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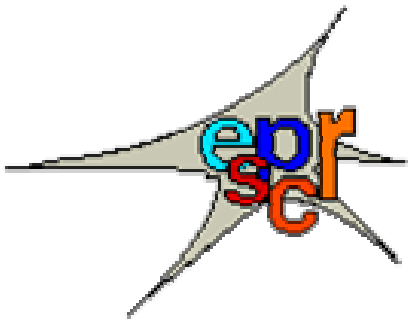

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

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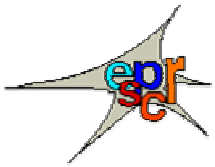

LETTER TO THE EDITOR
DISCUSSION, REVIEW,
SHORT COMMUNICATION, ...

Editor's Selection

MSH / MSH AGONISTS FOR SKIN PHOTOPROTECTION - REVISITED

Melanoma prevention strategy based on using tetrapeptide alpha-MSH analogs that protect human melanocytes from UV-induced DNA damage and cytotoxicity.
Abdel-Malek ZA, Kadekaro AL, Kavanagh RJ, Todorovic A, Koikov LN, McNulty JC, Jackson PJ, Millhauser GL, Schwemberger S, Babcock G, Haskell-Luevano C, Knittel JJ.
FASEB J. 20(9):1561-3, 2006.
Melanoma is the deadliest form of skin cancer, with no cure for advanced disease. We propose a strategy for melanoma prevention based on using analogs of alpha-melanocyte stimulating hormone (alpha-MSH) that function as melanocortin 1 receptor (MC1R) agonists. Treatment of human melanocytes with alpha-MSH results in stimulation of eumelanin synthesis, reduction of apoptosis that is attributable to reduced hydrogen peroxide generation and enhanced repair of DNA photoproducts. These effects should contribute to genomic stability of human melanocytes, thus preventing their malignant transformation to melanoma. Based on these findings, we synthesized and tested the effects of 3 tetrapeptide alpha-MSH analogs, Ac-His-D-Phe-Arg-Trp-NH2, n-Pentadecanoyl- and 4-Phenylbutyryl-His-D-Phe-Arg-Trp-NH2, on cultured human melanocytes. The latter two analogs were more potent than the former, or alpha-MSH, in stimulating the activity of tyrosinase, thus melanogenesis, reducing apoptosis and release of hydrogen peroxide and enhancing repair of DNA photoproducts in melanocytes exposed to UV radiation (UVR). The above analogs are MC1R agonists, as their effects were abrogated by an analog of agouti signaling protein, the physiological MC1R antagonist, and were absent in melanocytes expressing loss-of-function MC1R. Analogs, such as 4-Phenylbutyryl-His-D-Phe-Arg-Trp-NH2 with prolonged and reversible effects, can potentially be developed into topical agents to prevent skin photocarcinogenesis, particularly melanoma.

Effect of MELANOTAN, [Nle(4), D-Phe(7)]-alpha-MSH, on melanin synthesis in humans with MC1R variant alleles.
Fitzgerald LM, Fryer JL, Dwyer T, Humphrey SM.
Peptides. 2006 Feb;27(2):388-94
MELANOTAN (NDP-MSH) binds the MC1 receptor to significantly increase the eumelanin content of human skin cells. In this study of 77 Caucasian individuals, we investigated the effects of MELANOTAN in individuals with variant MC1R genotypes, as it has been suggested through in vitro studies that variant alleles decrease MELANOTAN binding efficacy, which would subsequently affect the synthesis of melanin. Administration of MELANOTAN produced a significant ($p<0.001$) increase in melanin density in treated, compared to placebo, individuals. Importantly, MELANOTAN increased the melanin density to a greater extent in individuals carrying the variant alleles Val60Leu, Asp84Glu, Val92Met, Arg142His, Arg151Cys, and Arg160Trp than in individuals with no variant alleles. This study demonstrates that MELANOTAN effectively increases the melanin content of skin in those individuals with MC1R variant alleles and therefore, those most in need of photoprotection.

[Nle(4)-D-Phe(7)]-alpha-Melanocyte-Stimulating Hormone Significantly Increased Pigmentation and Decreased UV Damage in Fair-Skinned Caucasian Volunteers.
Barnetson RS, Ooi TK, Zhuang L, Halliday GM, Reid CM, Walker PC, Humphrey SM, Kleinig MJ.
J Invest Dermatol. 2006 Aug;126(8):1869-78.

Epidermal melanin reduces some effects of UV radiation, the major cause of skin cancer. To examine whether induced melanin can provide protection from sunburn injury, 65 subjects completed a trial with the potent synthetic melanotropin, [Nle(4)-D-Phe(7)]-alpha-melanocyte-stimulating hormone ([Nle(4)-D-Phe(7)]-alpha-MSH) delivered by subcutaneous injection into the abdomen at 0.16 mg/kg for three 10-day cycles over 3 months. Melanin density, measured by reflectance spectroscopy, increased significantly in all [Nle(4)-D-Phe(7)]-alpha-MSH-treated subjects. The highest increases were in volunteers with lowest baseline skin melanin levels. In subjects with low minimal erythemal dose (MED) skin type, melanin increased by an average of 41% (from 2.55 to 3.59, $P<0.0001$ vs placebo) over eight separate skin sites compared with only 12% (from 4.18 to 4.70, $P<0.0001$ vs placebo) in subjects with a high-MED skin type. Epidermal sunburn cells resulting from exposure to 3 MED of UV radiation were reduced by more than 50% after [Nle(4)-D-Phe(7)]-alpha-MSH treatment in the volunteers with low baseline MED. Thymine dimer formation was also shown to be reduced by 59% ($P=0.002$) in the epidermal basal layer. This study has shown for the first time the potential ability of a synthetic hormone that augments melanin production to provide photoprotection to people who normally burn in direct sunlight.

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Dr. A. Napolitano)

The properties of natural and synthetic melanins are currently the focus of increasing interest. The scattering of "well solubilized" eumelanin solution was measured and the contribution to the optical density spectra was estimated as very small or negligible over the whole UV-Vis region (Riesz et al *Biophys. J*). Other studies deal with: the effects of thermal treatment on the structural and electronic properties of synthetic melanins (Goncalves, P.J. *J. Appl. Phys*.); the structural, optical and electronic properties of synthetic and natural melanins as investigated by X-ray diffraction and photocurrent techniques (Capozzi et al *Thin Solid Films*); the effect of the solvent used in melanin preparation on the assembling mechanisms of melanin films (Lorite et al . *J. Appl. Phys*.).
A comparable interest has been directed toward pheomelanins either natural or synthetic. Quantitative fluorescence spectra and quantum yield map of synthetic pheomelanins were determined and discussed in comparison with those previously obtained for eumelanins (Nighswander-Rempel, *Biopolymers*). In spite of similarities in the absorption spectra, model pheomelanins and eumelanins were found to possess very different excitation and emission characteristics. (Riesz et al. *J. Phys. Chem. B*). The very low radiative quantum yield found for both type of melanins would indicate that most of the energy absorbed is dissipated via non radiative pathways.
The ability of synthetic pheomelanins to exacerbate the effects of UVA in terms of generation of reactive oxygen species and alteration of the properties of antioxidant enzymes superoxide dismutase and catalase was shown in a study presented by Picardo's group. (Maresca et al *J. Invest. Dermatol.*)
A variety of melanogenesis inhibitors were described including notably the metabolites occurring in the extracts of *Ephedrae Herba* (Kim et al. *Phytother. Res.*) and *Spatholobus suberectus Dunn* (Lee et al, *Phytochemistry*), both of which are traditional herbal medicines used to treat colds and inflammatory processes including rheumatisms. The depigmenting activity of compounds extracted from other plants originarting from China was also described. (Jin et al. *Chem. Res. Chin. Univ.;* Wang, K-H *J. Ethnopharmacol.)* Of particular interest is the long lasting hypopigmentation observed in the hair shafts of zinc ions treated C57BL/6 mice which can be rationalized in the light of the inhibition of tyrosinase activity and stimulation of dopachrome tautomerase activity previously documented by in vivo studies (Plonka et al. *Br. J. Dermatol.*) .
Stimulation of melanogenesis was reported in the case of the citrus flavonoid naringenin (Ohguchi et al. *Biosci. Biotechnol. Biochem.*), as well as the metabolites in the aqueous ethanolic extracts of Piper species. (Matsuda et al. *Biol. Pharm. Bull.*)
Dopaquinone cyclization, a key step of the melanogenetic process was investigated using *N*-substituted dopamines as model compounds with a view to developing strategies of melanogenesis control of therapeutic value. The cyclization rates were determined by pulse radiolysis and oxymetric techniques. Cyclization of amides, ureas and carbamates of the type NHCO-X; X=R, NHR or OR) was not observed suggesting that these compounds may represent a viable approach to the formation of tyrosinase-activated antimelanoma prodrugs. (Borowansky et al. *Pigment Cell Res.*).
Methods previously developed for simultaneous analysis of eumelanin and pheomelanin were applied to human skin biopsies with the aim to assess whether a correlation exhist with skin reflectance measurements. (Kongshoj et al *Photodermatol. Photoimmunol. Photomed.*) A statistical treatment of the reliability of pyrrole-2,3-5-tricarboxylic acid as eumelanin marker and candidate for identification of high risk melanoma individuals was provided by the group of the Piedmont Cancer Registry using data from 100 melanoma cases vs 100 controls. (Zanetti et al. *Med. Sci. Monit.*)

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2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

Abdel-Malek and co-workers propose a strategy for melanoma prevention based on using teratepeptide analogs of alpha-MSH, that functions as MC1R agonists. This strategy is based on the previous evidences which demonstrated that the treatment of human melanocytes with alpha-MSH results in stimulation of eumelanin synthesis, reduction of apoptosis, that is attributable to reduced hydrogen peroxide generation and enhanced DNA repair. These beneficial effects should contribute to genomic stability of human melanocyte, thus preventing their malignant transformation to melanoma.
Bivik and co-workers studied UV-mediated apoptosis in primary coltures of human melanocytes. The obtained results emphasize translocation of Bcl-2 family proteins to have a central regulatory functions of UV-induced apoptosis in melanocytes and suggest cathepsins A and D to be proapoptotic mediators operating upstream of Bax. These effector proteins were released from lysosomes to the cytosol after irradiation and induced nuclear fragmentation.
Fecker and co-workers performed a retrospective study on patients affected by melanoma. Tumour sections were analysed by immunohistochemistry for the expression of regulators of the cell cycle, of the intrinsic or extrinsic proapoptotic pathways and of Bcl-2-related proteins, which regulate the common mitochondrial apoptotic pathway. The study show that regulators of apoptosis may candidate for independent prognostic markers for primary melanomas. The work underlines the particular role of the mitochondrial apoptosis pathway and proapoptotic Bcl-2-related proteins for melanoma progression.
Conditions that might result in epidermal oxidative stress and consequently damage to pigment cells have been reported in the skin of vitiligo patients, including low catalase activity and increases in hydrogen peroxide levels. However, the cause of the decrease in catalase activity as not been equivocally determined. **Gavalas and co-workers** assessed the relevance of catalase gene variants in patients with vitiligo. Even if the authors describe a C/T genotype which was significantly over-represented in the vitiligo patients compared with the control, they conclude that the low catalase activity in vitiligo patient epidermis is more likely to result from environmental conditions such as inhibitory levels of hydrogen peroxide rather than allelic variations in the catalase gene which affect either expression or function of the enzyme.
The review by **Grimes and co-workers** focuses on development in pigmentary disorders. Their review considers the basic biochemistry, pharmacology, and physiology of the melanocortin system, melanosome development, genetic diseases associated with pigmentary disorders, pigmentary disorders secondary to and primary disorders of hyperpigmentation. This review also reports on the new health-related quality-of-life instrument (MELAAQOL) tha has been developed for women with melasma.
Susceptibility genes influence melanoma risk, one of which is MC1R, the main regulator of the melanogenic pathway. Certain MC1R alleles are strongly associated with melanoma. **Hauser and co-workers** present experimental evidence for the role of two melanoma risk factors: constitutive pigmentation and MC1R genotype, respectively, in determining the induction and repair of DNA photoproducts in primary human cultures of melanocytes, after irradiation with increasing doses of UV.
TRAIL-R1 and TRAIL-R2 are targets of drugs in clinical development and receptor expression levels may be important determinants of sensitivity to receptor agonists**. McCarthy and co-workers,** assessed the expression pattern of both receptor in a large cohort of melanomas and benign nevi by tissue macroarrays. The expression levels for both receptors was higher in malignant melanocytes with respect to their benign counterpart, suggesting that these receptors might be effective therapeutic targets in melanoma. More in detail, expression was higher in early-stage disease than in metastatic specimens, and expression exceedingthat found in nevi was found in a substantially larger fraction of melanomas for TRAIL-R2 compared with TRAIL-R1. Assesment of baseline tumor TRAIL receptor expression may be important in analysis of clinical trials involving TRAIL receptor agonists.
Melanoma is responsible for 80 percent of death from skin cancer and only 14 percent of patients with metastatic melanoma survive for five years. The intractability of advanced tumour shows how much we have to learn about the progression and about the mechanisms that block the effectiveness of chemotherapy. The Clark model of the progression of melanoma emphasizes the stepwise transformation of meelanocytes to melanoma. Several molecular events,many of them revealed by genomic and proteomic methods, have been associated with the development of melanoma. **Miller and Mihim**, focus on the possible connections between molecular pathways and risk factors for melanoma, the different steps of neoplastic transformation, and the patterns of molecular changes in melanoma.
On binding on the cell surface receptor tyrosine kinase known as c-Met, hepatocyte growth factor (HGF) stimulates pleiotropic effects in many cell types, including melanocytes, which are essential during development, homeostasis, and tissue regeneration. HGF signalling also contributes to oncogenesis and tumour progression in several human cancers and promotes aggressive cellular invasiveness that is strongly linked to tumour metastasis. The hypothesis from **Peruzzi and Bottaro** support at least three avenues of pathway selective anticancer drug development: antagonism of ligand/receptor interaction, inhibition of tyrosine kinasase catalytic activity, and blockade of intracellular receptor/effector interactions. Potent and selective preclinical drug candidates have been developed using all three strategies, and human clinical trials in two of the three areas are now under way. **Schallreuter and co-workers** show for the first time the expression and the function of methionine sulfoxide reductase A and B, together with thioredoxin reductase, in the cytosol as well as in the nucleus of epidermal melanocytes, which are especially sensitive to ROS. Since these cells reside in the basal layer of the epidermis and their number and functions are reduced upon ageing, and for instance also in depigmentation processes, the authors believe that this discovery adds an intricate repair mechanism to melanocyte homeostasis and survival. The paper by **Thomas** considers BRAF mutants, which act as transforming oncogenes in NIH-3T3 cells and immortalized murine

melanocytes. The origin of these acquired mutations remains unknown, but melanomas have a different BRAF mutational spectrum from other tumours, possibly resulting from unique environmental exposure. They act stimulating constitutive RAF/MEK/ERK pathway activation. The author concludes affirming that preclinical and early studies predict that RAF/MEK/ERK pathway inhibitors will have therapeutic activity towards melanoma, but that tumour sub-classification by BRAF/NRAS mutational status may be necessary to evaluate their efficacy.
To date, a role for agouti signalling protein (ASIP) in human pigmentation has not been well characterized. It is known that agouti plays a pivotal role in the pigment switch from the dark eumelanin to the light pheomelanin in the mouse. However, because humans do not have an agouti banded hair pattern, its role in human pigmentation has been questioned. **Voisey and co-workers** identified a polymorphism in the agouti signalling protein, in the humans, which was associated with decreased levels of mRNA and with a dark phenotype. The authors suggest that this polymorphism in the 3'-UTR region result in decreased levels of ASIP and therefore less pheomelanin production.
Starting from cutaneous biopsies, **Wang and co-workers** obtained and cell lines from a patient with metastatic cutaneous melanoma who showed several melanoma recurrences over a decade of observation. A rare mutation of the beta-catenin gene and an unbalance metylation of the androgen receptor were documented in all cell lines. Karyotype analyses and comparative genomic hybridization identified consistent genetic traits in spite of divergent phenotypes, suggesting that all the metastases were derived from the same primary tumor, although they were each probably not derived from the most recent previous metastasis in a sequential manner. Thus, metastatic melanoma recurs from a common progenitor cell and phenotypic changes occur around a central core of genetic stability. This observation may bear significancefor the development of target anticancer therapies.
Melanin exert a key role in the protection of the skin against the deleterious effects of UV. A higher incidence skin cancers was seen in individual with a fair complexion. Starting from a previous observations in which the some authors reported an inverse correlation between melanin levels and amount of UV- induced DNA damage in the human skin, **Yamaguchi and co-workers** analysed separately DNA damage in the upper and lower epidermal layers in various type of skin before and after UV-exposure and subsequent apoptosis and phosporylation of p53. Obtained results show that two major mechanisms underlie the increased photocarcinogenesis in fair skin. First, UV-induced DNA damage in the lower epidermis is more effectively prevented in darker skin, suggesting that the pigmented epidermis is an efficient UV filter. Second, UV-induced apoptosis is significantly greater in darker skin, which suggests that UV-damaged cells may be removed more efficiently in pigmented epidermis. The combination of decreased DNA damage and more efficient removal of UV-damaged cells may play a critical role in the decreased photocarcinogenesis seen in individuals with darker skin.
Using human embryonic stem cell culture conditions, **Yu and co-workers** isolated a population of adult stem cells from human hair follicles that are distinctively different from known epithelial or melanocytic stem cells. These cells do not express squamous or melaocytic markers but express neural crest and neuron stem cell markers as well as the embryonic stem cell transcription factors Nanog and Oct4. These precursor cells proliferate as spheres, are capable of self-renewal and can differentiate into multiple lineages. The results obtained suggest that human embryonic stem cell medium can be used to isolate and expand human adult stem cells obtained from human hair follicles and that human hair follicles may provide an accessible autologous source of adult stem cells for therapeutic application.

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Isolation of a novel population of multipotent adult stem cells from human hair follicles. Am J Pathol 168: 1879-1888, 2006.

3. MSH, MCH, other hormones, differentiation

(Dr. R. Morandini)

In a recent paper (FASEB, 2006) Abdel-Malek propose a strategy for melanoma prevention based on using analogs of alpha-melanocyte stimulating hormone that function as melanocortin 1 receptor agonists. Treatment of human melanocytes with alpha-MSH results in stimulation of eumelanin synthesis, reduction of apoptosis that is attributable to reduced hyhdrogen peroxide generation and enhanced repair of DNA photoproducts. These effects should contribute to genomic stability of human melanocytes, thus preventing their malignant transformation to melanoma. To this aim Abdel-Malek have synthetized a tetrapeptide alpha-MSH analogs that is more potent than alpha-MSH and can be used as topical agents to prevent skin photocarcinogenesis.

Vitiligo is a common depigmenting skin disorder resulting from the loss of melanocytes in the cutaneous epidermis. Although the cause of the disease remains obscure, autoimmune mechanisms are thought to be involved. Recently, melanin-concentrating hormone receptor (MCHR)-binding autoantibodies have been identified in vitiligo patients. A recent study made by Gottumukkala (Lab Invest. 2006) aimed to determine if MCHR autoantibodies could also affect receptor function either by direct activation or by blocking its response to melanin-concentrating hormone. However, this could only be demonstrated in two vitiligo patient sera. Overall, this work has provided additional evidence that MCHR is a B-cell autoantigen in vitiligo and has demonstrated the existence of MCHR function-blocking autoantibodies further to the receptor-binding autoantibodies previously reported. Synthesis of small-molecule antagonists of the melanin-concentrating hormone receptor-1 by Rowbottom (Bioorg Med Chem Lett, 2006) is very important and can be applied in this study.

Regulation and signal transduction

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4. Photobiology

(Dr. N. Smit)

The paper by Rhodes points out that the public messages emphasizing the role of UVR in tumour development has resulted in a general believe that melanoma is caused by UVR exposure. The author indicates that we do not know the exact contribution of UVR on melanoma and prevention strategies should not only be directed towards protection against UVR exposure. Skin awareness and e.g. total skin screening, especially for patients with high melanoma risk (historically and phenotypically) could have a more immediate positive impact on melanoma mortality. Familial melanoma, as reviewed by Pho et al, develops early and patients are prone to develop multiple primary tumours. Also for the familial melanomas the exact contribution of UVR is unclear. The familial melanoma could serve as an excellent model to improve the understanding of genotype-phenotype and environmental relationships in the pathogenesis of melanoma. Niendorf and Tsao also mention genetic determinants as modulators of melanoma risk that are probably more important than environmental (UVR) exposure. Another paper by Berwick and Wiggins also discusses the modest role of UVR with only a 1.7 fold risk for developing melanoma, thus focus is now on genotypic and phenotypic factors.
The paper by Wang et al is another example of a recent study on mutagen sensitivity that once more demonstrates the role of UV in NMSC but not in melanoma.
The idea of Rhodes about the general believe that melanoma is caused by UVR exposure is confirmed in the paper by Roussaki-Schulze et al. After they described that no significant relationship between melanoma and HPV infection could be demonstrated, UVR exposure is still considered as the main cause of melanoma in the region. Nevertheless five out of 28 patients were positive for HPV DNA (and 0 out of 6 controls). Although this was not significant it does not rule out a role for HPV in the positive cases. In the paper by Akgul et al the literature is reviewed on the role of HPV infection and UV in skin carcinogenis (mostly NMSC). In case of the non-melanoma skin cancers, especially the immunosupressed renal transplant patient have a markedly increased (~ 200 –fold) incidence of SCC, especially on sunexposed body sites. Unfortunately, the immunosuppressive role of UVR exposure is not discussed in this review. Several papers may give some more insight in the immunosuppressive mechanisms caused by UVR exposure.
In the paper by Hernandez-Pigeon et al it is shown that next to melanocytes also T-lymphocytes are targets for granzyme B and perforin dependent apoptosis. Expression of GrB and PFN is induced in keratinocytes by UV-B and influenced by redox signalling. Pedeux et al have studied the sensitivity of peripheral lymphocytes for UV-B as risk factor for melanoma. They show that the lymphocytes in melanoma patients are more sensitive to UV-B induced apoptosis. McGee et al aimed to shed some light on the link between UV-B irradiation in childhood and melanoma at later age. In mice they found that neonatal Langerhans cells (LC) were more susceptible to depletion by UV-B than adult LC. At maturity however an enhanced immune response was observed in the mice system. Fourtanier et al show the protective effects of different sunscreens on various skin cell types and skin equivalent model systems. In humans they found a depletion of LC after solar simulated radiation (SSR) and protection by sunscreens which was much better for the sunscreen with high UV-A protection factor (PF). Also for the delayed type hypersensitivity (DTH) response improved protection was obtained for the sunscreen with better UVA-PF. Results indicate an important role for UVA in immunosupression. Earlier melanocyte studies showed a higher sensitivity to DNA breaks than unpigmented cells (shown by the comet assay). Also for the melanocytes it was shown that better protection against the photooxidative damage was provided by the sunscreen with higher UVA-PF. Papers by Maresca et al and Haywood et al may give some explanation on the roles of melanin in the UVA induced photooxidation reactions. Maresca et al nicely demonstrated the effects of cysteinyl-DOPA melanin on UVA induced changes in the catalase electrophoretic properties indicating the free radical mediated protein oxidation in myeloid U937 cells. In a commentary in the same issue of the J. Invest. Dermatol. Wood and Schallreuter support the data on photooxidation of catalase and expand on them by clarifying the structural modelling of the enzyme and showing oxidation of the methionione and tryptophan residues.
Haywood et al used different sources of melanin to show the production of hydroxyl and hydroperoxyl radicals using DMPO as a spin trap. In the case of natural eumelanins one might question the purity of the eumelanin polymer and other compounds could interfere. In case of synthetic eumelanin however also superoxide was produced as a photooxidation product. This suggests that not only pheomelanin may act as a photosensitizer.

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5. Neuromelanins

(Prof. M. d'Ischia)

Literature on neuromelanin in the first months of 2006 comprises two reviews and two research papers. One review by Keller (2006) addresses the molecular and cellular processes that occur during brain aging and during age-related disorders of the central nervous system, dealing only peripherally with neuromelanin. Another review, provided by Tribl et al. (2006) is focused on the analysis of neuromelanin granules and their function as Fe accumulator in the human brain.
Kim et al. (2006) investigated age dependent changes in the dopaminergic neurones in mice comparing 7- and 50-wk-old animals. They demonstrated dramatic increases in tyrosine hydroxylase (TH) immunodensity as early as middle age along with an increase in the number of neuromelanin-containing neurones, volume of neuromelanin per cell, and number of degenerating neurones.
Fedorow et al. (2006) studied the age-related development and regulation of neuromelanin within dopamine neurons by examining the ventral substantia nigra neurons from 29 people spanning the ages of 24 wk to 95 years old. Three developmental phases were demonstrated, associated with the appearance of the pigment (3 years of age), an increase in the number of pigment granules and pigment granule coloration until 20 years of age, followed by a period of darkening without apparent growth in pigment volume. A regulatory mechanism of neuromelanin production and turnover, possibly through enzymic processes, was suggested, but verification of this suggestion awaits a more in-depth understanding of certain critical aspects of neuromelanin biogenesis, roles and functions.

- Fedorow H., Halliday G. M., Rickert C. H., Gerlach M., Riederer P., Double K. L.
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  Abstract: Neuromelanin is a dark-colored pigment which forms in the dopamine neurons of the human midbrain. The age-related development and regulation of neuromelanin within these dopamine neurons has not been previously described. Optical d. and area measurements of unstained neuromelanin in ventral substantia nigra neurons from 29 people spanning the ages of 24 wk to 95 years old, demonstrated three developmental phases. Neuromelanin was not present at birth and initiation of pigmentation began at approx. 3 years of age, followed by a period of increasing pigment granule no. and increasing pigment granule coloration until age 20. In middle and later life the color of the pigment granules continued to darken but was not assocd. with any substantial growth in pigment vol. The identification of three phases and changes in the rate of neuromelanin prodn. over time suggests the regulation of neuromelanin prodn. and turnover, possibly through enzymic processes.

- Keller Jeffrey N.
  Age-related neuropathology, cognitive decline, and Alzheimer's disease. Ageing Research Reviews, 5(1), 1-13, 2006.
  Abstract: In the last 20 years, there have been tremendous strides made in the understanding of the mol. and cellular processes that occur during brain aging, as well as our understanding of age-related disorders of the central nervous system (CNS). Aging is assocd. with a decline in cognitive performance, and is the biggest risk factor for the development of Alzheimer's disease (AD), although the underlying basis for both of these observations is poorly defined. Both normal aging and AD are assocd. with overlapping and increased levels of pathol. Numerous reports have now linked elevations in pathol. as potential mediators of cognitive decline in the elderly, with most studies focusing on the role of AD-related pathol. However, it is important to point out that there are numerous other pathol. features obsd. in the aging brain including corpora amylacea, argyrophilic grains, neuromelanin, and lipofuscin. In this review, I discuss the decreased cognitive performance obsd. during normal aging, the potential for pathol. to alter neuronal function and neuronal viability during normal brain aging, and the potential for common pathologies to either inhibit or promote the development of age-related disorders such as AD.

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  Increases in TH immunoreactivity, neuromelanin and degeneration in the substantia nigra of middle aged mice. Neuroscience Letters 396(3), 263-268, 2006.
  Abstract: The dopaminergic (DArgic) neurons in the substantia nigra (SN) are particularly vulnerable to oxidative stress and during aging. The present study was undertaken in order to det. whether aging is assocd. with changes in the DA synthesizing enzyme tyrosine hydroxylase (TH) as early as middle age by comparing 7- and 50-wk-old mice. Quant.anal performed by measuring the d. of TH-immunopos. neurons, revealed that in the older animals, the no. of DArgic neurons was decreased by 10% while TH immunodensity was 24 3% higher compared to the younger animals. Based on Masson-Fontana staining for neuromelanin (NM), the no.of NM-contg. neurons in the SN and the vol. of NM per NM-pos. neurons in the older animals were 5- and 11.6 0.1-fold higher, resp. The silver stain-pos. fibers, indicative of degeneration, were higher in the SN and striatum of the older animals, with the optical d. 3.3 0.1- and 5.4 0.2-fold of the younger animals. The present study demonstrates that aging is assocd. with changes in the DA synthesizing enzyme TH as early as middle age and that this is assocd. with dramatic increases in the no. of NM-contg. neurons, vol. of NM per cell, and degeneration.

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Neuromelanin granula. Bioforum 28(11), 28-30, 2005.

Abstract: A review is given on the anal. of neuromelanin granules by sub-cellular proteome anal. The biol. of neuromelanin granules and their function as Fe accumulator in the human brain are described. Pathways of biogenesis involving endosomal compartments are discussed.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

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Of mice and men: tyrosinase modification of congenital glaucoma in mice but not in humans. Invest Ophthalmol Vis Sci 47(4):1486-1490, 2006.

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Retrotransposon insertion in SILV is responsible for merle patterning of the domestic dog. Proc Natl Acad Sci U S A 103(5):1376-1381, 2006.
Summary: Merle is a pattern of coloring observed in the coat of the domestic dog and is characterized by patches of diluted pigment. This trait is inherited in an autosomal, incompletely dominant fashion. Dogs heterozygous or homozygous for the merle locus exhibit a wide range of auditory and ophthalmologic abnormalities, which are similar to those observed for the human auditory-pigmentation disorder Waardenburg syndrome.
The results obtained in this report show that SILV is responsible for merle patterning and is associated with impaired function of the auditory and ophthalmologic systems. Although the mutant phenotype of SILV in the human is unknown, these results make it an intriguing candidate gene for human auditory-pigmentation disorders.

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Oncogenic MITF dysregulation in clear cell sarcoma: defining the MiT family of human cancers. Cancer Cell 9(6):473-484, 2006.

Abstract: Clear cell sarcoma (CCS) harbors a pathognomonic chromosomal translocation fusing the Ewing's sarcoma gene (EWS) to the CREB family transcription factor ATF1 and exhibits melanocytic features. We show that EWS-ATF1 occupies the MITF promoter, mimicking melanocyte-stimulating hormone (MSH) signaling to induce expression of MITF, the melanocytic master transcription factor and an amplified oncogene in melanoma. Knockdown/rescue studies revealed that MITF mediates the requirement of EWS-ATF1 for CCS survival in vitro and in vivo as well as for melanocytic differentiation. Moreover, MITF and TFE3 reciprocally rescue one another in lines derived from CCS or pediatric renal carcinoma. Seemingly unrelated tumors thus employ distinct strategies to oncogenically dysregulate the MiT family, collectively broadening the definition of MiT-associated human cancers.

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Conclusion: In summary, we have shown for the first time the differentiation of hESCs into melanocytes. This method provides a novel in vitro system for studying the development biology of human melanocytes.

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Abstract: We identified a new spontaneous recessive mutation in the mouse, mhyp (mosaic hypopigmentation), in a screen for novel proviral integration sites in a multiple ecotropic provirus mapping stock. Integration of an 8.4-kb retrovirus results in mosaic loss of coat pigment in mhyp homozygotes. Patchy loss of pigmentation in the retinal pigmented epithelial layer of the eye with abnormal melanosomes is also evident. We mapped mhyp to mouse chromosome 7 and cloned the underlying gene. mhyp is a defect in the Trappc6a gene. Expression of Trappc6a is markedly diminished in mhyp homozygotes. The normal protein, TRAPPC6A, is a subunit of the TRAPP (transport protein particle) I and II complexes. While TRAPP complexes are essential for ER-to-Golgi and intra-Golgi vesicle trafficking in yeast, TRAPP subunits participate in additional, including post-Golgi, transport events in mammals. The data implicate mammalian TRAPPC6A in vesicle trafficking during melanosome biogenesis.

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Comment: This study localizes the merle locus to the same chromosomal region of the dog genome as in the study by Clark et al. (2006), see above.

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A single amino acid mutation contributes to adaptive beach mouse color pattern. Science 313(5783):101-104, 2006.
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Interspecies difference in the regulation of melanocyte development by SOX10 and MITF. Proc Natl Acad Sci U S A 103(24):9081-9085, 2006.
Shortened abstract: Mutations in either Sox10 or Mitf impair pigment cell development. In Sox10-mutant zebrafish, experimentally induced expression of Mitf fully rescues pigmentation. Using lineage-directed gene transfer, the authors show , that, in the mouse, Mitf can rescue Sox10-mutant precursor cells only partially. In fact, retrovirally mediated, Sox10-independent Mitf expression in mouse melanoblasts leads to cell survival and expression of a number of pigment biosynthetic genes but does not lead to expression of tyrosinase, the rate-limiting pigment gene which critically depends on both Sox10 and Mitf. Hence, compared with fish, mice have evolved a regulation of tyrosinase expression that includes feed-forward loops between Sox10 and tyrosinase regulatory regions. The results may help to explain how some embryos, such as zebrafish, can achieve rapid pigmentation after fertilization, whereas others, such as mice, become pigmented only several days after birth.

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Albinism in the domestic cat (Felis catus) is associated with a tyrosinase (TYR) mutation. Anim Genet 37(2):175-178, 2006.

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Ascidian neural crest-like cells: phylogenetic distribution, relationship to larval complexity, and pigment cell fate. J Exp Zoolog B Mol Dev Evol 17(17, 2006.

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Replacement of the Sox10 transcription factor by Sox8 reveals incomplete functional equivalence. Development 21(21, 2006.
Summary: The authors have replaced Sox10 by Sox8 using a knock-in approach in the mouse. Whereas Sox8 can fully rescue development of glial cells and neurons in the sensory and sympathetic parts of the peripheral nervous system, melanocyte development was as defective as in Sox10 knockout mice. The authors conclude that "the extent of functional equivalence depends on the tissue and that, despite their relatedness, Sox8 and Sox10 have more unique functions than previously appreciated."

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Comparative Oncogenomics Identifies NEDD9 as a Melanoma Metastasis Gene. Cell 125(7):1269-1281, 2006.
Commentary: The authors analysed chromosomal amplifications occuring in an inducible model of mouse melanoma, and compared these findings to human melanomas. This allowed to identify NEDD9, which is an adaptor protein related to p130CAS, as gene implicated in melanoma metastasis. Moreover, as stated in the last sentence of the Discussion, "This has illustrated the power of the GEM models ...." (GEM = genetically engineered mouse).

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Varied expression of functionally important genes of RPE and choroid in the macula and in the periphery of normal human eyes. Graefes Arch Clin Exp Ophthalmol 6(6), 2006.

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Vertebrate DNA Transposon as a Natural Mutator: The Medaka Fish Tol2 Element Contributes to Genetic Variation without Recognizable Traces. Mol Biol Evol 23(7):1414-1419, 2006.

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Regulation of pigmentation in zebrafish melanophores. Pigment Cell Res 19(3):206-213, 2006.

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Identification of genes expressed in retinal progenitor/stem cell colonies isolated from the ocular ciliary body of adult mice. Gene Expr Patterns 7(7), 2006.

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Notch signaling via Hes1 transcription factor maintains survival of melanoblasts and melanocyte stem cells. J Cell Biol 173(3):333-339, 2006.
Abstract: Melanoblasts (Mbs) are thought to be strictly regulated by cell-cell interactions with epidermal keratinocytes, although the precise molecular mechanism of the regulation has been elusive. Notch signaling, whose activation is mediated by cell-cell interactions, is implicated in a broad range of developmental processes. We demonstrate the vital role of Notch signaling in the maintenance of Mbs, as well as melanocyte stem cells (MSCs). Conditional ablation of Notch signaling in the melanocyte lineage leads to a severe defect in hair pigmentation, followed by intensive hair graying. The defect is caused by a dramatic elimination of Mbs and MSCs. Furthermore, targeted overexpression of Hes1 is sufficient to protect Mbs from the elimination by apoptosis. Thus, these data provide evidence that Notch signaling, acting through Hes1, plays a crucial role in the survival of immature Mbs by preventing initiation of apoptosis.

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A golden clue to human skin colour variation. Bioessays 28(6):578-582, 2006.

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Development and evolution of the migratory neural crest: a gene regulatory perspective. Curr Opin Genet Dev 19(19), 2006.

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Mouse coat color mutations: From fancy mice to functional genomics. Dev Dyn 11(11), 2006.

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Pocket protein function in melanocyte homeostasis and neoplasia. Pigment Cell Res 19(4):260-283, 2006.

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Color reversion of the albino medaka fish associated with spontaneous somatic excision of the Tol-1 transposable element from the tyrosinase gene. Pigment Cell Res 19(3):243-247, 2006.

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7. Tyrosinase, TRPs, other enzymes

(Prof. J.C. Garcia-Borron)

- Anderson MG, Libby RT, Mao M, Cosma IM, Wilson LA, Smith RS, John SW.
Genetic context determines susceptibility to intraocular pressure elevation in a mouse pigmentary glaucoma. BMC Biol. 4(1):20, 2006.
BACKGROUND: DBA/2J (D2) mice develop an age-related form of glaucoma. D2 eyes progressively develop iris pigment dispersion and iris atrophy followed by increased intraocular pressure (IOP) and glaucomatous optic nerve damage. Mutant alleles of the Gpnmb and Tyrp1 genes are necessary for the iris disease, but it is unknown whether alleles of other D2 gene(s) are necessary for the distinct later stages of disease. Here, we initiate a study of congenic strains to further define the genetic requirements and disease mechanisms of the D2 glaucoma. RESULTS: To further understand D2 glaucoma, we created congenic strains of mice on the C57BL/6J (B6) genetic background. B6 double congenic mice carrying D2-derived Gpnmb and Tyrp1 mutations develop a D2-resembling iris disease. B6 single congenics with only the Gpnmb and Tyrp1 mutations develop milder forms of iris disease. Genetic epistasis experiments introducing a B6 tyrosinase mutation into the congenic strains demonstrate that both the single and double congenic iris diseases are rescued by interruption of melanin synthesis. Importantly, our experiments analyzing mice at ages up to 27 months, indicate that the B6 double congenic mice are much less prone to IOP elevation and glaucoma than D2 mice. CONCLUSIONS: As demonstrated here, the Gpnmb and Tyrp1 iris phenotypes are both independently dependent on tyrosinase function. These results support involvement of abnormal melanosomal events in the diseases caused by each gene. In the context of the inbred D2 mouse strain, the glaucoma phenotype is clearly influenced by more genes than just Gpnmb and Tyrp1. Despite the outward similarity of pigment dispersing iris disease between D2 and the B6 double congenic mice, the congenic mice are much less susceptible to developing high IOP and glaucoma. These new congenic strains, provide a valuable new resource for further studying the genetic and mechanistic complexity of this form of glaucoma.

- Aquaron R, Berge-Lefranc J, Badens C, Roche J, Fite A, Sainte-Marie D, Piquion N, Cartault F. **[Oculocutaneous albinism in French overseas territories (Reunion, French Guyana, Martinique) and Mayotte. Study of 21 cases in 16 families]** Med Trop 65(6):584-91, 2005.
The dual purpose of this study was to determine the genotype of patients with oculocutaneous albinism type 1 and 2 based on analysis of tyrosinase and P gene mutations and to attempt to establish a correlation between phenotype and genotype. This study included a total of 21 Caucasian, Indian and Black African patients from La Reunion, la Martinique, French Guyana and Mayotte. PCR-sequencing of genomic DNA was performed to detect tyrosinase gene mutations and PCR-separation of PCR products by agarose gel electrophoresis was performed to detect 2.7kb deletion allele of the P gene. Tyrosinase gene mutations were identified in two cases, i.e., on eheterozygous guanine "g" deletion (c.572 delG) with a frameshift (Gly191fs) resulting in apremature termination signal at codon 225 in a Caucasian patient from La Reunion and one homozygous missense mutation, Glycine419Arginine, in an Indian patient from La Reunion. The 2.7-kb deletion allele of the P gene was detected in three Black African patients, i.e. two in the homozygous state in siblings from Mayotte and one in the heterozygous state in a girl from la Martinique. The latter patient whose mother was from la Martinique inherited the mutation from her father who was from Cameroon. This study shows that characterization of tyrosinase and P gene mutations in albinos patients is crucial to (a) differentiate subjects with oculocutaneous albinism types 1 and 2 and establish a correlation between phenotype and genotype, (b) identify healthy heterozygous carriers among the patient's immediate family (parents and siblings) and (c) allow prenatal diagnosis during subsequent pregnancies in couples who have already engendered albino children with severe visual phenotype and documented mutation(s).

- Arung ET, Shimizu K, Kondo R.
Inhibitory Effect of Isoprenoid-Substituted Flavonoids Isolated from Artocarpus heterophyllus on Melanin Biosynthesis. Planta Med. 2006.
Isoprenoid-substituted flavonoids were isolated from the wood of ARTOCARPUS HETEROPHYLLUS by means of activity-guided fractionation. Artocarpin (1), cudraflavone C (2), 6-prenylapigenin (3), kuwanon C (4), norartocarpin (5) and albanin A (6) inhibited melanin biosynthesis in B16 melanoma cells without inhibiting tyrosinase. A structure-activity investigation indicated that the presence of the isoprenoid-substituted moiety enhanced the inhibitory activity on melanin production in B16 melanoma cells.

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Of mice and men: tyrosinase modification of congenital glaucoma in mice but not in humans. Invest Ophthalmol Vis Sci. 47(4):1486-90, 2006.
PURPOSE: Primary congenital glaucoma (PCG) is an autosomal recessive ocular trait caused by mutations in the gene for cytochrome P4501B1 (CYP1B1). Although PCG is often considered to be fully penetrant, the disease shows 50% penetrance in some Saudi Arabian families. The familial segregation of the nonpenetrance suggests a genetic modifier. Recently, tyrosinase (Tyr) deficiency was found to worsen the drainage structure/ocular dysgenesis phenotype of Cyp1b1-/- mice, suggesting that Tyr is a modifier of the phenotype. In the current study, tyrosinase (TYR) was investigated in human PCG. METHODS: A genome-wide screen, a single nucleotide polymorphism (SNP) analysis in

the TYR chromosomal region 11q13-q21, and sequencing of the TYR gene was performed with individuals from Saudi Arabian families with multiple, clinically confirmed, molecularly proven, nonpenetrant members. RESULTS: The study outcome did not support TYR as a modifier of the PCG phenotype in this population. The sequencing data showed no TYR mutations in the nonpenetrant family members and no difference in polymorphism frequencies between nonpenetrant or fully penetrant families. CONCLUSIONS: TYR is not a modifier of the CYP1B1-associated PCG phenotype in the Saudi Arabian population.

- Chavan B, Gillbro JM, Rokos H, Schallreuter KU.
GTP Cyclohydrolase Feedback Regulatory Protein Controls Cofactor 6-Tetrahydrobiopterin Synthesis in the Cytosol and in the Nucleus of Epidermal Keratinocytes and Melanocytes. J Invest Dermatol. 2006.
(6R)-L-Erythro 5,6,7,8 tetrahydrobiopterin (6BH(4)) is crucial in the hydroxylation of L-phenylalanine-, L-tyrosine-, and L-tryptophan-regulating catecholamine and serotonin synthesis as well as tyrosinase in melanogenesis. The rate-limiting step of 6BH(4) de novo synthesis is controlled by guanosine triphosphate (GTP) cyclohydrolase I (GTPCHI) and its feedback regulatory protein (GFRP), where binding of L-phenylalanine to GFRP increases enzyme activities, while 6BH(4) exerts the opposite effect. Earlier it was demonstrated that the human epidermis holds the full capacity for autocrine 6BH(4) de novo synthesis and recycling. However, besides the expression of epidermal mRNA for GFRP, the presence of a functioning GFRP feedback has never been shown. Therefore, it was tempting to investigate whether this important mechanism is present in epidermal cells. Our results identified indeed a functioning GFRP/GTPCHI axis in epidermal keratinocytes and melanocytes in the cytosol, adding the missing link for 6BH(4) de novo synthesis which in turn controls cofactor supply for catecholamine and serotonin biosynthesis as well as melanogenesis in the human epidermis. Moreover, GFRP expression and GTPCHI activities have been found in the nucleus of both cell types. The significance of this result warrants further investigation.

- Chen KG, Valencia JC, Lai B, Zhang G, Paterson JK, Rouzaud F, Berens W, Wincovitch SM, Garfield SH, Leapman RD, Hearing VJ, Gottesman MM.
Melanosomal sequestration of cytotoxic drugs contributes to the intractability of malignant melanomas. Proc Natl Acad Sci U S A. 103(26):9903-7, 2006.
Multidrug resistance mechanisms underlying the intractability of malignant melanomas remain largely unknown. In this study, we demonstrate that the development of multidrug resistance in melanomas involves subcellular sequestration of intracellular cytotoxic drugs such as cis-diaminedichloroplatinum II (cisplatin; CDDP). CDDP is initially sequestered in subcellular organelles such as melanosomes, which significantly reduces its nuclear localization when compared with nonmelanoma/KB-3-1 epidermoid carcinoma cells. The melanosomal accumulation of CDDP remarkably modulates melanogenesis through a pronounced increase in tyrosinase activity. The altered melanogenesis manifested an approximately 8-fold increase in both intracellular pigmentation and extracellular transport of melanosomes containing CDDP. Thus, our experiments provide evidence that melanosomes contribute to the refractory properties of melanoma cells by sequestering cytotoxic drugs and increasing melanosome-mediated drug export. Preventing melanosomal sequestration of cytotoxic drugs by inhibiting the functions of melanosomes may have great potential as an approach to improving the chemosensitivity of melanoma cells.

- de la Serna IL, Ohkawa Y, Higashi C, Dutta C, Osias J, Kommajosyula N, Tachibana T, Imbalzano AN.
The microphthalmia-associated transcription factor (MITF) requires SWI/SNF enzymes to activate melanocyte specific genes. J Biol Chem. 2006.
The Microphthalmia Transcription Factor (Mitf) activates melanocyte specific gene expression, is critical for survival and proliferation of melanocytes during development, and has been described as an oncogene in malignant melanoma. SWI/SNF complexes are ATP dependent chromatin remodeling enzymes that play a role in many developmental processes. To determine the requirement for SWI/SNF enzymes in melanocyte differentiation, we introduced Mitf into fibroblasts that inducibly express dominant negative versions of the SWI/SNF ATPases, BRM or BRG1. These dominant negative SWI/SNF components have been shown to inhibit gene activation events that normally require SWI/SNF enzymes. We found that Mitf-mediated activation of a subset of endogenous melanocyte specific genes required SWI/SNF enzymes but that cell cycle regulation occurred independently of SWI/SNF function. Activation of tyrosinase related protein 1 (Trp1), a melanocyte specific gene, correlated with SWI/SNF dependent changes in chromatin accessibility at the endogenous locus. Both BRG1 and Mitf could be localized to the Trp1 and tyrosinase promoters by chromatin immunoprecipitation (ChIP), while immunofluorescence and immunoprecipitation experiments indicate that Mitf and BRG1 co-localized in the nucleus and physically interacted. Together these results suggest that Mitf can recruit SWI/SNF enzymes to melanocyte specific promoters for the activation of gene expression via induced changes in chromatin structure at endogenous loci.

- Desentis-Mendoza RM, Hernandez-Sanchez H, Moreno A, Rojas Del C E, Chel-Guerrero L, Tamariz J, Jaramillo-Flores ME.
Enzymatic Polymerization of Phenolic Compounds Using Laccase and Tyrosinase from Ustilago maydis. Biomacromolecules. 7(6):1845-1854, 2006.
Flavonoids are a big group of polyphenols of low molecular weight with in Vitro antioxidant properties. In this study, the laccase and tyrosinase from Ustilago maydis were partially characterized and their effect on the antioxidant activity of some phenolic compounds was investigated. Since enzymatic polymerization of the phenolic compounds was

detected, the size of the aggregates was determined and related to their antioxidant activity. Morphology of the polymers was analyzed by atomic force microscopy. The results showed that the laccase- and tyrosinase-catalyzed polymerization of quercetin produced aggregates with relatively low molecular weight and higher antioxidant activity than the monomeric quercetin. In the case of kaempferol, the aggregates reached higher sizes in the first 2 h of reaction and their antioxidant activity was increased. In the last case, the aggregates adopted fractal-ordered shapes similar to coral in the case of the kaempferol-laccase system and to fern in the case of the kaempferol-tyrosinase system. The kaempferol and quercetin polymers at low concentration had strong scavenging effect on Reactive oxygen species (ROS) andinhibition of lipoperoxidation in human hepatic cell line WRL-68.

- Freddi G, Anghileri A, Sampaio S, Buchert J, Monti P, Taddei P.
Tyrosinase-catalyzed modification of Bombyx mori silk fibroin: Grafting of chitosan under heterogeneous reaction conditions. J Biotechnol. 2006.
The capability of mushroom tyrosinase to catalyze the oxidation of tyrosine residues of Bombyx mori silk fibroin was studied under heterogeneous reaction conditions, by using a series of silk substrates differing in surface and bulk morphology and structure, i.e. hydrated and insoluble gels, mechanically generated powder and fibre. Tyrosinase was able to oxidize 10-11% of the tyrosine residues of silk gels. The yield of the reaction was very low for the powder and undetectable for fibres. FT-Raman spectroscopy gave evidence of the oxidation reaction. New bands attributable to vibrations of oxidized tyrosine species (o-quinone) appeared, and the value of the I(853)/I(829) intensity ratio of the tyrosine doublet changed following oxidation of tyrosine. The termal behaviour of SF substrates was not affected by enzymatic oxidation. o-Quinones formed by tyrosinase onto gels and powder were able to undergo non-enzymatic coupling with chitosan. FT-IR and FT-Raman spectroscopy provided clear evidence of the formation of silk-chitosan bioconjugates under heterogeneous reaction conditions. Chitosan grafting caused a beta-sheet-->random coil conformational transition of silk fibroin and significant changes in the thermal behaviour. Chitosan grafting did not occur, or occurred at an undetectable level on silo fibres. The results reported in this study show the potential of the enzymatically initiated protein-polysaccharide grafting for the production of a new range of bio-based, environmentally friendly polymers.

- Gasowska B, Frackowiak B, Wojtasek.
Indirect oxidation of amino acid phenylhydrazides by mushroom tyrosinase. H.Biochim Biophys Acta. 2006.
We have investigated oxidation of amino acid phenylhydrazides by mushroom tyrosinase in the presence of 4-tert-butylcatechol and N-acetyl-l-tyrosine. Spectrophotometric measurements showed gradual disappearance of 4-tert-butyl-o-benzoquinone, generated by oxidation of 4-tert-butylcatechol with sodium periodate, after addition of amino acid phenylhydrazides. However, the presence of the phenylhydrazides did not influence the concentration of 4-tert-butyl-o-benzoquinone formed during enzymatic oxidation. Oxygen consumption measurements demonstrated that in a mixture both compounds were oxidized but the reaction rate was proportional to the concentration of the catechol. In the oxidation of N-acetyl-l-tyrosine addition of phenylhydrazides shortened the lag period, indicating that they acted as reducing agents, converting N-acetyl-l-dopaquinone to N-acetyl-l-dopa. In HPLC analysis of the oxidation 4-tert-butylcatechol and the phenylhydrazide of Boc-tryptophan only the N-protected amino acid and 4-tert-butyl-o-benzoquinone were detected as final products. In the presence of the natural substrates the oxidation of amino acid phenylhydrazides required much smaller amounts of the enzyme and was up to 40 times faster than the reaction carried out without these compounds. These results demonstrate that tyrosinase can oxidize phenylhydrazides indirectly through o-quinones. This reaction explains the inhibitory effect of agaritine, a natural amino acid hydrazide, on melanin formation and the inhibitory effects of other hydrazine derivatives on tyrosinase described in the literature.

- Hasegawa T, Matsuzaki-Kobayashi M, Takeda A, Sugeno N, Kikuchi A, Furukawa K, Perry G, Smith MA, Itoyama Y.
Alpha-synuclein facilitates the toxicity of oxidized catechol metabolites: implications for selective neurodegeneration in Parkinson's disease. FEBS Lett. 580(8):2147-52, 2006.
Free radicals, including dopamine (DA)-oxidized metabolites, have long been implicated in pathogenesis of Parkinson's disease (PD). However, the relationships between such oxidative stresses and alpha-synuclein (alpha-S), a major constituent of Lewy bodies, remain unknown. In this study, we established neuronal cells that constitutively express alpha-S and tetracycline-regulated tyrosinase. While tyrosinase overexpression induced apoptosis, co-expression of wild type or A53T mutant human alpha-S with tyrosinase further exacerbated cell death. In this process, the formation of alpha-S oligomers and the reduction in mitochondrial membrane potential were demonstrated. This cellular model may reconstitute the pathological metabolism of alpha-S in the synucleinopathy and provide a useful tool to explore possible pathomechanisms of nigral degeneration in PD.

- Hassan Khan MT, Iqbal Choudhary M, Atta-Ur-Rahman, Mamedova RP, Agzamova MA, Sultankhodzhaev MN, Isaev MI.
Tyrosinase inhibition studies of cycloartane and cucurbitane glycosides and their structure-activity relationships. Bioorg Med Chem. 2006.
In the present paper, tyrosinase inhibition studies and structure-activity relationship of eight cycloartane glycosides and one cucurbitane glycoside and its genin, which were isolated from Astragalus (Leguminoseae) and Bryonia (Cucurbitaceae) plants, have been discussed. The activities are compared with two reference tyrosinase inhibitors,

kojic acid and l-mimosine. These studies and the SAR showed that the askendoside B which exhibited highly potent (IC(50)=13.95muM) tyrosinase inhibition could be a possible lead molecule for the development of new medications of several skin diseases related with the over-expression of the enzyme tyrosinase, like hyperpigmentation. The molecule also may be interesting for the cosmetic industries as a skin whitening agent.

- Haugarvoll E, Thorsen J, Laane M, Huang Q, Koppang EO.
Melanogenesis and evidence for melanosome transport to the plasma membrane in a CD83 teleost leukocyte cell line. Pigment Cell Res. 19(3):214-25, 2006.
Visceral organs of ectothermic vertebrates harbour melanin-containing leukocytes termed melanomacrophages. These cells are thought to participate in immune reactions and free-radical trapping. In teleosts, the melanin-producing ability of melanomacrophages has hitherto not been confirmed by molecular techniques. Here, a leukocyte marker and the apparatus for melanosome production and transport were investigated in an Atlantic salmon (Salmo salar) pronephros-derived mononuclear leukocyte (SHK-1) cell line. The SHK-1 cells expressed transcripts specific for a mammalian CD83 homologue, a standard surface marker for activated or differentiated dendritic cells, and dopachrome tautomerase/tyrosinase-related protein-2, a melanocyte specific enzyme essential for melanin production. Reduction potential of melanin or its precursors was demonstrated histochemically after prolonged cultivation. Ultrastructural investigations revealed tyrosinase and acid phosphate activity in identical organelles and BSA-gold co-localized with multilamellar melanosomes after 2 h internalization. Apparently, melanosomes were transported and released through periodically occurring tubules fusing with the plasma membrane. Video monitoring revealed filopodia and macropinocytosis. These results showed that the SHK-1 cell line is capable of melanogenesis and melanosome secretion. Melanin-producing cells in teleost pronephros may represent a distinct CD83(+) leukocyte population consisting of phylogenetically relict multifunctional cells. This is the first report of a melanin-producing leukocyte cell-line.

- Hirobe T, Wakamatsu K, Ito S, Kawa Y, Soma Y, Mizoguchi M.
The slaty mutation affects eumelanin and pheomelanin synthesis in Mouse melanocytes. Eur J Cell Biol. 85(6):537-49, 2006.
The slaty (Dct(slt)) mutation is known to reduce the activity of dopachrome tautomerase (DCT) in melanocytes. However, it is unknown whether the reduced DCT activity leads to a defect in the proliferation and differentiation of mouse melanocytes. To address this point, the proliferation and differentiation of neonatal melanocytes from Dct(slt)/Dct(slt) congenic mice in serum-free primary culture were investigated in detail. The proliferation of slaty epidermal melanoblasts/melanocytes in culture did not differ from that of wild-type mice. However, the differentiation was greatly inhibited. Tyrosinase (TYR) activity detected by dopa reaction as well as staining of DCT in slaty melanocytes was greatly reduced. The content of eumelanin in cultured slaty melanocytes was reduced, whereas the content of pheomelanin in media derived from cultured 7.5-day-old slaty melanocytes was greatly increased. The contents of eumelanin and pheomelanin in the neonatal slaty epidermis and dermis were reduced, except that the pheomelanin content in 3.5-day-old dermis was increased. These results suggest that the slaty mutation affects both eumelanin and pheomelanin synthesis in developmental stage-specific and skin site-specific manners, and, in addition, the gene controls the differentiation of melanocytes via the regulation of activity of TYR in addition to its own DCT.

- Hoashi T, Muller J, Vieira WD, Rouzaud F, Kikuchi K, Tamaki K, Hearing VJ.
The repeat domain of the melanosomal matrix protein Pmel17/gp100 is required for the formation of organellar fibers. J Biol Chem. 2006.
Over 125 pigmentation-related genes have been identified to date. Of those, Pmel17/gp100 has been widely studied as a melanoma specific antigen as well as a protein required for the formation of fibrils in melanosomes. Pmel17 is synthesized, glycosylated, processed and delivered to melanosomes which allows them to mature from amorphous round vesicles to elongated fibrillar structures. In contrast to other melanosomal proteins, such as tyrosinase and tyrosinase related protein-1, the processing and sorting of Pmel17 is highly complex. The monoclonal antibody HMB45 is commonly used for melanoma detection but has the added advantage that it specifically reacts with sialylated Pmel17 in the fibrillar matrix in melanosomes. In this study, we generated mutant forms of Pmel17 to clarify the subdomain of Pmel17 required for formation of the fibrillar matrix, a process critical to pigmentation. The internal proline/serine/threonine-rich repeat (RPT) domain of Pmel17 undergoes variable proteolytic cleavage. Deletion of the RPT domain abolished its recognition by HMB45 and its capacity to form fibrils. Truncation of the C-terminal domain did not significantly affect the processing or trafficking of Pmel17, but in contrast, deletion of the N-terminal domain abrogated both. We conclude that the RPT domain is essential for its function in generating the fibrillar matrix of melanosomes, and that the luminal domain is necessary for its correct processing and trafficking to those organelles.

- Imes DL, Geary LA, Grahn RA, Lyons LA.
Albinism in the domestic cat (Felis catus) is associated with a tyrosinase (TYR) mutation. Anim Genet. 37(2):175-8, 2006.
Albino phenotypes are documented in a variety of species including the domestic cat. As albino phenotypes in other species are associated with tyrosinase (TYR) mutations, TYR was proposed as a candidate gene for albinism in cats. An Oriental and Colourpoint Shorthair cat pedigree segregating for albinism was analysed for association with TYR by linkage and sequence analyses. Microsatellite FCA931, which is closely linked to TYR and TYR sequence variants

were tested for segregation with the albinism phenotype. Sequence analysis of genomic DNA from wild-type and albino cats identified a cytosine deletion in TYR at position 975 in exon 2, which causes a frame shift resulting in a premature stop codon nine residues downstream from the mutation. The deletion mutation in TYR and an allele of FCA931 segregated concordantly with the albino phenotype. Taken together, our results suggest that the TYR gene corresponds to the colour locus in cats and its alleles, from dominant to recessive, are as follows: C (full colour) > c(b) (burmese) > or = c(s) (siamese) > c (albino).

- Kauser S, Slominski A, Wei ET, Tobin DJ.
Modulation of the human hair follicle pigmentary unit by corticotropin-releasing hormone and urocortin peptides. FASEB J. 20(7):882-95, 2006.
Human skin is a local source of corticotropin-releasing hormone (CRH) and expresses CRH and CRH receptors (CRH-R) at mRNA and protein levels. Epidermal melanocytes respond to CRH by induction of cAMP with up-regulation of pro-opiomelanocortin gene expression and subsequent production of adrenocorticotropin hormone. However, the role of CRH/CRH-R in melanocyte biology is complicated by the significant heterogeneity of cutaneous melanocyte subpopulations, from continuously active and UV-responsive melanocytes in epidermis to UV nonresponsive, hair growth cycle-coupled melanogenesis in hair follicles. In the present study we report that normal human scalp hair follicle melanocytes express CRH at the mRNA level. Furthermore, CRH, urocortin and CRH-R 1 and 2 were differentially expressed in follicular melanocytes, fibroblasts, and keratinocytes depending on anatomic location and differentiation status in situ and in vitro. Stimulation of follicular melanocytes with CRH and CRH peptides, modified for selectivity for CRH-R1 and/or CRH-R2, variably induced cell melanogenesis, dendricity, and proliferation. CRH-peptides also stimulated the expression and activity of Tyrosinase, and expression of Tyrosinase-related protein-1 and-2. However, a modified urocortin peptide highly selective for CRH-R2 down-regulated melanocyte differentiation phenotype. This study indicates that CRH peptides can differentially influence hair follicle melanocyte behaviour not only via CRH-R1 signaling but also by complex cross-talk between CRH-R1 and CRH-R2.

- Ko CF, Chiou TT, Vaseeharan B, Lu JK, Chen JC.
Cloning and characterisation of a prophenoloxidase from the haemocytes of mud crab Scylla serrata. Dev Comp Immunol. 2006.
A prophenoloxidase (proPO) cDNA was cloned from the haemocytes of mud crab Scylla serrata using oligonucleotide primers and RT-PCR. Both 3'- and 5'-regions were isolated by rapid amplification of cDNA end (RACE) method. Analysis of the nucleotide sequence revealed that the cDNA clone has a full length of 2663bp, with an open reading frame of 2019bp, a 124-bp 5'-untranslated region, and a 520-bp 3'-untranslated region containing a poly A signal. It encodes a protein of 673 amino acids with a predicted molecular weight of 77.5kDa and with an estimated pI of 5.96. It contains two putative tyrosinase copper-binding motifs with six histidine residues (copper A, 185, 189, 211, and copper B, 346, 350, 386). The proPO has thiol-ester-like motif (GCGWPQHM), which showed similar structural features of proPOs from other decapod crustaceans. It also contains five possible glycosylation sites, and a conserved C-terminal region common to all known proPOs. Sequence comparison showed that the proPO-deduced amino acid of mud crab S. serrata has an overall similarity of 78%, 57%, 56%, 51-55%, 54%, 53%, 52%, 52%, and 52% to that of Dungeness crab Cancer magister, American lobster Homarus americanus, European lobster Homarus gammarus, kuruma prawn Marsupenaeus japonicus, crayfish Pacifastacus leniusculus, white shrimp Litopenaeus vannamei, tiger shrimp Penaeus monodon, green tiger shrimp Penaeus semisulcatus, and giant freshwater prawn Macrobrachium rosenbergii, respectively. The proPO was strongly expressed in haemocytes, but not in heart, eyestalk, gill, muscle, ovary, hepatopancreas, stomach, and intestine. The proPO transcript of mud crab S. serrata increased significantly in 12 and 24h post-lipopolysaccharide (LPS) injection, but returned to the original values in 72h post injection.

- Ma HJ, Yue XZ, Wang DG, Li CR, Zhu WY.
A modified method for purifying amelanotic melanocytes from human hair follicles. J Dermatol. 33(4):239-48, 2006.
We describe a modified method for establishing long-term pure cultures of amelanotic melanocytes (AMMC) derived from human hair follicles. Normal human corpse scalp (just after death, 1 h) was transected 1 mm below the epidermis, and hair follicles in the remaining dermis were isolated by a two-step enzyme treatment. Hair follicle cell suspensions were prepared by 0.50% trypsin treatment for 30 min and cultured in an optimized melanoblast proliferation nature mitogen medium. Cells attached to the substratum were mostly amelanotic melanocytic in character with small, bipolar shapes in the early stage; only a few keratinocytes and rare fibroblasts were observed. Keratinocytes were easily removed by differential trypsinization. After the third passage, the proliferating cells were all amelanotic melanocytes as confirmed by immunostaining with polyclonal antibodies to alphaPEP7h, which recognized the tyrosinase protein located on melanosomes and NKI/beteb, which is a pre-melanosomal antigen against synthetic peptides corresponding to the carboxyl termini of human melanosomal protein GP100. Cultured AMMC were highly positive to L-dopa reactivity after the addition of IBMX to the culture medium for 7 days. Many stage I and II melanosomes and occasional stage III melanosomes without stage IV melanosomes were found in the cytoplasm by transmission electron microscope. This modified technique is potentially more suitable for cultivating amelanotic melanocytes. The availability of pure cultures of hair-follicle amelanotic melanocytes will facilitate investigations of the roles of those cells in migration and differentiation during treatment of vitiligo.

- Plonka PM, Handjiski B, Michalczyk D, Popik M, Paus R.
Oral zinc sulphate causes murine hair hypopigmentation and is a potent inhibitor of eumelanogenesis in vivo. Br J Dermatol. 155(1):39-49, 2006.
Background C57BL/6 a/a mice have been widely used to study melanogenesis, including in electron paramagnetic resonance (EPR) studies. Zinc cations modulate melanogenesis, but the net effect of Zn(2+)in vivo is unclear, as the reported effects of Zn(2+) on melanogenesis are ambiguous: zinc inhibits tyrosinase and glutathione reductase in vitro, but also enhances the activity of dopachrome tautomerase (tyrosinase-related protein-2) and has agonistic effects on melanocortin receptor signalling. Objectives To determine in a C57BL/6 a/a murine pilot study whether excess zinc ions inhibit, enhance or in any other way alter hair follicle melanogenesis in vivo, and to test the usefulness of EPR for this study. Methods ZnSO(4).7H(2)O was continuously administered orally to C57BL/6 a/a mice during spontaneous and depilation-induced hair follicle cycling (20 mg mL(-1); in drinking water; mean +/- SD daily dose 1.2 +/- 0.53 mL), and hair pigmentation was examined macroscopically, by routine histology and by EPR. Results Oral zinc cations induced a bright brown lightening of new hair shafts produced during anagen, but without inducing an EPR-detectable switch from eumelanogenesis to phaeomelanogenesis. The total content of melanin in the skin and hair shafts during the subsequent telogen phase, i.e. after completion of a full hair cycle, was significantly reduced in Zn-treated mice (P = 0.0005). Compared with controls, melanin granules in precortical hair matriz keratinocytes, hair bulb melanocytes and hair shafts of zinc-treated animals were reduced and poorly pigmented. Over the course of several hair cycles, lasting hair shaft depigmentation was seen during long-term exposure to high-dose oral Zn(2+). Conclusions High-dose oral Zn(2+) is a potent downregulator of eumelanin content in murine hair shafts in vivo. The C57BL/6 mouse model offers an excellent tool for further dissecting the as yet unclear underlying molecular basis of this phenomenon, while EPR technology is well suited for the rapid, qualitative and quantitative monitoring of hair pigmentation changes.

- Popescu. CI, Mares A, Zdrentu L, Zitzmann N, Dwek RA, Petrescu SM.
Productive folding of tyrosinase ectodomain is controlled by the transmembrane anchor. J Biol Chem. 2006.
Transmembrane domains (TMDs) are known as structural elements required for the insertion into the membrane of integral membrane proteins. We provide here an example showing that the presence of the TMD is compulsory for the productive folding pathway of a membrane anchored glycoprotein. Tyrosinase, a type I transmembrane protein whose insertion into the melanosomal membrane initiates melanin synthesis is misfolded and degraded when expressed as a truncated polypeptide. We used constructs of tyrosinase ectodomain fused with chimeric TMDs or glycosyl-phosphatidylinositol-anchor to gain insights into how the TMD enables the productive folding pathway of the ectodomain. We find that in contrast to the soluble constructs, the membrane anchored chimeras fold into the native conformation which allows their ER exit. They recruit calnexin to monitor their productive folding pathway characterized by the post-translational formation of buried disulfides. Lacking calnexin assistance, the truncated mutant is arrested in an unstable conformation bearing exposed disulfides. We show that the transmembrane anchor of a protein may crucially, albeit indirectly, control the folding pathway of the ectodomain.

- Rooryck C, Roudaut C, Robine E, Musebeck J, Arveiler B.
Oculocutaneous albinism with TYRP1 gene mutations in a Caucasian patient. Pigment Cell Res. 9(3):239-42, 2006.
Non-syndromic oculocutaneous albinism (OCA) is a clinically and genetically heterogeneous autosomal recessive disorder with mutations identified in several genes: OCA1 (tyrosinase, TYR), OCA2 (OCA2), OCA3 (tyrosinase-related protein 1, TYRP1), and OCA4 (membrane-associated transporter protein, MATP). OCA3 was thought to be restricted to black populations, where it was clinically described as rufous or brown albinism, until the recent report of a homozygous TYRP1 mutation in Caucasian patients from a consanguineous Pakistani family. Here, we describe a German patient of Caucasian origin, with a light-yellow skin, yellow-gold hair with orange highlights, fair eyelashes, several pigmented naevi, and no tendency to tan, only to burn. Eye-colour is blue-green with substance defects of the iris. Molecular analysis did not reveal any mutation in the TYR and OCA2 genes. Two mutations were found in the TYRP1 gene: a missense mutation (c.1066G>A/p.Arg356Glu) that was inherited from the mother, and a de novo single-base deletion (c.106delT/p.Leu36X). This finding suggests that mutation screening should be extended to the TYRP1 gene in patients from all ethnic origins, at least in cases where no mutations have been identified in the other OCA genes.

- Saha B, Singh SK, Sarkar C, Mallick S, Bera R, Bhadra R.
Transcriptional activation of tyrosinase gene by human placental sphingolipid. Glycoconj J. 23(3-4):259-68, 2006.
The sphingolipids, a class of complex bioactive lipids, are involved in diverse cellular functions such as proliferation, differentiation, and apoptosis as well as growth inhibition. Recently sphingosylphosphorylcholine (SPC), sphingosine-1-phosphate (S1P), and C2-ceramide (C2-Cer), sphingolipid containing acetic acid are emerging as melanogenic regulators. A bioactive sphingolipid (PSL) was isolated from hydroalcoholic extract of fresh term human placenta and it induced melanogenesis in an in vitro culture of mouse melanoma B16F10 cells. Tyrosinase, the rate-limiting enzyme for melanogenesis, is required to be upregulated for the increased melanin production. The expression of tyrosinase, both at protein as well as mRNA level, was higher in the PSL treated B16F10 cells as evidenced by Western blot and RT-PCR analysis. Actinomycin D and cycloheximide, inhibitors of transcription and translation, respectively, inhibited

PSL-induced tyrosinase activity and its protein expression showing decrease in melanogenesis, correspondingly. The activity of GFP coupled tyrosinase promoter was upregulated in transfected B16F10 cells after treating with PSL as determined by fluorescence microscopy, fluorometric analysis, and Western blot. These results, thus, suggested that PSL upregulated tyrosinase gene expression at transcription level through promoter activation to show increased melanogenesis. Therefore, PSL as an inducer of melanogenesis might account for the recovery of pigment in depigmentation disorder.

- Schraermeyer U, Kopitz J, Peters S, Henke-Fahle S, Blitgen-Heinecke P, Kokkinou D, Schwarz T, Bartz-Schmidt KU.
  Tyrosinase biosynthesis in adult mammalian retinal pigment epithelial cells. Exp Eye Res. 83(2):315-21, 2006.
  Tyrosinase (EC 1.14.18.1) is the rate limiting enzyme of melanogenesis and it is unclear whether it is synthesized in postnatal retinal pigment epithelium (RPE). Cultured RPE cells from cattle were fed with isolated rod outer segments (ROS). After phagocytosis, RPE cells were tested for tyrosinase presence and activity with three independent methods: (1) ultrastructural DOPA (l-3,4-dihydroxyphenylalanine) histochemistry (2) immunocytochemistry with anti-tyrosinase antibodies (3) measuring tyrosine hydroxylase activity using [(3)H]tyrosine. With all three methods tyrosinase was found in RPE cells alter ROS-feeding but was absent without feeding. In contrast to the classical hypothesis, we demonstrated with three independent methods that the expression of tyrosinase and its enzymatic activity are induced in cultured adult RPE by phagocytosis of rod outer segments (ROS) in vitro.

- Smaniotto A, Comai S, Bertazzo A, Costa CV, Allegri G, Seraglia R, Traldi P.
  A mass spectrometric investigation on the possible role of tryptophan and 7-hydroxytryptophan in melanogenesis. J Mass Spectrom 41(7):921-930, 2006.
  The activity of tyrosinase and peroxidase + H(2)O(2) in promoting melanogenesis from tryptophan (Trp) and 7-hydroxytryptophan (7-HTP) has been investigated. The reaction samples have been drawn at different reaction times and analysed by MALDI mass spectrometry.The data obtained showed that tryptophan undergoes, under tyrosinase and peroxidase action, an oligomerization process mainly due to the reaction of anthranilic acid (AA) and Trp. However, analysing the UV and fluorescence data, it is seen that the oligomers cannot belong to the melanin pattern, but their possible role in melanogenesis is not to be excluded. Once it reacts with the two enzymes, 7-hydroxytryptophan leads to dark brown products, indicating its possible role in melanin production. In contrast to what was observed in the case of 5-hydroxytryptophan, for which oligomers were constituted by 5-hydroxytryptophan (5-HTP) and 5-hydroxytryptamine (5-HT) units, the MALDI data indicate a sharply different behaviour for 7-HTP. In fact, in the case of 5-hydroxytryptophan, oligomerization takes place through the formation of 5-hydroxytryptamine and the oligomerization products are due to mixed 5-HTP-5-HT oligomers. In the case of 7-hydroxytryptophan, the formation of 7-hydroxytryptamine (7-HT) is also observed, but it does not seem to play any role; the only oligomerization products formed are due to the reaction of 7-hydroxytryptophan and AA. The data so obtained indicate that 7-hydroxytryptophan acts like an effective melanin precursor in the presence of both tyrosinase and peroxidase + H(2)O(2).

- Suderman RJ, Dittmer NT, Kanost MR, Kramer KJ.
  Model reactions for insect cuticle sclerotization: cross-linking of recombinant cuticular proteins upon their laccase-catalyzed oxidative conjugation with catechols. Insect Biochem Mol Biol. 36(4):353-65, 2006.
  The quinone-tanning hypothesis for insect cuticle sclerotization proposes that N-acylcatecholamines are oxidized by a phenoloxidase to quinones and quinone methides, which serve as electrophilic cross-linking agents to form covalent cross-links between cuticular proteins. We investigated model reactions for protein cross-linking that occurs during insect cuticle sclerotization using recombinant pupal cuticular proteins from the tobacco hornworm, Manduca sexta, fungal or recombinant hornworm laccase-type phenoloxidase, and the cross-linking agent precursor N-acylcatecholamines, N-beta-alanydopamine (NBAD) or N-acetyldopamine (NADA). Recombinant M. sexta pupal cuticular proteins MsCP36, MsCP20, and MsCP27 were expressed and purified to near homogeneity. Polyclonal antisera to these recombinant proteins recognized the native proteins in crude pharate brown-colored pupal cuticle homogenates. Furthermore, antisera to MsCP36, which contains a type-1 Rebers and Riddiford (RR-1) consensus sequence, also recognized an immunoreactive protein in homogenates of larval head capsule exuviae, indicating the presence of an RR-1 cuticular protein in a very hard, sclerotized and nonpigmented cuticle. All three of the proteins formed small and large oligomers stable to boiling SDS treatment under reducing conditions after reaction with laccase and the N-acylcatecholamines. The optimal reaction conditions for MsCP36 polymerization were 0.3mM MsCP36, 7.4mM NBAD and 1.0U/mul fungal laccase. Approximately 5-10% of the monomer reacted to yield insoluble oligomers and polymers during the reaction, and the monomer also became increasingly insoluble in SDS solution after reaction with the oxidized NBAD. When NADA was used instead of NBAD, less oligomer formation occurred, and most of the protein remained soluble. Radiolabeled NADA became covalently bound to the MsCP36 monomer and oligomers during cross-linking. Recombinant Manduca laccase (MsLac2) also catalyzed the polymerization of MsCP36. These results support the hypothesis that during sclerotization, insect cuticular proteins are oxidatively conjugated with catechols, a posttranslational process termed catecholation, and then become cross-linked, forming oligomers and subsequently polymers.

- Thorsen J, Hoyheim B, Koppang EO.

Isolation of the Atlantic salmon tyrosinase gene family reveals heterogenous transcripts in a leukocyte cell line. Pigment Cell Res.19(4):327-336, 2006.

In ectothermic vertebrates, visceral organs harbor melanin-containing cells. Their ability as pigment producers is nevertheless disputed. To address expression of the key genes for melanogenesis in Atlantic salmon (Salmo salar), a tyrosinase-positive leukocyte cell line (SHK-1) and skin were used to obtain full-length tyrosinase (Tyr), tyrosinase-like protein-1 (Tyrp1), and dopachrome tautomerase (Dct) mRNA transcripts. In the SHK-1 cells, two different Tyrp1 transcripts were identified, one lacking exon 1. However, only the full-length version of Tyrp1 was identified in the skin. Sequencing of Tyrp1 genomic region revealed that the two Tyrp1 transcripts might originate from two different loci, possibly a result of pseudo-tetraploidity of the Atlantic salmon genome.Expression of Tyr, Tyrp1 and Dct was investigated by quantitative real-time reverse transcriptase polymerase cain reaction showing highest expression in the SHK-1 cell line and skin, intermediate in pronephros, and negligible or absent in liver and muscle. Histological approaches were used to demonstrate melanin and revealed presence of melanized cells in skin, kidney and liver, and absence of such cells in muscle. In addition to verify melanin synthesis abilities of visceral-located cells, our results indicate loci-specific transcription differences between populations of melanin-producing cells in Atlantic salmon.

- Westerhof W.

  The discovery of the human melanocyte. Pigment Cell Res. 19(3):183-93, 2006.

  Around 2200 bc the first written description of a human pigmentation disorder, most likely vitiligo, was recorded, and from that moment the history of research into human pigmentation can be traced. For the following 4000 yr, the origins of human skin colour remained an enigma that was to generate a multitude of misconceptions. Even after European physicians began to dissect and compare dark and light coloured skin to reveal its underlying anatomy, the origins of skin and hair pigmentation were a matter of frequently erroneous speculation. The true source of human pigmentation was only finally revealed with the discovery of the melanocyte in the 19th century. Once tyrosinase was identified to be the key enzyme in pigment formation, attention focused on elucidating the chemical structure of melanin, an enterprise that remains incomplete. The developmental origins of the melanocyte were described from 1940 to 1960, and the concept of the epidermal melanin unit was introduced together with a description of the ultrastructure of the melanosome and melanosome transfer. With these advances came the realization that different skin types exhibit distinct differences at the histological level that relate to varying amounts of eumelanin and pheomelanin produced by the melanocytes. The foundation established over the past 4000 yr is the basis for all current research into this fascinating cell type.

- Wuyts N, De Waele D, Swennen R.

  Extraction and partial characterization of polyphenol oxidase from banana (Musa acuminata Grande naine) roots. Plant Physiol Biochem. 2006.

  Polyphenol oxidase activity (PPO, EC 1.14.18.1, monophenol monooxygenase, and EC 1.10.3.2, o-diphenoloxidase) has been extensively studied in banana fruit for its role in enzymatic browning. Rapid discolouration of leaf, stem and root tissue after injury and strong pigmentation of tissue extracts indicate that PPO and phenolic compounds are ubiquitous in vegetative tissue of banana as well. They hamper biochemical and molecular studies in banana, as cumbersome adaptations of extraction protocols are required. On the other hand, PPO and phenolic compounds could be an important part of the plant's defence system against pests and diseases, including root parasitic nematodes. To facilitate future studies in this area, extraction and assay conditions for PPO from roots of banana (Musa acuminata AAA, Grande naine) were optimized. Highest enzyme activities were obtained in a 0.2 M phosphate buffer at pH 7.0 with 5% insoluble polyvinylpyrrolidone and 0.25% Triton X-100. The lowest K(m) values were obtained for dopamine and D-catechin. Monophenolase activity was shown with p-cresol. Banana root PPO was strongly inhibited by dithiothreitol and sodium metabisulfite. In root sections, oxidation of dopamine strongly co-localized with aerenchyma in the cortex. The experiments revealed indications for the involvement of root PPO and dopamine in resistance of banana against the parasitic nematode Radopholus similis.

- Zafar KS, Siegel D, Ross D.

  A potential role for cyclized quinones derived from dopamine, DOPA and DOPAC in proteasomal inhibition. Mol Pharmacol. 2006.

  We examined the ability of oxidation products of dopamine, DOPA and DOPAC to inhibit proteasomal activity. Dopamine DOPA and DOPAC underwent tyrosinase catalyzed oxidation to generate aminochrome, dopachrome and furanoquinone, respectively. These cyclized-quinones were then incubated with rabbit reticulocyte lysate, and proteasomal activity was measured using a fluorescently labeled peptide. The results from these studies showed that the oxidation of dopamine by tyrosinase generated product(s) that inhibited the proteasome and that proteasomal inhibition correlated with the presence of the UV/vis spectrum of aminochrome. Proteasomal inhibition by aminochrome was not prevented by the addition of SOD and catalase suggesting that reactive oxygen species were not responsible for proteasomal inhibition which was prevented however by the addition of NADH and the quinone reductase, NQO1. Altough NQO1 protected against dopamine induced proteasomal inhibition it led to greater oxygen uptake due to the generation of a redox labile hydroquinone, further emphasizing the lack of involvement of oxygen radicals in proteasomal inhibition. DOPA underwent tyrosinase catalyzed oxidation to form dopachrome, and similar to aminochrome, proteasomal inhibition correlated with the presence of a dopachrome UV/vis spectrum. The inclusion of NQO1 did not protect against proteasomal inhibition induced by dopachrome. Oxidation of DOPAC by tyrosinase

generated furanoquinone which was a poor proteasome inhibitor and induced less than 20 percent inhibition at the highest concentration tested. These studies demonstrate that oxidation products including cyclized quinones derived from dopamine and related compounds rather than oxygen radicals have the ability to inhibit the proteasome. They also suggest an important protective role for NQO1 in protecting against dopamine-induced proteasomal inhibition. The ability of endogenous intermediates formed during dopaminergic metabolism to cause proteasomal inhibition provides a potential basis for the selectivity of dopaminergic neuron damage in Parkinson's disease.

8. Melanosomes

(Prof. J. Borovansky)

It is possible to state generally that ocular, especially RPE melanosomes , have received much attention recently (*Azarian et al, Cortese et al, Eibl et al, Faraggi et al, Futter, Runkel et al, Tolleson, Tolleson et al, Zareba et al*). There are four very good reviews (*Futter, Sarangajaran & Apte, Tolleson et al, Wei*) dealing with various aspects of melanosome and melanocyte research. *Aspengren et al, De Schepper et al, Futter, Zannelli et al* have brought new information on melanosome transport. Melanosome ultrastructure was studied in spontaneous uveal melanomas in transgenic mice (*Tolleson et al*), in animals with *Grey*, a novel mutation in the murine *Lyst* gene (*Runkel et al*) and in various mouse albinism models (*Cortese et al*). An interaction – between amyloid precursor protein, neurofibromin and melanosome was observed in neurofibromatosis (*De Schepper et al*). Modern techniques have brought new information on the elemental composition of RPE and choroid melanosomes (*Eibl et al*) and on proteins in porcine RPE melanosomes (*Azarian et al*). Melanosome genesis was reported in a glioblastoma cell line (*Bonfigli et al*). Sorting and trafficking of melanosomal proteins were central topics of several papers (*Katzmann, Ni-Komatsu & Orlow, Valencia et al, Wei*). *Cortese et al* have suggested that the *OA1* gene controls both the rate of melanosome biogenesis and organelle size. *Zareba et al* have concluded that if melanosome performs a cytoprotective role within a cell, its effect may be limited to local environment of the organelle and undetectable by conventional methods.

- Aspengren S, Hedberg D, Wallin M.
  Studies of pigment transfer between *Xenopus laevis* melanophores and fibroblasts in vitro and in vivo. Pigment Cell Res 19(2):136-145, 2006.
  Comments: Frog melanophores exist both in epidermis where keratinocytes are present and in dermis where fibroblasts dominate. Since no frog keratinocyte cell line exists, the authors studied whether release and transfer of melanosomes can be studied in a melanophore – fibroblast coculture. Evidence was found for exocytosis/endocytosis and cytophagocytosis of melanosomes. They were transferred as membrane-enclosed organelles in a process that was upregulated by α-MSH and that was different from that of latex beads. In vivo studies confirmed the presence of extracellular pigment in dermis and uptake of melanosomes by fibroblasts.

- Azarian SM, McLoad I, Lillo C, Gibbs D, Yates JR, Williams DS.
  Proteomic analysis of mature melanosomes from the retinal pigment epithelium. J Proteom Res 5(3):521-529, 2006.
  Comments: The protein spectrum of melanosomes prepared from the porcine RPE was studied by means of mass spectrometry and 102 proteins were found including several lysosomal enzymes. The authors conclude that melanosomes may contribute to the degradation of ingested photoreceptor outer segment discs. (A modification of RPE melanosome isolation procedure was introduced. Optiprep proved to be superior to Percoll or sucrose in gradient preparation).

- Bonfigli A, Zarivi O, Colafarina S, Cimini AM, Ragnelli AM, Aimola P, Natali PG, Ceru MP, Amicarelli F, Miranda M.
  Human glioblastoma ADF cells express tyrosinase, L-tyrosine hydroxylase and melanosomes and are sensitive to L-tyrosine and phenylthiourea. J Cell Physiol 207(3):675-682, 2006.
  Comments: The authors demonstrated that glioblastoma cells possessed tyrosinase as well as tyrosine hydroxylase activities and synthesized melanosomes. They also found that L-tyrosine downregulated the expression of the peroxisomal proliferators activated receptor α transcription factor expression in glioblastoma ADF cells and induced (similarly to phenylthiourea) apoptosis in glioblastoma and neuroblastoma cells.

- Cortese K, Giordano F, Surace EM, Venturi C, Ballabio A, Tacchetti C, Marigo V.
  The ocular albinism type 1 (*OA1*) gene controls melanosome maturation and size. Invest Ophthalmol Vis Sci 46(12):4358-4364, 2005.
  Comments: Immunohistochemical and ultrastructural study of both tyrosinase activity and OA1 protein expression in various albinism mouse models ($Oa1^{-/-}$/ $Oa1^{-/-}$; $Tyr^{c2\text{-}J}/Tyr^{c2\text{-}J}$ and $Oa1^{-/-}$; $Matp^{uw}$ / $Matp^{uw}$) indicated that OA1, mutated in ocular albinism type 1, controls the rate of melanosome biogenesis at early stage of organellogenesis but at later stages the size of melanosomes.

- De Schepper S, Boucneau JM, Westbroek W, Mommaas M, Onderwater J, Messiaen L, Naeyaert JM, Lambert JL.
  Neurofibromatosis type 1 protein and amyloid precursor protein interact in normal human melanocytes and colocalize with melanosomes. J Invest Dermatol 126(3):547-550, 2006.
  Comments: A novel interaction between the amyloid precursor protein and neurofibromin (gene product of neurofibromatosis gene) was identified. In addition, a colocalization of amyloid precursor protein and neurofibromin with melanosomes was observed. Amyloid precursor protein has been proposed to function as a vesicle cargo receptor for the motor protein kinesin-1 in neurons. The authors suggest that a complex between amyloid precursor protein, neurofibromin and melanosome might be important for melanosome transport.

- Eibl O, Schultheiss S, Blitgen-Heinecke P, Schraermeyer U.
  Quantitative chemical analysis of ocular melanosomes in the TEM. Micron 37(3): 262-276, 2006.
  Comments: Chemical composition of melanosomes in RPE and choroid of a human, monkey and rat was analyzed by means of EDX (energy dispersion X-ray microanalysis). The presence of C, O, Na, Mg, K, Si, P, S, Cl and Ca was demonstrated. Ca was bound to oxygen rich sites in the melanin.As for the transition metals, a mole fraction ratio of less than 0.1 at.% was found for Fe, whereas the mole fractions of Zn and Cu were clearly beyond the minimum detectable mass only in the RPE melanosomes of human eye.

- Faraggi E, Gerstman BS, Sun JM.
  Biophysical effects of pulsed lasers in the retina and other tissues containing strongly absorbing particles: shockwave and explosive bubble generation. J Biomed Optics 10(6): 64029-64029, 2005.

- Futter CE.
  The molecular regulation of organelle transport in mammalian retinal pigment epithelial cells. Pigment Cell Res 19(2): 104-111, 2006.
  Comments: A review summarizing what we know about the molecular regulation of melanosome movement and phagosome maturation within RPE cells and discussing the potential roles of defects of organelle transport in RPE in the pathogenesis of eye diseases. A contribution of mouse model studies in this respect has not been negligible.

- Katzmann DJ.
  No ESCRT to the melanosome: MVB sorting without ubiquitin. Developmental Cell 10(3): 278-280, 2006.
  Summary: Multivesicular bodies (MVBs) are critical for a variety of cellular functions ranging from lysosomal degradation to the budding of HIV. To date, delivery into MVBs has been dependent on the ESCRT (**E**ndosomal **S**orting **C**omplex **R**equired for **T**ransport) machinery.However, analysis of a melanosomal protein has uncovered an an alternative pathway for the MVB sorting.

- Ni-Komatsu L, Orlow SJ.
  Heterologous expression of tyrosinase recapitulates the misprocessing and mistrafficking in oculocutaneous albinism type 2: Effects of altering intracellular pH and pink-eyed dilution gene expression. Exp Eye Res 82(3): 519-528, 2006.

- Runkel F, Büssow H, Seburn KL, Cox GA, Mc Vey Ward D, Kaplan J, Franz T.
  ***Grey*, a novel mutation in the murine *Lyst* gene, causes the *beige* phenotype by skipping of exon 25.** Mammalian Genome 17(3): 203-210, 2006.
  Comments: A novel mutation in the mouse Lyst gene – $Lyst^{bg\text{-}grey}$ was analyzed both histologically and molecularly. Melanosomes of melanocytes associated with hair follicles and the choroid as well as RPE melanosomes were larger and irregularly shaped in homozygous mutants compared with those of wild animals. The grey phenotype may be caused by the absence or marked reduction of LYST protein due to its degradation as a consequence of a deletion in the area of exon 25.

- Sarangajaran R, Apte SP.
  The polymerization of melanin: A poorly understood phenomena with egregious biological implications. Melanoma Res 16(1): 3-10, 2006.
  Comments: An interesting review presenting several lines of evidence to support a hypothesis that the degree of melanin polymerization may be causative or correlative to its redox status and proposing a model explaining the heterogeneity in the molecular weight and structure of melanin. A cycle of increasing oxidative stress and proliferation may lead to the leakage of melanin monomers outside the melanosome , thereby causing cytotoxicity.

- Tolleson WH.
  Human melanocyte biology, toxicology and pathology. J Environ Sci Health 23(2): 105-161, 2005.
  Comments: A modern review dealing with four populations of melanocytes located in the skin, eyes, inner ear and covering of the brain. A decent attention was paid to melanosome assembly, maturation and properties. Text is accompanied by 14 excellent figures.

- Tolleson WH, Doss JC, Latendresse J, Warbritton AR, Melchior Jr WB, Chin L.
  Spontaneous uveal amelanotic melanoma in transgenic *Tyr-RAS+ Ink4a/Arf-/-* mice. Arch Ophthalmol 123(8):1088-1094, 2005.
  Comments: In transgenic *Tyr-RAS+ Ink4a/Arf-/-* mice spontaneous melanomas occur that arise within the choroid or ciliary body and share histopathological features typical of human uveal melanomas. As for melanosomes, electron microscopy revealed the presence of ovoid premelanosomes (of 100-400nm size) containing haphazardly arranged inner membranes and limiting membranes with defects. Some of the organelles were electron-dense.

- Valencia JC, Watabe H, Chi A, Rouzaud F, Chen KG, Vieira WD, Takahashi K, Yamaguchi Y, Berens W, Nagashima K, Shabanowitz J, Hunt DF, Appella E, Hearing VJ.

Sorting of Pmel17 to melanosomes through the plasma membrane by AP1 and AP2: evidence for the polarized nature of melanocytes. J Cell Sci 119(6): 1080-1091, 2006.
Comments: Processing and trafficking of melanosome protein Pmel17 via adaptor protein complexes within melanocytic cells was characterized. Modern proteomics and molecular biology analyses revealed that the Pmel17 is sorted from Golgi to stage I melanosomes directly or indirectly according to whether adaptor protein 1 isoform μ1B is expressed (e.g. in melanocytes) or not (e.g. in metastsasizing melanoma cells) – elegantly summarized in Fig.8. Moreover, it was shown that the expression of μ1B is regulated physiologically by UV radiation or DKK1, an inhibitor of Wnc signalling.

- Wei ML.
Hermansky-Pudlak syndrome: A disease of protein trafficking and organelle function. Pigment Cell Res 18(1): 19-42, 2006.
Comments: An excellent review summarizing recent molecular, biochemical and cell biological findings and clinical studies. All eight human HPS subtypes are characterized in detail and corresponding mouse models are mentioned. Superb colour schemes explaining trafficking pathways in melanocytes mediated by HPS proteins and function of HPS protein complexes along the pathway of melanosome biogenesis.

- Zannolli R, Buoni S, Macucci F, Santi MM, Miracco F, Pierluigi M, Mogni M, Piomboni P, Massafra MR, Galluzzi P, Liwi W, Cuccia A, Margollicci MA, Pucci L, Sacco P, Molinelli M, Burlina AB, Swift JA, Fimiani M, Zappella M, Miracco C.
Global developmental delay, osteopenia and ectodermal defect: A new syndrome. Brain Dev 28(3): 155-161, 2006.
Comments: Skin defects in children affected by global developmental delay comprise various abnormalities – abnormal keratinocyte differentiation, sweat gland and melanocyte abnormalities. The latter displayed both morphological (reduced number and size without evident dendritic processes) and functional changes (defects of the melanosome migration into keratinocytes).

- Zareba M, Raciti MW, Henry MM.
Oxidative stress in ARPE-19 cultures: Do melanosomes confer cytoprotection? Free Rad Biol Med 40(1): 87-100, 2006.
Comments: To examine whether melanosomes can confer cytoprotection against oxidative stress induced chemically or photically in human amelanotic retinal pigment epithelium cells ARPE19, the cells were incubated with melanosomes isolated from bovine and porcine RPE and for comparison also with ozonated charcoal particles and silica particles. None of the phagocytized particles affected ARPE19 cell viability or levels of catalse and glutathione peroxidase neither any evidence was obtained to indicate that melanosomes confer a cytoprotection against oxidative stress. It is concluded that if melanosome performs a cytoprotective role within cell, its effect may be limited to local environment of the organelle and undetectable by conventional methods. (However, since the isolation procedure included sonication, temporary pH 1!? and repeated freezing and thawing, the melanosomes used, were not native according to my experience, and hence their behaviour did not have to be identical to a physiological situation).

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2006 4th Summer Academy of Dermatopathology Meeting

July 31 - August 04, Graz, Austria
Contact : Department of Dermatology, Medical University Graz
Lorenzo Cerroni, MD
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8036 Graz , Austria
Phone: +43-316-385-2423
Fax: +43-316-385-2466
Email: lorenzo.cerroni@meduni-graz.at

2006 36th Annual ESDR Meeting

September 7-9, Paris, France
Contact: European Society for Dermatological Research
7 Rue Cingria
1205 Geneva, Switzerland
Phone: 41-22-321-48-90
Fax: 41-22-321-48-92
E-mail: office@esdr.org
Web: www.esdr.org

2006 International Dermoscopy Course and Conference

September 7-9, Warsaw, Poland
Contact: Dept. Dermatology CSK MSWiA
Lidia Rudnicka
Woloska 137
00-768Warsaw, Poland
Phone: +48 22 824 22 00
Fax: +48 22 508 14 92
Email: lidiarudnicka@yahoo.com
Website: www.derm.pl

2006 13th meeting of the Pan American Society for Pigment Cell Research

September 7-10, Cincinnati, Ohio, USA
Contact: Zalfa Abdel-Malek
E-mail: paspcr13@uc.edu
Congress Web page: http://www.conferencing.uc.edu/Details.asp?ConferenceID=239
Web : paspcr.med.umn.edu/

2006 Perspectives in Melanoma X

September 13-16, Amsterdam, Netherlands
Contact: Imedex
Phone: 770-751-7332
Fax: 770-751-7334
Email: meetings@imedex.com
Website: www.imedex.com

2006 6th Congress of BADV

September 14-16, Riga, Latvia
Contact: Andris Y. Rubins, MD
Riga Stadins University
Kr. Valdemara Street 76-75
Riga LV-1013, Latvia
Fax: 371-737-0395
Email: arubins@apollo.lv
Website: www.badv.lv

2006 XIIIth Meeting of the ESPCR

September 24-27, Barcelona, Spain
Contact: Dr. L. Montoliu
E-mail: montoliu@cnb.uam.es
Web: www.cnb.uam.es/~espcr06/

2006 15th Congress of the European Academy of Dermatology and Venereology - EADV

October 04-08, Rhodes Island, Greece
Contact: Mrs. Penelope Mitroyianni
Phone: 30-2-107-257-693
Fax: 30-2-107-257-532
E-Mail: info@eadv2006.com
Website: www.eadv.org

2006 XXVII Symposium of the ISDP

November 09-11, Malaga, Spain
Contact: ISDP - Cathy Klapak
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NC 27113-5717 USA
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Fax: 336-788-0742
Email: intsocdermpath@aol.com
Website: www.intsocdermpath.org

2006 20th Annual Meeting of the Japanese Society for Pigment Cell Research (JSPCR)

November 25-26, Matsumoto City, Japan
Contact: Prof. Toshiaki Saita of Shinsyu University
Web: wwwsoc.nii.ac.jp/jspcr/

2007 2nd Conference of the Asian Society for Pigment Cell Research (ASPCR)

July 6-8, Singapore
Contact: Conference Secretariat, Mrs Alice Chew
National Skin Centre
1 Mandalay Road
Singapore 308205
Tel: (65) 6350 8405 ; Fax: (65) 6253 3225
E-mail: training@nsc.gov.sg
Web: http://www.aspcr.org/ASPCR2007

2007 37th Annual ESDR Meeting

September 6-8, Zurich, Switzerland
Contact: E-mail: office@esdr.org
Web: www.esdr.ch

2007 14th meeting of the PanAmerican Society for Pigment Cell Research

September 13-16, Chicago, IL, USA
Contact: Caroline LePoole
E-mail: ilepool@lumc.edu
Web : paspcr.med.umn.edu/

2007 XIVth Meeting of the ESPCR

September, Bari, Italy
Organizer: Prof. Rosa Cicero

2007 21st World Congress of Dermatology

October 1-5, Buenos Aires, Argentina
Contact: E-mail: info@dermato2007.org
Web: www.dermato2007.org

2007 21th Annual Meeting of the Japanese Society for Pigment Cell Research (JSPCR)

December 8-9, Toyoake City, Japan
Contact: Prof. Kazumasa Wakamatsu
Web: wwwsoc.nii.ac.jp/jspcr/

2008 20th International Pigment Cell Conference (IPCC)

May 7–12 Sapporo, Japan
Contact: Secretariat Office
Toshiharu YAMASHITA (Sapporo Medical University, Japan)
Minami 1-jo, Nishi 16-chome Chuo-ku, Sapporo, Japan 060-8543
Phone: +81-11-611-2111
Fax: +81-11-613-3739
E-mail: **ipcc-imrc2008@sapmed.ac.jp**
Web: http://**www.e-convention.org/ipcc-imrc2008**

2008 International Investigative Dermatology (Joint Meeting of the ESDR, SID and JSID)

May 14-17 , Kyoto, Japan
Contact: E-mail: office@esdr.org
Web: www.esdr.ch

NEXT ESPCR GENERAL ASSEMBLY

The ESPCR General Assembly at the Barcelona meeting will take place:
Tuesday 26 sept, at 19:15
at the Auditorium Sant Joan de Deu

NEW ELECTED ESPCR COUNCIL MEMBERS

Lluis Montoliu
Alessandra Napolitano
Anja Bosserhoff
Alain Taieb
Miguel Seabra

after the barcelona meeting the ESPCR council members will be:

Anja Bosserhoff
Jose Carlos Garcia-Borron as ex officio
Colin Goding
Ghanem Ghanem as non-voting permanent (Bulletin/Web site)
Jo Lambert as Treasurer
Lionel Larue as Secretary
Lluis Montoliu
Alessandra Napolitano
Mauro Picardo as President
Miguel Seabra
Alain Taieb

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

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