereby blocking melanogenesis via the downstream Mitf target genes Tyrosinase, Tyrp1 and Dct. The data suggest that NDRG2 impairs melanogenesis by interfering with both the TCF/beta-catenin and cAMP/CREB pathways that are known to stimulate Mitf expression in melanocytes and have major implications for the role of NDRG2 in pigmentation and melanoma progression. Taken together, the results not only identify NDRG2 as a novel regulator of pigmentation, but also potentially a key factor in regulating melanoma progression via Mitf.

- Kim S, Lee J, Jung E, Lee J, Huh S, Hwang H, Kim Y, Park D.
6-Benzylaminopurine stimulates melanogenesis via cAMP-independent activation of protein kinase A. Arch Dermatol Res.301(3):253-8, 2009.
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 designed to determine the effects of 6-benzylaminopurine (6-BAP) on melanogenesis and elucidate the molecular events of melanogenesis induced by 6-BAP. To elucidate the pigmenting effect of 6-BAP and its mechanism, several experiments were performed in B16 melanoma cells. Melanin content, tyrosinase activity, cAMP production, and Western blots for proteins which are involved in melanogenesis were introduced in this study. Melanin content and tyrosinase activity increased in response to treatment with 6-BAP in a concentration-dependent manner. The tyrosinase, TRP-1, TRP-2 and MITF protein levels were found to increase significantly in response to 6-BAP in a time-dependent manner. In addition, Western blot analysis revealed that 6-BAP increased the phosphorylated level of CRE-binding protein. The increased melanin synthesis that was induced by treatment with 6-BAP treatment was reduced significantly in response to co-treatment with H-89 [a protein kinase A (PKA) inhibitor], whereas co-treatment with SB203580 (a p38 MAPK inhibitor) and Ro-32-0432 (a PKC inhibitor) did not attenuate the increase in melanin content levels that was induced by 6-BAP. In a cAMP production assay, 6-BAP did not increase the intracellular cAMP level. These findings suggest that 6-BAP activates PKA via a cAMP-independent pathway and subsequently stimulates melanogenesis by up-regulating MITF and tyrosinase expression.

- Kupán A, Kaizer J, Speier G, Giorgi M, Réglier M, Pollreisz F.
Molecular structure and catechol oxidase activity of a new copper(I) complex with sterically crowded monodentate N-donor ligand. J Inorg Biochem. 103(3):389-95, 2009. Epub 2008 Dec 6.
The attempted alkylation of 1,3-bis(2'-pyridylimino)isoindoline (indH) by the use of n-BuLi and subsequent alkyl halides led to quaternization of the pyridine nitrogens and the zwitterionic monodentate N-ligand (Me(2)ind)I was formed. By the use of the ligand the copper(I) complex [Cu(I)(Me(2)ind)I(2)] was prepared and its structure determined. It was found to be good catalyst for the oxidation of 3,5-di-tert-butylcatechol (DTBCH(2)) to 3,5-di-tert-butyl-1,2-benzoquinone (DTBQ) and H(2)O(2) by dioxygen. Detailed kinetic studies revealed first-order dependence on the catalyst and dioxygen concentration and saturation type behavior with respect to the substrate.

- Land EJ, Ramsden CA, Riley PA, Stratford MR.
Evidence consistent with the requirement of cresolase activity for suicide inactivation of tyrosinase. Tohoku J Exp Med. 216(3):231-8, 2008.
Tyrosinase is a mono-oxygenase with a dinuclear copper catalytic center which is able to catalyze both the ortho-hydroxylation of monophenols (cresolase activity) and the oxidation of catechols (catecholase activity) yielding ortho-quinone products. Tyrosinases appear to have arisen early in evolution and are widespread in living organisms where they are involved in several processes, including antibiosis, adhesion of molluscs, the hardening of the exoskeleton of insects, and pigmentation. Tyrosinase is the principal enzyme of melanin formation in vertebrates and is of clinical interest because of the possible utilization of its activity for targeted treatment of malignant melanoma. Tyrosinase is characterised by an irreversible inactivation that occurs during the oxidation of catechols. In a recent publication we

proposed a mechanism to account for this feature based on the ortho-hydroxylation of catecholic substrates, during which process Cu(II) is reduced to Cu(0) which no longer binds to the enzyme and is eliminated (reductive elimination). Since this process is dependent on cresolase activity of tyrosinase, a strong prediction of the proposed inactivation mechanism is that it will not be exhibited by enzymes lacking cresolase activity. We show that the catechol oxidase readily extracted from bananas (Musa cavendishii) is devoid of cresolase activity and that the kinetics of catechol oxidation do not exhibit inactivation. We also show that a species with the molecular mass of the putative cresolase oxidation product is formed during tyrosinase oxidation of 4-methylcatechol. The results presented are entirely consistent with our proposed mechanism to account for suicide-inactivation of tyrosinase.

- Le Pape E, Passeron T, Giubellino A, Valencia JC, Wolber R, Hearing VJ.
Microarray analysis sheds light on the dedifferentiating role of agouti signal protein in murine melanocytes via the Mc1r. Proc Natl Acad Sci U S A.106(6):1802-7, 2009.
The melanocortin-1 receptor (MC1R) is a key regulator of pigmentation in mammals and is tightly linked to an increased risk of skin cancers, including melanoma, in humans. Physiologically activated by alpha-melanocyte stimulating hormone (alphaMSH), MC1R function can be antagonized by a secreted factor, agouti signal protein (ASP), which is responsible for the lighter phenotypes in mammals (including humans), and is also associated with increased risk of skin cancer. It is therefore of great interest to characterize the molecular effects elicited by those MC1R ligands. In this study, we determined the gene expression profiles of murine melan-a melanocytes treated with ASP or alphaMSH over a 4-day time course using genome-wide oligonucleotide microarrays. As expected, there were significant reductions in expression of numerous melanogenic proteins elicited by ASP, which correlates with its inhibition of pigmentation. ASP also unexpectedly modulated the expression of genes involved in various other cellular pathways, including glutathione synthesis and redox metabolism. Many genes up-regulated by ASP are involved in morphogenesis (especially in nervous system development), cell adhesion, and extracellular matrix-receptor interactions. Concomitantly, ASP enhanced the migratory potential and the invasiveness of melanocytic cells in vitro. These results demonstrate the role of ASP in the dedifferentiation of melanocytes, identify pigment-related genes targeted by ASP and by alphaMSH, and provide insights into the pleiotropic molecular effects of MC1R signaling that may function during development and may affect skin cancer risk.

- Maida I, Arciuli M, Guida G, Zanna PT, Cicero R.
Seasonal variations of Rana esculenta L. skin tyrosinase. Comp Biochem Physiol B Biochem Mol Biol.
Various enzymes are known to be involved in melanin biosynthesis, but the key role appertains to tyrosinase. In amphibians this enzyme displays peculiar characteristics: i) it requires an activation process; ii) its level of enzymatic activity in the animal skin changes depending on the season. In this work, by using chymotrypsin, subtilisin and SDS as putative activators, we studied the activation process of the skin pro-tyrosinase of Rana esculenta L. in different seasons over a period of two years. We found that chymotrypsin and subtilisin were able to yield an active enzyme, but not SDS. The maximum levels of tyrosinase activity were recorded in winter and the minimum in summer. We detected tyrosinase activity in the melanosomal fraction, where the enzyme form was least sensitive to proteolytic activation, probably corresponding to a "mature" tyrosinase. The enzyme forms found in the microsomal and soluble fractions were more sensitive to proteolytic activation, probably corresponding to "immature" tyrosinase. On SDS-PAGE, the tyrosinase activity assays showed a dopa-positive band at 200 kDa and a second aggregated band with a still higher molecular mass. The significance of these results in frog melanogenesis regulation is discussed.

- Muñoz-Muñoz JL, García-Molina F, García-Ruiz PA, Molina-Alarcón M, Tudela J, García-Cánovas F, Rodríguez-López JN.
Phenolic substrates and suicide inactivation of tyrosinase: kinetics and mechanism. Biochem J. 416(3):431-40, 2008.
The suicide inactivation mechanism of tyrosinase acting on its substrates has been studied. The kinetic analysis of the proposed mechanism during the transition phase provides explicit analytical expressions for the concentrations of o-quinone against time. The electronic, steric and hydrophobic effects of the substrates influence the enzymatic reaction, increasing the catalytic speed by three orders of magnitude and the inactivation by one order of magnitude. To explain the suicide inactivation, we propose a mechanism in which the enzymatic form E(ox) (oxy-tyrosinase) is responsible for such inactivation. A key step might be the transfer of the C-1 hydroxyl group proton to the peroxide, which would act as a general base. Another essential step might be the axial attack of the o-diphenol on the copper atom. The rate constant of this reaction would be directly related to the strength of the nucleophilic attack of the C-1 hydroxyl group, which depends on the chemical shift of the carbon C-1 (delta(1)) obtained by (13)C-NMR. Protonation of the peroxide would bring the copper atoms together and encourage the diaxial nucleophilic attack of the C-2 hydroxyl group, facilitating the co-planarity with the ring of the copper atoms and the concerted oxidation/reduction reaction, and giving rise to an o-quinone. The suicide inactivation would occur if the C-2 hydroxyl group transferred the proton to the protonated peroxide, which would again act as a general base. In this case, the co-planarity between the copper atom, the oxygen of the C-1 and the ring would only permit the oxidation/reduction reaction on one copper atom, giving rise to copper(0), hydrogen peroxide and an o-quinone, which would be released, thus inactivating the enzyme.

- Munoz-Munoz JL, Garcia-Molina F, García-Ruiz PA, Varon R, Tudela J, García-Cánovas F, Rodriguez-Lopez JN.

Stereospecific inactivation of tyrosinase by L- and D-ascorbic acid. Biochim Biophys Acta. 1794(2):244-53, 2009. Epub 2008 Oct 29.
A kinetic study of the inactivation of tyrosinase by L- and D-ascorbic acid isomers has been carried out. In aerobic conditions, a suicide inactivation mechanism operates, which was attributed to the enzymatic form oxytyrosinase. This suicide inactivation is stereospecific as regards the affinity of the enzyme for the substrate but not as regards the speed of the process, which is the same for both isomers, reflecting the influence of the chemical shift of the carbon C-2 (delta(2)) and C-3 (delta(3)) as seen by (13)C-NMR. The inactivation of deoxytyrosinase and mettyrosinase observed in anaerobic conditions, is irreversible and faster than the suicide inactivation process, underlining the fact that the presence of oxygen protects the enzyme against inactivation.

- Murase D, Hachiya A, Amano Y, Ohuchi A, Kitahara T, Takema Y.
The essential role of p53 in hyperpigmentation of the skin via regulation of paracrine melanogenic cytokine receptor signaling. J Biol Chem. 284(7):4343-53, 2009. Epub 2008 Dec 18.
Hyperpigmentation of the skin is characterized by increases in melanin synthesis and deposition. Although considered a significant psychosocial distress, little is known about the detailed mechanisms of hyperpigmentation. Recently, the tumor suppressor protein p53 has been demonstrated to promote ultraviolet B-induced skin pigmentation by stimulating the transcription of a melanogenic cytokine, POMC (pro-opiomelanocortin), in keratinocytes. Given that p53 can be activated by various kinds of diverse stresses, including sun exposure, inflammation, and aging, this finding led us to examine the involvement of p53 in cytokine receptor signaling, which might result in skin hyperpigmentation. Immunohistochemical and reverse transcription-PCR analyses revealed the increased expression and phosphorylation of p53 in the epidermis of hyperpigmented spots, accompanied by the higher expression of melanogenic cytokines, including stem cell factor, endothelin-1, and POMC. The involvement of p53 in hyperpigmentation was also indicated by the significantly higher expression of p53 transcriptional targets in the epidermis of hyperpigmented spots. Treatment of human keratinocytes and melanocytes with known p53 activators or inhibitors, including pifithrin-alpha (PFT), demonstrated significant increases or decreases, respectively, in the expression of melanogenic factors, including cytokines and their receptors. Additionally, PFT administration abolished stem cell factor-induced phosphorylation of mitogen-activated protein kinase in human melanocytes. Furthermore, when organ-cultured hyperpigmented spots, in vitro human skin substitutes, and mouse skin were treated with PFT or p53 small interfering RNA, the expression of melanogenic cytokines and their receptors was significantly decreased, as were levels of tyrosinase and melanogenesis. Taken together, these data reveal the essential role of p53 in hyperpigmentation of the skin via the regulation of paracrine-cytokine signaling, both in keratinocytes and in melanocytes.

- Oetting WS, Pietsch J, Brott MJ, Savage S, Fryer JP, Summers CG, King RA.
The R402Q tyrosinase variant does not cause autosomal recessive ocular albinism. Am J Med Genet A. 149A(3):466-9, 2009.
Mutations in the gene for tyrosinase, the key enzyme in melanin synthesis, are responsible for oculocutaneous albinism type 1, and more than 100 mutations of this gene have been identified. The c.1205G > A variant of the tyrosinase gene (rs1126809) predicts p.R402Q and expression studies show thermolabile enzyme activity for the variant protein. The Q402 allele has been associated with autosomal recessive ocular albinism when it is in trans with a tyrosinase gene mutation associated with oculocutaneous albinism type 1. We have identified 12 families with oculocutaneous albinism type 1 that exhibit segregation of the c.1205G > A variant with a known pathologic mutation on the homologous chromosome, and demonstrate no genetic association between autosomal recessive oculocutaneous albinism and the Q402 variant. We conclude that the codon 402 variant of the tyrosinase gene is not associated with albinism.

- Saeed M, Rogan E, Cavalieri E.
Mechanism of metabolic activation and DNA adduct formation by the human carcinogen diethylstilbestrol: the defining link to natural estrogens. Int J Cancer. 124(6):1276-84, 2009.
Diethylstilbestrol (DES) is a human carcinogen, based on sufficient epidemiological evidence. DES is mainly metabolized to its catechol, 3'-hydroxyDES (3'-OH-DES), which can further oxidize to DES-3',4'-quinone (DES-3',4'-Q). Similarly to estradiol-3,4-quinone, the reaction of DES-3',4'-Q with DNA would form the depurinating 3'-OH-DES-6'-N3Ade and 3'-OH-DES-6'-N7Gua adducts. To prove this hypothesis, synthesis of DES-3',4'-Q by oxidation of 3'-OH-DES with Ag(2)O was tried; this failed due to instantaneous formation of a spiro-quinone. Oxidation of 3'-OH-DES by lactoperoxidase or tyrosinase in the presence of DNA led to the formation of 3'-OH-DES-6'-N3Ade and 3'-OH-DES-6'-N7Gua adducts. These adducts were tentatively identified by LC-MS/MS as 3'-OH-DES-6'-N3Ade, m/z = 418 [M+H](+), and 3'-OH-DES-6'-N7Gua, m/z = 434 [M+H](+). Demonstration of their structures derived from their oxidation by MnO(2) to the DES quinone adducts and subsequent tautomerization to the dienestrol (DIES) catechol adducts, which are identical to the standard 3'-OH-DIES-6'-N3Ade, m/z = 416 [M+H](+), and 3'-OH-DIES-6'-N7Gua, m/z = 432 [M+H](+), adducts. The reaction of DIES-3',4'-Q or lactoperoxidase-activated 3'-OH-DIES with DNA did not produce any depurinating adducts, due to the dienic chain being perpendicular to the phenyl planes, which impedes the intercalation of DIES into the DNA. Enzymic oxidation of 3'-OH-DES suggests that the catechol of DES intercalates into DNA and is then oxidized to its quinone to yield N3Ade and N7Gua adducts. These results suggest that the common denominator of tumor initiation by the synthetic estrogen DES and the natural estrogen estradiol is formation of their catechol quinones, which react with DNA to afford the depurinating N3Ade and N7Gua adducts.

- Saha B, Singh SK, Mallick S, Bera R, Datta PK, Mandal M, Roy S, Bhadra R.
  Sphingolipid-mediated restoration of Mitf expression and repigmentation in vivo in a mouse model of hair graying. Pigment Cell Melanoma Res. 22(2):205-18, 2009.
  Recent advances in the identification and characterisation of stem cell populations has led to substantial interest in understanding the precise triggers that would operate to induce activation of quiescent stem cells. Melanocyte stem cells (MSCs) reside in the bulge region of the hair follicles and are characterised by reduced expression of the microphthalmia-associated transcription factor (Mitf) and its target genes implicated in differentiation. Vitiligo is characterised by progressive destruction of differentiated melanocytes. However, therapies using UV irradiation therapy can induce a degree of repigmentation, suggesting that MSCs may be activated. As Mitf is implicated in control of proliferation, we have explored the possibility that inducing Mitf expression via lipid-mediated activation of the p38 stress-signalling pathway may represent a re-pigmentation strategy. Here we have isolated from placental extract a C18:0 sphingolipid able to induce Mitf and tyrosinase expression via activation of the p38 stress-signalling pathway. Strikingly, in age-onset gray-haired C57BL/6J mice that exhibit decaying Mitf expression, topical application of placental sphingolipid leads to increased Mitf in follicular melanocytes and fresh dense black hair growth. The results raise the possibility that lipid-mediated activation of the p38 pathway may represent a novel approach to an effective vitiligo therapy.

- Sánchez-Campillo M, Gabaldon JA, Castillo J, Benavente-García O, Del Baño MJ, Alcaraz M, Vicente V, Alvarez N, Lozano JA.
  Rosmarinic acid, a photo-protective agent against UV and other ionizing radiations. Food Chem Toxicol. 47(2):386-92, 2009. Epub 2008 Nov 30.
  Solar UV and other ionizing radiations cause a generation of reactive oxygen species, induce cellular DNA damage and alter skin homeostasis. The use of exogenous antioxidants is increasingly frequents, we attempt to demonstrate that a rosmarinic acid extract acts as photo-protector; both free radical scavenger as an inducer of the body's own endogenous defence mechanisms by regulating tyrosinase activity and stimulating melanin production. Malonyldialdehyde formation (TBARS) was delayed when RA was used. The protection factor was 3.24 times vs AA. TEAC value for RA was 1.6 times vs AA. The radioprotective-antimutagenic effects of RA were measure using the micronucleus test. The level of micronucleous for treatments before irradiation was: RA [14]<AA [22]<DMSO [28]<Control [32], and after irradiation was: RA [23]<AA [25]<DMSO [31]<Control [32]. RA increased the Tyr activity and its expression level in B16 melanoma cells after stimulation lasting 48 h compared with the negative control. In vivo experiments show the capacity of RA orally administered to inhibit cutaneous alterations caused by UVA exposure (skin photocarcinogenesis). Therefore, according all these experiences, RA can be proposed as a proper photo-protective agent.

- Shimada Y, Tai H, Tanaka A, Ikezawa-Suzuki I, Takagi K, Yoshida Y, Yoshie H.
  Effects of ascorbic acid on gingival melanin pigmentation in vitro and in vivo. J Periodontol. 80(2):317-23, 2009.
  BACKGROUND: Gingival melanin pigmentation may cause esthetic concerns, even if no serious medical problem is present. As an inhibitor of melanin formation, ascorbic acid is often used to treat skin melanin pigmentation. Thus, the present study investigated the effects of ascorbic acid on gingival melanin pigmentation in vitro and in vivo. METHODS: The effects of ascorbic acid on melanin formation were evaluated in vitro in B16 mouse melanoma cells and three-dimensional human skin models. In addition, a clinical trial was performed to investigate the inhibitory effects of a gel containing ascorbic acid 2-glucoside (AS-G gel) on gingival melanin pigmentation. This study used a double-masked, split-mouth design on 73 subjects with symmetric gingival melanin pigmentation. AS-G gel was applied to one side of the gingiva for 12 weeks, whereas placebo gel was applied to the other side as a control. Luminance (L*)-value, which describes the lightness of gingiva, was determined by spectrophotometry to obtain an objective measure of melanin pigmentation every 4 weeks. RESULTS: Ascorbic acid significantly inhibited tyrosinase activity and melanin formation in B16 mouse melanoma cells ($P < 0.01$ and $P < 0.05$, respectively). The inhibitory effects of ascorbic acid on melanin formation were also significant in three-dimensional human skin models ($P < 0.01$). Moreover, in the clinical trial, a significant relative change in pigmentation was seen after 4 weeks with the application of AS-G gel compared to placebo (L*-value ratio). CONCLUSION: Ascorbic acid (AS-G) has potential for the treatment of gingival melanin pigmentation.

- Slominski A, Zbytek B, Slominski R.
  Inhibitors of melanogenesis increase toxicity of cyclophosphamide and lymphocytes against melanoma cells. Int J Cancer. 124(6):1470-7, 2009.
  High mortality rate for metastatic melanoma is related to its resistant to the current methods of therapy. Melanogenesis is a metabolic pathway characteristic for normal and malignant melanocytes that can affect the behavior of melanoma cells or its surrounding environment. Human melanoma cells in which production of melanin pigment is dependent on tyrosine levels in medium were used for experiments. Peripheral blood mononuclear cells were derived from the buffy cotas purchased from Lifeblood Biological Services. Cell pigmentation was evaluated macroscopically, and tyrosinase activity was measured spectrophotometrically. Cell proliferation and viability were measured using lactate dehydrogenase release MTT, [(3)H]-thymidine incorporation and DNA content analyses, and gene expression was

measured by real time RT-PCR. Pigmented melanoma cells were significantly less sensitive to cyclophosphamide and to killing action of IL-2-activated peripheral blood lymphocytes. The inhibition of melanogenesis by either blocking tyrosinase catalytic site or chelating copper ions sensitized melanoma cells towards cytotoxic action of cyclophosphamide, and amplified immunotoxic activities of IL-2 activated lymphocytes. Exogenous L-DOPA inhibited lymphocyte proliferation producing the cell cycle arrest in G1/0 and dramatically inhibited the production of IL-1beta, TNF-alpha, IL-6 and IL-10. Thus, the active melanogenesis could not only impair the cytotoxic action of cyclophosphamid but also has potent immunosuppressive properties. This resistance to a chemotherapeutic agent or immunotoxic activity of lymphocytes could be reverted by the action of tyrosinase inhibitors. Thus, the inhibition of melanogenesis might represent a valid therapeutic target for the management of advanced melanotic melanomas.

- Spencer JD, Schallreuter KU.
Regulation of pigmentation in human epidermal melanocytes by functional high-affinity beta-melanocyte-stimulating hormone/melanocortin-4 receptor signaling. Endocrinology. 150(3):1250-8, 2009. Epub 2008 Oct 30.
To date, the principal receptor considered to regulate human pigmentation is the melanocortin-1 receptor (MC1-R) via induction of the cAMP/protein kinase A pathway by the melanocortins alpha-MSH and ACTH. In this context, it is noteworthy that beta-MSH can also induce melanogenesis, although it has a low affinity for the MC1-R, whereas the preferred receptor for this melanocortin is the MC4-R. Because beta-MSH is present in the epidermal compartment, it was of interest to ascertain whether functioning MC4-Rs are present in human epidermal keratinocytes and melanocytes. Our results provide evidence that the MC4-R is expressed in situ and in vitro throughout the human epidermis at the mRNA and protein level using RT-PCR, Western blotting, and double immunofluorescence staining. Moreover, radioligand binding studies yielded high-affinity receptors for beta-MSH on epidermal melanocytes (3600 receptors per cell), undifferentiated keratinocytes (7200 receptors per cell), and differentiated keratinocytes (72,700 receptors per cell), indicating that MC4-R expression correlates with epidermal differentiation. Importantly, increased melanogenesis after stimulation of the beta-MSH/cAMP/microphthalmia-associated transcription factor/tyrosinase cascade proved the functionality of this signal in melanocytes, which was attenuated in the presence of the specific MC4-R antagonist HS014. In summary, our results imply an important role for the beta-MSH/MC4-R cascade in human melanocyte biology, although the function and purpose of this signal in keratinocytes needs further elucidation.

- Vad NM, Yount G, Moore D, Weidanz J, Moridani MY.
Biochemical mechanism of acetaminophen (APAP) induced toxicity in melanoma cell lines. J Pharm Sci. 98(4):1409-25, 2009.
In this work, we investigated the biochemical mechanism of acetaminophen (APAP) induced toxicity in SK-MEL-28 melanoma cells using tyrosinase enzyme as a molecular cancer therapeutic target. Our results showed that APAP was metabolizad 87% by tyrosinase at 2 h incubation. AA and NADH, quinone reducing agents, were significantly depleted during APAP oxidation by tyrosinase. The IC(50) (48 h) of APAP towards SK-MEL-28, MeWo, SK-MEL-5, B16-F0, and B16-F10 melanoma cells was100 microM whereas it showed no significant toxicity towards BJ, Saos-2, SW-620, and PC-3 nonmelanoma cells, demonstrating selective toxicity towards melanomacells. Dicoumarol, a diaphorase inhibitor, and 1-bromoheptane, a GSH depleting agent, enhanced APAP toxicity towards SK-MEL-28 cells. AA and GSH were effective in preventing APAP induced melanoma cell toxicity. Trifluoperazine and cyclosporin A, inhibitors of permeability transition pore in mitochondria, significantly prevented APAP melanoma cell toxicity. APAP caused time and dose-dependent decline in intracellular GSH content in SK-MEL-28, which preceded cell toxicity. APAP led to ROS formation in SK-MEL-28 cells which was exacerbated by dicoumarol and 1-bromoheptane whereas cyslosporin A and trifluoperazine prevented it. Our investigation suggests that APAP is a tyrosinase substrate, and that intracellular GSH depletion, ROS formation and induced mitochondrial toxicity contributed towards APAP's selective toxicity in SK-MEL-28 cells.

- Wood JM, Decker H, Hartmann H, Chavan B, Rokos H, Spencer JD, Hasse S, Thornton MJ, Shalbaf M, Paus R, Schallreuter KU.
Senile hair graying: H2O2-mediated oxidative stress affects human hair color by blunting methionine sulfoxide repair. FASEB J. 2009 Feb 23. [Epub ahead of print]
Senile graying of human hair has been the subject of intense research since ancient times. Reactive oxygen species have been implicated in hair follicle melanocyte apoptosis and DNA damage. Here we show for the first time by FT-Raman spectroscopy in vivo that human gray/white scalp hair shafts accumulate hydrogen peroxide (H2O2) in millimolar concentrations. Moreover, we demonstrate almost absent catalase and methionine sulfoxide reductase A and B protein expression via immunofluorescence and Western blot in association with a functional loss of methionine sulfoxide (Met-S=O) repair in the entire gray hair follicle. Accordingly, Met-S=O formation of Met residues, including Met 374 in the active site of tyrosinase, the key enzyme in melanogenesis, limits enzyme functionality, as evidenced by FT-Raman spectroscopy, computer simulation, and enzyme kinetics, which leads to gradual loss of hair color. Notably, under in vitro conditions, Met oxidation can be prevented by L-methionine. In summary, our data feed the long-voiced, but insufficiently proven, concept of H2O2-induced oxidative damage in the entire human hair follicle, inclusive of the hair shaft, as a key element in senile hair graying, which does not exclusively affect follicle melanocytes. This new insight could open new strategies for intervention and reversal of the hair graying process

- Xie T, Nguyen T, Hupe M, Wei ML.
  Multidrug resistance decreases with mutations of melanosomal regulatory genes. Cancer Res. 2009 Feb 1;69(3):992-9.
  Whereas resistance to chemotherapy has long impeded effective treatment of metastatic melanoma, the mechanistic basis of this resistance remains unknown. One possible mechanism of drug resistance is alteration of intracellular drug distribution either by drug efflux or sequestration into intracellular organelles. Melanomas, as well as primary melanocytes from which they arise, have intracellular organelles, called melanosomes, wherein the synthesis and storage of the pigment melanin takes place. In this study, comparisons of congenic cells with and without functional molecules regulating melanosome formation show that sensitivity to the chemotherapeutic agent cis-diaminedichloroplatinum II (cis-platin) significantly increases with the mutation of genes regulating melanosome formation, concomitant disruption of melanosome morphology, and loss of mature melanosomes. Absence of the melanosomal structural protein gp100/Pmel17 causes increased cis-platin sensitivity. Independent mutations in three separate genes that regulate melanosome biogenesis (Dtnbp1, Pldn, Vps33a) also result in increased cis-platin sensitivity. In addition, a mutation of the gene encoding the integral melanosomal protein tyrosinase, resulting in aberrant melanosome formation, also causes increased cis-platin sensitivity. Furthermore, sensitivity to agents in other chemotherapeutic classes (e.g., vinblastine and etoposide) also increased with the mutation of Pldn. In contrast, a mutation in another melanosomal regulatory gene, Hps1, minimally affects melanosome biogenesis, preserves the formation of mature melanosomes, and has no effect on cis-platin or vinblastine response. Together, these data provide the first direct evidence that melanosomal regulatory genes influence drug sensitivity and that the presence of mature melanosomes likely contributes to melanoma resistance to therapy.

- Zanna PT, Maida I, Arciuli M, Jimenez-Cervantes C, Garcia-Borron JC, Cicero R, Guida G.
  Molecular cloning and biochemical characterization of the skin tyrosinase from Rana esculenta L. Comp Biochem Physiol B Biochem Mol Biol. 152(3):234-42, 2009.
  Amphibian tyrosinases display unique and poorly understood properties such as seasonal activity variations, different activities in dorsal and ventral skin and the occurrence as inactive forms requiring proteolytic activation. For the first time we have sequenced and characterized Rana esculenta L. tyrosinase by functional expression of the cloned cDNA, and compared it with frog skin extracts. R. esculenta tyrosinase ORF is well conserved compared with tyrosinases of various sources. The amino acid similarities between the tyrosinases from R. esculenta and other amphibia range from 85% to 98%. Homology remains high with mammalian tyrosinases (65% identity with Homo sapiens, and 63% with Mus musculus) and with bird orthologues (66% identity with Gallus gallus). Tyrosinase was expressed in HEK293T cells as an active enzyme. Activity staining on non reducing SDS-PAGE revealed two bands around 63 and 68 kDa. R. esculenta skin extracts were mildly active and reached maximal activity upon protease treatment, revealing a high molecular weight dopa-positive band in the 200 kDa range and one of higher MW, after nagarse treatment, in activity stainings. The different behaviour of recombinant tyrosinase compared to skin extracts suggests formation in vivo of a multimeric complex.

8. Melanosomes

(Pr J. Borovansky)

Reviews: An extensive review by Aspengren et al examines in detail the roles of melanin, melanophores and melanocytes with a particular emphasis on the molecular motor proteins involved in the melanosome transport. In their brief review, Wasmeier et al concentrated on the melanosome biogenesis and transport and on the relationship between the melanosomes and the endosomes. On the plus side, the review contains a beautiful colour illustration, on the minus side is the absence of genuine primary citations (e.g. the knowledge of the stages of the melanosome biogenesis dates back to 1968 and not to 2007). Sturm published a review which examined the impact of the genes on the melanosome biogenesis as well as on the melanin biosynthetic pathway. The central role of melanosome formation in human pigmentation diversity was pointed out. Park et al have summarized melanin biological properties, melanogenic enzymes and regulatory proteins, the melanosome biogenesis and the transport and the regulation of melanocyte function. Fruehauf and Trapp discussed a potential exploitation of radicals leaking from the disorganized melanosomes in the melanoma treatment.

Melanosome biogenesis: With the use of the modern techniques of the high-pressure freezing and the electron tomography Hurbain et al describe how and where the amyloid-like fibrils are formed. Their formation begins in multivesicular compartments. In the stage II melanosomes, the fibrils get organized into sheet-like arrays which might exert pressure resulting in ellipsoid shape of the melanosomes. Sitaram et al studied the localization of the OCA2 protein: within the melanocytes, the exogenously expressed OCA2 is contained in the melanosomes and in an analogy to other melanosomal proteins it localizes to lysosomes when expressed in non-pigment cells (cf. Arch Derm Res 289: 145-150,1997). Steady state melanosomal localization requires a conserved consensus acidic dileucine-based sorting motif within the cytoplasmic N-terminal region of OCA2.
In addition to the findings mentioned in the article title, Gunathilake et al observed that dendrites from the type IV-V melanocytes were more acidic than those from the type I-II subjects , and they transferred more melanosomes to the stratum corneum. Xie et al have demonstrated that the mutations in three separate genes that regulate melanosome biogenesis (*Dtnbp1, Pldn, Vps33a)* as well as the absence of the melanosomal structural protein Pmel17, resulted in a significantly increased sensitivity to cis-platin; the presence of mature melanosomes seems to contribute to melanoma resistance to therapy.
Inulavosin, a melanogenesis inhibitor from Inula nervosa, reduced the melanin content affecting neither the activity nor the transcription of tyrosinase and Tyrp1 in the mouse B16 melanoma cells (Fujita et al). The EM revealed that inulavosin impaired the development of the stage III and IV melanosomes; the melanogenesis was inhibited by an accelerated tyrosinase degradation and by its mistargetting to the lysosomes.

Melanosome properties: Gidanian et al examined the effect of a combination of UV treatment and metal ion exposure on melanosomes within melanocytes, as well as their ability to act as pro-oxidants in ex situ experiments. UVB exposure caused morphologic changes of the cells and bleaching of melanosomes in normal melanocytes, both significantly enhanced in Cu(II) and Cd(II)-treated cells. The promoted bleaching by Cu(II) was due to its ability to redox cycle under oxidative conditions, generating reactive oxygen species; verified by the observed enhancement of hydroxyl radical generation when isolated melanosomes were treated with both Cu(II) ions and UVB. Vinther et al demonstrated that a fossil feather and a fossil bird skull with preserved feathers and an eye contained structures that are melanosomes, previously incorrectly reported as bacteria. The authors believe that melanosomes are the main source of carbon in the fossil feathers as the eumelanin inside the melanosome remains after the keratin enclosing melanosomes degraded. This confirms again the previously reported resistance of melanins (cf. Melanins, Hermann, Paris, pp.1-130, 1968).

Ultrastructure of melanosomes in medical studies: Brooks et al. characterized melanosomes in a keratoacanthoma. According to Shirazi et al. the detection of melanosomes is an important criterion in a differential diagnostics between clear cell sarcoma and epitheloid sarcoma. The number of melanosomes within the melanocytes in naevus depigmentosus was largely decreased under EM compared to the normal skin. In the keratinocytes aggregated melanosomes were often observed (Xu et al).

Other studies dealing with melanosomes: Ogiwara and Hata defined two steps in the melanoma cell differentiation induced by triterpene lupeol. A short term treatment induced an rearrangement of the actin cytoskeleton , while a longer exposure (48hrs) increased the expression of the tyrosinase and the MITF , as well as of the Rab27a and myosin-Va (but not Slac-2a); the total amount of melanosomes increased. Ortolani-Machado et al presented beautiful EM pictures of stage I-IV melanosomes in the dermal melanocytes which had the same characteristics as those in the epidermal melanocytes of Japanese Silky chicken embryos. Dermal melanophages were observed but the final destiny of melanosomes remained unclear.

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Inulavosin, a melanogenesis inhibitor, leads to mistargeting of tyrosinase to lysosomes and accelerates its degradation. J Invest Dermatol - advance online publication, 25 December 2008; doi:10.1038/jid.2008.376.

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Melanosomal damage in normal human melanocytes induced by UVB and metal uptake -a basis for the pro-oxidant state of melanoma. Photochem Photobiol. 84(3):556-64, 2008.

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Melanoma cell differentiation induced by lupeol separates into two stages: morphological and functional changes. J Nat Med (Tokyo). 2009 Feb 13. [Epub ahead of print]

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Melanogenesis in dermal melanocytes of Japanese Silky chicken embryos. Tissue Cell. 2009 Jan 9. [Epub ahead of print]

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Localization to mature melanosomes by virtue of cytoplasmic dileucine motifs is required for human OCA2 function. Mol Biol Cell 20(5): 1464-1477, 2009.

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Molecular genetics of human pigmentation diversity. Human Mol Genetics 18(1): R9-R17, 2009.

9. Melanoma experimental, cell culture

(Dr R. Morandini)

Culturing cells on flat plasticware results in artificial two-dimensional sheets of cells and is very useful to initiate cell culture or direct study of cell signaling. In contrast, normal or tumor cells in the human body experience a three-dimensional environment, completely surrounded by other cells, membranes, fibrous layers, and adhesion proteins. This microenvironment is increasingly recognized as a critical component in tumor progression and metastases. As such, the bi-directional signaling of extracellular mediators that promote tumor growth within the microenvironment is a focus of intense activity.

Abbott demonstrates the epigenetic influence of the hESC (human embryonic stem cell) microenvironment on the reprogramming of aggressive melanoma cells using an innovative 3-D model. This model are compact colonies of human embryonic stem cells (hESCs) or cancer cells that are seeded onto a 3-D matrix (composed of Matrigel or type I collagen) for 3 to 4 days, then removed, resulting in a conditioned 3-D matrix onto which other cell types such as cancer cells, or normal melanocytes are seeded and incubated for 3–4 days. Studies with the aggressive melanoma conditioned matrix microenvironment demonstrated the transdifferentiation of normal melanocytes into melanoma-like cells exhibiting a vasculogenic phenotype.

In the same fields of 3D culture, Tzukert has developed a 3D cell culture dynamic matrix detachment (DMD) model with a turbulent-free laminar flow in order to produce a dynamic fluid circulation to avoid cell aggregates in contrast with the static matrix detachment (SMD). The only aim of this system was to study difference in apotosis between the 2 systems. DMD shows an increased in apoptotic cells than with the SMD. The DMD model may be a useful matrix deprivation model to identify necrotic vs. apoptotic cell death pathways.

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B. Melanin and cell culture

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C. 3D cell culture and/or skin reconstitution

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E. Melanoma Experimental

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ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events
NEW MEMBERS

2009 6th EADV Spring Symposium
April 23 – 26, Bucharest, Romania

2009 Annual Meeting for the Society for Investigative Dermatology
May 6-9, Montreal, Quebec, Canada
Contact: Website: www.sidnet.org

2009 7th World Congress on Melanoma and 5th Congress of the European Association of Dermato-Oncology
May 12-16, Vienna, Austria
Contact: E-mail: pehamberger@worldmelanoma2009.com
Website: www.worldmelanoma2009.com

2009 12th World Congress on Cancers of the Skin
May 3-6, 2009, Tel Aviv
Contact:
E-mail: wccs2009@kenes.com
Website: kenes.com/skin-cancer

2009 3rd ASPCR Meeting
June 11-13, Seoul, Korea
Contact: Chang-Hun Huh, MD, PhD
Dept of Dermatology
Seoul National University Bundang Hospital 300
Gumi-dong, Bundang-gu, Seongnam
Gyeonggi, 463-707, Korea
Phone : 82-31-787-7319
Fax : 82-31-787-4058
E-mail: aspcr2009@gmail.com

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 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

NEW MEMBERS

The ESPCR is delighted to welcome the following colleagues to membership and hope they will play a full and active part in the Society

Geneviéve AUBIN-HOUZELSTEIN
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Developmental Biology
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