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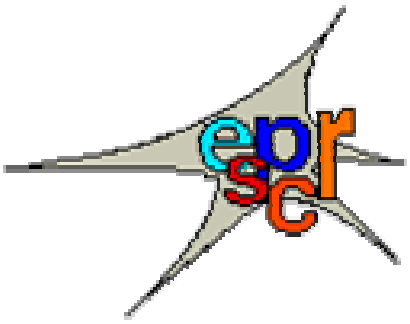

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

N° 51 - Jan 2005

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CONTENTS

ESPCR GENERAL ASSEMBLY

Friday, September 24, 2004, 17:45
Institut Curie, Paris, France

1. Opening of the General Assembly.

The Assembly was opened by J.-C. García-Borrón who welcomed all attendants.

2. Approval of the minutes of the ESPCR General Assembly in Ghent.

The minutes of the previous General Assembly, held in Ghent, were approved and signed by the President.

3. Secretary's Report.

The report was delivered by JM Naeyaert. His relevant activities were the organization of a ballot for various awards (*vide infra*) and the preparation of a meeting report of the Paris meeting to be published in the ESPCR Bulletin in December 2004.

The report dealt with the following topics:

- Elections during the year:

The term of C. Goding expires in 2005 and that of L. Larue in 2004. Both are eligible for re-election. The elections will be held in the coming months, before the end of 2004. Therefore, a call for nominations will be issued immediately, according to current constitutional regulations.

- Nominations and ballots:
A ballot was organized in order to choose nominees for the various awards, to be decided before the IPCC 2005.

Myron Gordon Award: among the nominees proposed by the ESPCR membership the ESPCR Council selected **B. Gilchrest**. This decision was communicated in due time to the IFPCS President, to be forwarded to the IFPCS Awards Committee.

Seiji Memorial Lecturer: S. Shibahara was selected by the ESPCR Council among nominees made by the ESPCR membership. His name was also forwarded to the IFPCS President who should pass this information to the IFPCS Awards Committee.

Takeuchi Medal: C. Goding will be proposed to the JSPCR Awards Committee.

H.S. Raper Medal: S. Pavel is the nominee to be proposed to the ESPCR Awards Committee.

XIIth ESPCR Meeting report:
Chairs of the different sessions in Paris were contacted by the Secretary in order to write a meeting report for the December issue of the ESPCR Bulletin. They will be asked to provide their report before the end of November.
L. Larue, the Congress Organizer, will be asked to write down his general impression of the XIIth ESPCR Meeting in Paris.

The President thanked the Secretary and moved on to the next item.

4. Treasurer's Report.

The ESPCR bank account in Paris and the bank account and savings account in London were closed after transfer of remaining credit onto a newly opened ESPCR account in Belgium. The society now has one account.

For 2004, 100 members paid for their subscription with 90 regular members, 10 student members, and 8 honorary members. Of these, 19 were new subscribers, with 15 new regular members, and 4 new student members.

The Treasurer delivered the following report of incomes and outgoings (in Euros) during this year.

Number of members on 9/2004 *(2003):*	**199**
New members in 2004:	19
Regular members	15
Student members	4
Members who paid subscription up to 9/2004:	99
Regular members	89
Student members	10
Honorary members	8

Balance carried over from 2003:	**3,971.56**
Income 10/2003-09/2004	
Member subscriptions 2004	5,516
Pigment Cell Research subscriptions	2,990
Donations	115
Total income (3971,56 + 8621)	**12,592.56**

Outgoings 9/2003-9/2004	
International Federation of Pigment Cell Societies	
Subscriptions for 2004 (106 x $28 = $2940)	2,030
Paris meeting + Fritz Anders Memorium	4,000
Bulletin and web costs (Prof. Ghanem)	210
Bank charges Centea	
cheques	43.45
visa/MC/EC/Amex	122.32
bank costs	8.65

ESPCR 4 Travel Awards	1,000
Pigment Cell Research Subscriptions (26 x115)	2,990
Two plaques for honorary members	0,000
HS Raper medal	-
Credit card machine (running budget)	312.35
Total outgoings	**10,716.77**

Balance on 15/09/03	**1,875.79**

This balance is approximate, pending minor changes due to the exact exchange rates when payments in dollars will be done, as well as the possible reception of late subscription fees.

The report was approved by the Assembly, and the President thanked Jo Lambert for her work.

5. Web site and ESPCR Bulletin Report.

G. Ghanem reported that the Web site has been regularly updated and improved. New links were added to free electronic journals. Links with the Pigment Cell Research website will be optimized. Moreover, L. Montoliu is willing to provide colour pictures of mouse coat colour mutants, which would be available through the ESPCR Web page. The President will further discuss this issue with L. Montoliu. The President thanked G. Ghanem for his excellent work on the site and the Bulletin.

6. Financial Matters and Legal Status of the ESPCR.

The President summarized the current financial situation of the Society, with higher expenses than incomes during 2004. Since this situation is not sustainable, the Society needs to decrease the outgoings and/or increase the incomes. The ESPCR needs more paying members. This can be achieved by increasing the percentage of current members that actually pay their fee in due time and by recruiting new ones, and all efforts should be made in these directions. A discussion followed on how to proceed.

A short discussion followed on the pertinence of updating the membership fee, which remains unchanged from 2001. Since the expenses in the next two years will probably be lower, it was decided to maintain the current fees and to reconsider the situation in 2005.

The legal status of the Society was then discussed. As of today, the ESPCR is not legally registered, because the original register in Italy is no longer valid. Several options were considered. The goal is to create an international Charity Organization (french: ASBL; dutch: VZW). The Secretary and the Treasurer will seek out how to proceed in order to get a legal registration of the ESPCR in Belgium, since this country was considered more appropriate, given the nationality of the current Officers.

A related issue is the lack of a VAT number for the Society. A VAT number is now absolutely required to pay invoices such as those from Blackwell corresponding to Pigment Cell Research subscriptions. This year, the payment has been arranged through the IFPCS, and J.-C. García-Borrón thanked the IFPCS President, D. Bennett for her help in this respect. It was decided to find out the requirements for obtaining a VAT number in Belgium. If obtaining a VAT number in Belgium is easy, this would be preferable than in any other European country, in view of the nationality of the Officers. The Secretary and the Treasurer will try to get the necessary information. If there is any problem with this arrangement, the President will obtain a VAT number in Spain, where the procedure seems quite straightforward. It was agreed to proceed as quick as possible in order to solve this irregular situation.

7. Venues for forthcoming Meetings.

The venues of the 2005 IPCC (Virginia, USA, organized by Vince Hearing) and the 2006 XIIIth ESPCR Meeting (Barcelona, Spain, organized by L. Montoliu) were already decided.
The venue of the 2007 XIVth ESPCR Meeting needs to be decided. Several possibilities are considered, including Edinburgh (I. Jackson), Münster (M. Böhm) or Iceland (E. Steingrimsson), and others.

8. Any other business.

No items were raised.

9. Close of the General Assembly.

With no other matters to discuss, the General Assembly was closed by J.-C. García-Borrón.

ADDENDUM

The election process to cover the two vacancies in the ESPCR Council mentioned in the Secretary's report was actually completed at the end of October 2005. The (re)elected candidates were:

- Dr. Colin Goding, from the MCRI, Oxted.
- Dr. Lionel Larue, from the Institut Marie Curie, Paris.

The new composition of the Council will be presented for approval to the next General Assembly, to be held in Reston, Virginia during the XIXth IPCC.

This new composition will be:

OFFICERS

President: J. C. García-Borrón (Murcia)
Secretary: J.M. Naeyaert (Ghent)
Treasurer: J. Lambert (Ghent)

COUNCIL

F. Beermann (Lausanne)
Dorothy Bennett (London)
G. Ghanem (Brussels)
C.R. Goding (Oxted)
Lionel Larue (Paris)
M. Picardo (Rome)
N. Smit (Leiden)
A. Taïeb (Bordeaux)

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Dr. A. Napolitano)

Inductively coupled plasma mass spectrometry is employed by Hong et al to quantify the binding of EDTA-washed sepiomelanin to different metal ions. Differences were observed in the ability of melanins to cause breaks to DNA strand depending on the metal ion bound. A further investigation by Liu and Simon on sepiomelanin provided evidence for a role of metal ions in templating the three dimensional substructure distributed along the protein scaffold within the granule. Different spectroscopic techniques were applied to the examination of various physicochemical properties of melanin pigments. Thus magic angle spinning NMR spectroscopy allowed to compare the binding affinity of synthetic melanin pigments toward different drugs (Borel et al), while optical absorption of eumelanin oligomers was used to examine stacking phenomena and redox state, the experimental results being corroborated and interpreted by computational studies (Stark et al.). Patents on the use of melanins as sunscreen or in photochromic lenses have also appeared.
Another paper by the group of the Korea Research Institute at Tajeon describes a synthetic approach to the new melanogenesis inhibitor terrein. The inibitory effects of 4,4'-diidroxybiphenyl on tyrosinase activity is described by Kim et al. An interesting paper by Garcia-Molina et al focuses on the long debated role of the peroxidase-hydrogen peroxide system in melanogenesis. It is concluded that peroxidase may contribute to the development of melanogenesis and the oxidant environment within the melanosome.

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Melanogenesis: a photoprotective response to DNA damage? Mutation Res. 571(1-2), 121-132, 2005.

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Isolation, purification and physicochemical characterization of water-soluble Bacillus thuringiensis melanin. Pigment Cell Res. 18(2), 130-135, 2005.

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Characterization of melanin produced by a wild-type strain of Bacillus thuringiensis. J. Gen. App. Microbiology 50(4), 183-188, 2004.

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Microstructure of Cell Wall-Associated Melanin in the Human Pathogenic Fungus Cryptococcus neoformans. Biochemistry 44(10), 3683-3693, 2005.

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Melanization of Penicillium marneffei in vitro and in vivo. Microbiology 151(Pt 1),291-299, 2005.

2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

For this update of literature we aimed to focuse the attention on a pigmentation disorder (vitiligo) and basic biology.
The need of improving diagnostic accuracy in melanocytic skin tumors has led to the development of imaging tools. The **Pellacani** group illustrated the clinical use of in vivo confocal laser scanning microscopy (CLSM) and its prognostic value. CSLM allows a fine characterization of melanocytic lesions and among the wide spectrum of features previous described by confocal images the cellular atypia appears to be the most sensitive feature for melanoma diagnosis. **Gerger and co-workers** validated CLSM in diagnosing melanocytic skin tumors in an observed-blinded manner. The results of this study indicates that CLSM can provide useful diagnostic information and can also represent an opportunity to spare some patients a biopsy or excision procedure and save time and costs. **Yamashita et al** tested the applicability of reflectance-mode confocal microscopy for non-invasive visualization of melanin *in vivo*. The assessment of melanin distribution in the basal layer and the changes in the UV-exposed human skin has been performed.
A further update on melanoma genesis was provided by **Gordon-Thomson**. The western blot analysis of the expression of three different receptors (ErbB2, ErbB3, and ErbB4) for epidermal growth factors and of the downstream kinases modulation showed a different answer by normal melanocytes and melanoma cells. The first difference depends on the lack of expression of ErbB4 by melanoma cells. In addition, in normal melanocytes the growth factor heregulin beta1 (HRGbeta1) binds ErbB3 and ErbB4 allowing the cell migration whereas the same growth factor determines the cell proliferation in melanoma cell line. The switch between the two different answers could be responsible for the malignant degeneration.
Several studies focused on the different biological response induced on melanocytes following UVA or UVB irradiation. **Larsson et al** compared cellular damage in normal human keratinocytes and melanocytes after UVA and UVB exposure. They found reduction of proliferation, membrane destabilization, and altered redox balance in both melanocytes and keratinocytes immediately after UVA irradiation. These findings can reflect the activation of signal transduction cascades and intracellular stress response mechanism. Similar responses were noted in keratinocytes irradiated with high doses of UVB, whereas melanocytes showed a marked resistance to UVB irradiation and no oxidative damage was detected. The authors observed that UVA as well as UVB induce apoptosis at a similar frequency in both melanocytes and keratinocytes. The readiness for melanocytes to undergo apoptosis in this pure cell culture system might be the disruption of the interplay between melanocytes and keratinocytes inside the epidermis. The high sensitivity of melanocytes to the pro-oxidant effect of UVA can support the hypothesis that UVA is more involved that UVB in the induction of melanoma.
Bohm et al demonstrated regulation of UVB-induced apoptosis in human melanocytes by the α-MSH. This neuropeptide naturally expressed within the epidermis was found to be able to reduce the UVB-mediated DNA damage, as demonstrated by reduction of cyclobutane dimers and induction of nucleotide excision repair system. These findings, together with the well-kown capacity of α-MSH to induce the synthesis of eumelanin, suggested a relevant role of α-MSH in the protection of genomic stability of epidermal cells against harful effects of UV radiation. As regard to UVB and Bcl2 pattern, the experimental data are contrasting. This biological puzzle, as suggested by **Bivik**, could be due to a wavelength-dependent effect of the UVB on Bcl2/Bax ratio and apoptotic process. An intriguing hypothesis is that an intracellular redistribution of bax could be more relevant than total content. In addition the presence of keratinocytes in a co-culture system can be responsible for a melanocytic lack of susceptibility to apoptosis. The link between UV (B and/or A1) and melanocyte proliferation and the molecular pathway undelying the process was studied by **van Schanke**. He demostrated that the proliferative effect of erythemagenic dose of UVB, but not of UVA1, is mediated by the generation of thymidine cyclobutane dimers. The level of DNA damage appears to be correlated to the proliferation index. **Wu et al** provided possible mechanisms for the effectiveness of narrow-band UVB irradiation in treating vitiligo by investigating the direct and keratinocyte-mediated effects on melanocyte proliferation and migration. They found that narrow-band UVB stimulated the release of bFGF and ET-1 from keratinocytes, leading to melanocyte proliferation. The effect of narrow-band UVB on melanocyte migration seemed to be mediated by the expression of $p125^{FAK}$ and MMP-2 in melanocytes.
Another contribute to the knowledge of Bcl2, Fas and SCF/KIT role in growth and death of melanocytes and melanocyte precursors was provided by **Kimura**. Based on an in vitro study performed by means of DNA gel electrophoresis, immunohistochemistry, electron microscopy, western blotting, and RT-PCR, he demonstrated that SCF/KIT through ERK and RSK promotes cell survival in melanocyte precursors and that the Fas-L expression does not correlate necessarily with the susceptibility to apoptosis. The reciprocal interaction between Bcl2 members and Fas pathway could be relevant in the regulation of cell survival. On the other hand, **von Willebrand** through a simple in vitro model (melanocyte colture on collagen gel and wetern blotting and FACS analyses) demonstrated that pro-apoptotic and anti-apoptotic effects of TGF-□1 and FGF2, respectively, appear to be mediated by a Bcl2-dependent mechanism. On the contrary, the phosphorylation level of the TGF-b1 downstream kinases SMAD is not affected by the presence of FGF2.
Hearing reviewed the mechanisms that regulate the biogenesis of melanin pigments. He well described the current opinions on melanosome functions and its regulation, melanogenic enzymes, melanosomal proteins, transport of melanosomes to surrounding keratinocytes, and pigmentary diseases related to dysfunction of melanocome proteins. **Hirobe** described the role played by keratinocyte-derived factors in the regulation of proliferation and differentiation of epidermal melanocytes. **Herzog** proposed a new and interesting role for APP (amyloid precursor protein because of its involvement in melanosome transfer and melanine release. It could take part in prolifertive process of melanocytes and keratinocytes playing a role in psoriatic dysregulation.

Slominski provides further evidences for an involvement of the seratoninergic/melatoninergic pathway in the protection against oxidative stress in the skin.
The regulation of pigmentation in senescent melanocytes has been examinated by **Schwahn et al.** They observed dramatic changes in the expression of differentiation-related proteins, with absence of MITF and dopachrome tautomerase proteins in senescent melanocytes. Such alterations may result in altered melanin chemistry and increased UV damage in aged skin.
Employing intrisically aged A1 guinea pigs, **Tobiishi and co-workers** provided evidence that the irregular pigmentation observed in this animal model is induced in absence of UV irradiation, through a process that differs from that elicited by UV irradiation. As observed in young animals that received UV irradiation, they showed an increase in the number of melanocytes in the skin of intrisically aged A1 guinea pigs. However, in aged guinea pigs, a clustering of melanocytes was also found, suggesting the involvement of another factor in the accumulation of melanocytes.
A constant wide production of papers on vitiligo underlines the relelvance of the topic whitin the pigmentation disorders and continuous experimental and therpautical upgrading. The surgical and phototherapeutical approaches obtained a consistent attention even for the recent development of new surgical methods (autologous melanocytes, cultured melanocytes and melanocyte-keratinocyte transplantation) and new UV or excimer sources. On the other hand there is a continuous experimentalstudy on the pathogenetic mechanisms. Among these the immunological aspect and the melanocyte susceptibility to apoptotic process are up to now the most investigated. **Lee** reports an increased number of apoptotic keratinocytes in depigmented areas and suggests that a loss of keratinocytes, with the subsequent lower growth factors production, affetcs the melanocytes survival and function. The same author, in another work, suggests the possible molecular mechanism leading to the melanocytes and keratinocytes impairment. He suggests an involvement of the retinoic acid receptors and of the trnslation factor eIF4A1. A separated section of suggested papers is focused on vitiligo.

Suggested papers

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The cutaneous serotoninergic/melatoninergic system: securing a place under the sun. FASEB J 19(2): 176-94, 2005.

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FGF-2 blocks TGF-b1-mediated suppression of Bcl2 in normal melanocytes. Exp Dermatol 14: 202-8, 2005.

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Vitiligo suggested papers

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3. MSH, MCH, other hormones, differentiation

(Dr. R. Morandini)

It is well known that brain melanocortins (MCs), derived from pro-opiomelanocortin (POMC) by enzymatic processing, are involved in learning and memory. It is also well known that stress and brain melanocortins such as α-MSH and ACTH enhance anxiogenic-like behavior in the social interaction test.
In a recent paper (Pharmacol Biochem Behav. 2005 Mar;80(3):395-400), using Male Sprague–Dawley rats as a model, Shimazaki describes a possible role for MC4 receptor and social interaction using Ac-[Nle4,Asp5,D-Phe7,Lys10]alpha-MSH-(4-10)-NH2 (MT II), an MC4 receptor agonist. These data also indicate a sub-chronic blockade of MC4 receptor produces anxiolytic effects. There are five known melanocortin receptors expressed in different tissues. MC-1 receptor is mainly expressed in melanocytes.
In a recent paper (Exp Dermatol. 2005 Feb;14(2):157); the authors show that MC1-R are also expressed on various human fibroblastic skin cells and that alpha-MSH is able to suppress collagen synthesis induced by TGF-beta. Alpha-MSH has an effect on the regulation of tyrosinase and melanogenesis. In the same way, Mallick et al. (Pigment Cell Res. 2005 Feb;18(1):25-33.) shows that in B16F10 melanoma cells, human placental lipid induces melanogenesis by increasing the expression of tyrosinase and its related proteins in vitro.
There are few papers establishing the potentiality of the therapeutic efficacy of alpha-MSH. The last is a paper from Miao (J Nucl Med. 2005 Jan;46(1):121-9) who examined the therapeutic efficacy of (188)Re-(Arg(11))[Cys(3,4,10),d-Phe(7)]alpha-melanocyte-stimulating hormone(3-13) in a human melanoma-bearing mouse model. The results suggest a promising radiolabeled peptide for targeted radionuclide therapy of human melanoma.

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4. Photobiology

(Dr. N. Smit)

The JBC paper by Bohm et al describes the effect of MSH on UV-B induced apoptosis in cultured human melanocytes, keratinocytes and fibroblasts. The culture systems are deprived of brain pituitary extract 3 days before the UVB treatment in order to test the influence of MSH (10-6M). Under these conditions a 6 hours preincubation of melanocytes with MSH will have a strongly influence on the overall stimulation of the cultures and it is not so surprising that UVB induced apoptosis is lower and surival is much better. Nevertheless, it is a very interesting finding that the UVB induced CPD's are reduced for the MSH stimulated cells. Thus, the MSH mediated signal transduction pathway seems to effect nucleotide excision repair. A strong argument for this is the demonstration that NER deficient XPA fibroblasts did not show the improved survival after irradiation. As mentioned by the authors fibroblasts also express receptors for MSH and genotyping of the XPA fibroblasts suggested that normal signalling via MC1R is possible in these XPA cells. Interesting in relation to this, is the idea that allelic variants of MC1R associated with red hair colour phenotype may cause a reduced ability of the epidermis to respond to UVR induced DNA damage (Rouzaud et al). With some further speculation one might even think of a connection between the reduced DNA repair and MC1R variants and the nevus development at young age described in the paper by Bauer et al. These authors investigated 1,232 German children for three years to clarify which factors are important for the development of melanocytic nevi (MN), being the most important risk factor for cutaneous melanoma. In the group of children from 2-7 years of age the number of newly acquired nevi increased with age. Total cumulative sun exposure was found to be the most crucial environmental risk factor for the nevus development next to host factors like fair skin complexion and parents with high nevus counts.
Some other papers describe the possible negative effects of avoiding sunlight resulting in vitamin D3 deficiency. A relative inability to induce skin pigmentation by UVR is reported to be protective for prostate cancer (Wood et al). Possible positive effects of sunlight exposure are also described by Egan et al in the J Natl Canc Inst. They refer to the papers by Berwick et al and Smedby et al in the same issue reporting increased survival rates for those patients with early-stage melanoma who showed signs of solar elastosis (as biomarker of sun exposure). Some interestig data on vitamin D in relation to melanoma in in vitro and epidemiological studies seem consistent with the positive effects. The effects of sunlight on NHL as described by Smedby et al is of particular interest since NHL is suspected to have a common etiology with skin cancer (and melanoma). Interestingly, in the paper by Hu et al current literature is reviewed on this link between NHL and skin cancer and the role of UV light.

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5. Neuromelanins

(Prof. M. d'Ischia)

Highlights of the literature on neuromelanin in the early 2005 include a paper by Fedorow et al. (2005) showing that dolichol is the major lipid component of human substantia nigra neuromelanin, amounting to ca. 14 % of the total neuromelanin mass. Dolichol is an isoprenoid compound synthesized by the same metabolic route as cholesterol and its identification in human neuromelanin may yield new insights into the origin, structure and function of this enigmatic pigment.
Confirmatory evidence about the potential neuroprotective value of free radical scavengers and iron chelating agents in Parkinson's disease comes from a paper by Youdim and coworkers (Mandel et al., 2004) showing that two polyphenolic compounds, R-apomorphine and green tea catechin polyphenol (-)-epigallocatechin-3-gallate, as well as iron chelators, prevent the accumulation of iron and □-synuclein in the Substantia nigra pars compacta of MPTP-treated mice, an animal model of parkinsonism.
A paper by Cintha and Andersen (2005) focused on the developmental factors involved in the generation of dopaminergic neurones in the brain, whereas two reviews by Riederer (2004) and Zucca et al. (2004) summarized the state-of-the-art in the fields of neurodegeneration and neuroprotection and the possible roles of neuromelanin.
Finally, mention goes to two abstracts by Tesema and Franz (2004) and Charkoudian and Franz (2004) dealing with low molecular weight complexes modelling metal-neuromelanin interaction, and to a patent by Nelson (2004) on the use of quinoline ring-containing neuromelanin-binding compounds to increase repiration of melanized neurones in Parkinson's disease and dyskinesias.

Literature search

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  Abstract: Neuromelanin is a dark brown pigment present at high concns. in dopaminergic neurons of the human substantia nigra (SN). Early electron microscopic examns. of neuromelanin fine structure revealed a significant neutral lipid component; however, the identity of this lipid has remained unknown. Here we show that the lipid component of neuromelanin pigment derived from human SN is the polyisoprenoid dolichol. Established methods were used to isolate the pigment from the SN of 32 brains and the lipid fraction was recovered in high purity and yield. Using reversed-phase HPLC, atm. pressure chem. ionization mass spectrometry, and 1H- and 13C-NMR techniques, we showed that the neuromelanin dolichol contained 17-23 isoprenoid units. Dolichol accounted for 14% of the mass of neuromelanin pigment; low levels of other hydrophobic compds. were detected (e.g. ubiquinone-10, □-tocopherol and cholesterol together accounted for < 0.5% of the neuromelanin lipid mass). This is the first time that dolichol has been identified in such a physiol. setting and significantly advances our understanding of neuromelanin pigment structure and biosynthetic pathways. Furthermore, these studies identity a potential novel role for the isoprenoid pathway in the regulation of neuromelanin function and neurodegeneration within the SN.

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Abstract: One of the prominent pathol. features of Parkinson's disease (PD) is the abnormal accumulation of iron in the substantia nigra pars compacta (SNpc), in the reactive microglia, and in assocn. with neuromelanin, within the melanin-contg. dopamine (DA) neurons. Lewy body, the morphol. hallmark of PD, is composed of lipids, redox-active iron, and aggregated □-synuclein, concg. in its peripheral halo and ubiquitinated, hyperphosphorylated, neurofilament proteins. The capacity of free iron to enhance and promote the generation of toxic reactive oxygen radicals has been discussed numerous times. Recent observations, that iron induces aggregation of inert □-synuclein to toxic aggregates, have reinforced the crit. role of iron in oxidative stress-induced pathogenesis of DA neuron degeneration and protein degrdn. via ubiquitination. N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- and 6-hydroxydopamine-induced neurodegeneration in rodents and nonhuman primates is assocd. with increased presence of iron and □-synuclein in the SNpc. The accumulation of iron in MPTP-induced neurodegeneration has been linked to nitric oxide-dependent mechanism, resulting in degrdn. of prominent iron regulatory proteins by ubiquitination. Radical scavengers such as R-apomorphine and green tea catechin polyphenol (-)-epigallocatechin-3-gallate, as well as the recently developed brain-permeable VK-28 series deriv. iron chelators, which are neuroprotective against these neurotoxins in mice and rats, prevent the accumulation of iron and □-synuclein in SNpc. This study supports the notion that a combination of iron chelation and antioxidant therapy, as emphasized on several occasions, might be a significant approach to neuroprotection in PD and other neurodegenerative diseases.

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Abstract: A review. Neuromelanin (NM) accumulates as a function of age in normal human substantia nigra (SN) but is relatively depleted in the SN of patients with Parkinson disease (PD). Several studies have been performed to further our understanding of the role of NM in neuronal aging and neurodegenerative mechanisms of PD. To this purpose, NM from human SN was isolated and its structure and mol. interactions were investigated. Cysteinyl-dopamine was shown to be one precursor of NM synthesis. A striking affinity of NM for specific metals, lipids, drugs and pesticides was found in vitro, and in animal and human brain postmortem studies. Because of these affinities, NM seems to play a protective role in the human brain by blocking toxic mols. On the other hand, expts. in cell culture indicate that NM can activate microglia, eliciting the release of cytotoxic factors that can induce neurodegeneration.

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Patent Family Information

Patent No.		Kind	Date	Application No.		Date
US	2004229908	A1	20041118	US	2003-616692	20030709
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US	2002198231	A1	20021226	US	2002-192414	20020709

Priority Application

US	1999-143767P	P	19990713
US	2000-175051P	P	20000107
US	2000-202140P	P	20000505
US	2000-615639	A2	20000713
US	2002-192414	A2	20020709
US	2003-479748P	P	20030619

Abstract: This invention provides compns. and methods for increasing cellular respiration of melanized catecholamine neurons, and methods for alleviating symptoms or stopping appearance and/or progression of symptoms of Parkinson's disease and related conditions, characterized by nigrostriatal degeneration, as well as drug-induced dyskinesias, tardive dyskinesia, Neuroleptic Malignant Syndrome, and neg. symptoms of schizophrenia. An effective amt. of a neuromelanin-binding compn. having a quinoline ring in a suitable pharmaceutical carrier is administered to patient in need of such treatment. Preferably the compn. comprises (-)-chloroquine diphosphate. Selected adjuvants are also provided as part of the compns. of this invention.

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Synthetic Inorganic Model Complexes of Iron Neuromelanin. Abstracts, 56th Southeast Regional Meeting of the American Chemical Society, Research Triangle Park, NC, United States, November 10-13 (2004). Publisher: American Chemical Society, Washington.
Abstract: Neuromelanin is an insol. biopolymer derived from the oxidn. of dopamine and cysteinyldopamine. Studies have shown that neuromelanin selectively deteriorates during the onset of Parkinson's disease. However, the limited availability of neuromelanin, the difficulty in extg. and purifying the pigment and the lack of an adequate model have resulted in an insufficient understanding of the biopolymer structure and function. In this study, we present methods to synthesize tractable coordination complexes formed between iron and neuromelanin precursor mols. Initial anal. of the phys. properties and reactivity profiles of the model compds. will be presented.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

The recent months saw the publication of two Nature papers (Carreira et al. 2005, by the group of C. Goding; Lang et al. 2005, by the group of J. Epstein) and a Science paper (Nishimura et al. 2005, by the group of D. Fisher), confirming that melanocytes can make it into the "big" journals.

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A Tyrosinase missense mutation causes albinism in the Wistar rat. Pigment Cell Res 18(2):144-145, 2005.

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Identification and cloning of genes displaying elevated expression as a consequence of metastatic progression in human melanoma cells by rapid subtraction hybridization. Gene 343: 191-201, 2004.

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Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. Nature 433(7027):764-769, 2005.
Abstract: The controls that enable melanoblasts and melanoma cells to proliferate are likely to be related, but so far no key regulator of cell cycle progression specific to the melanocyte lineage has been identified. The microphthalmia-associated transcription factor Mitf has a crucial but poorly defined role in melanoblast and melanocyte survival and in differentiation. Here we show that Mitf can act as a novel anti-proliferative transcription factor able to induce a G1 cell-cycle arrest that is dependent on Mitf-mediated activation of the p21(Cip1) (CDKN1A) cyclin-dependent kinase inhibitor gene. Moreover, cooperation between Mitf and the retinoblastoma protein Rb1 potentiates the ability of Mitf to activate transcription. The results indicate that Mitf-mediated activation of p21Cip1 expression and consequent hypophosphorylation of Rb1 will contribute to cell cycle exit and activation of the differentiation programme. The mutation of genes associated with melanoma, such as INK4a or BRAF that would affect either Mitf cooperation with Rb1 or Mitf stability respectively, would impair Mitf-mediated cell cycle control.

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Abstract: The genomic organization of the CDK2 gene, which overlaps the melanocyte-specific gene SILV/PMEL17, poses an interesting regulatory challenge. We show that, despite its ubiquitous expression, CDK2 exhibits tissue-specific regulation by the essential melanocyte lineage transcription factor MITF. In addition, functional studies revealed this regulation to be critical for maintaining CDK2 kinase activity and growth of melanoma cells. Expression levels of MITF and CDK2 are tightly correlated in primary melanoma specimens and predict susceptibility to the CDK2 inhibitor roscovitine. CDK2 depletion suppressed growth and cell cycle progression in melanoma, but not other cancers, corroborating previous results. Collectively, these data indicate that CDK2 activity in melanoma is largely maintained at the transcriptional level by MITF, and unlike other malignancies, it may be a suitable drug target in melanoma.

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Pax3 functions at a nodal point in melanocyte stem cell differentiation. Nature 433(7028):884-887, 2005.
Abstract: Most stem cells are not totipotent. Instead, they are partially committed but remain undifferentiated. Upon appropriate stimulation they are capable of regenerating mature cell types. Little is known about the genetic programmes that maintain the undifferentiated phenotype of lineage-restricted stem cells. Here we describe the molecular details of a nodal point in adult melanocyte stem cell differentiation in which Pax3 simultaneously functions to initiate a melanogenic cascade while acting downstream to prevent terminal differentiation. Pax3 activates expression of Mitf, a transcription factor critical for melanogenesis, while at the same time it competes with Mitf for occupancy of an enhancer required for expression of dopachrome tautomerase, an enzyme that functions in melanin synthesis. Pax3-expressing melanoblasts are thus committed but undifferentiated until Pax3-mediated repression is relieved by activated beta-catenin. Thus, a stem cell transcription factor can both determine cell fate and simultaneously maintain an undifferentiated state, leaving a cell poised to differentiate in response to external stimuli.

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Molecular basis of the extreme dilution mottled mouse mutation: a combination of coding and noncoding genomic alterations. J Biol Chem 280(6):4817-4824, 2005.

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MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. J Cell Biol 168(1):35-40, 2005.
Abstract: Cell cycle exit is required for proper differentiation in most cells and is critical for normal development, tissue homeostasis, and tumor suppression. However, the mechanisms that link cell cycle exit with differentiation remain poorly understood. Here, we show that the master melanocyte differentiation factor, microphthalmia transcription factor (MITF), regulates cell cycle exit by activating the cell cycle inhibitor INK4A, a tumor suppressor that frequently is mutated in melanomas. MITF binds the INK4A promoter, activates p16(Ink4a) mRNA and protein expression, and induces retinoblastoma protein hypophosphorylation, thereby triggering cell cycle arrest. This activation of INK4A was required for efficient melanocyte differentiation. Interestingly, MITF was also required for maintaining INK4A expression in mature melanocytes, creating a selective pressure to escape growth inhibition by inactivating INK4A. These findings demonstrate that INK4A can be regulated by a differentiation factor, establish a mechanistic link between melanocyte differentiation and cell cycle exit, and potentially explain the tissue-specific tendency for INK4A mutations to occur in melanoma.

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Short and highly efficient synthetic promoters for melanoma-specific gene expression. FEBS Lett 579(1):153-156. 2005.

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Sox10 overexpression induces neural crest-like cells from all dorsoventral levels of the neural tube but inhibits differentiation. Dev Dyn, Epub, 2005.

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Abstract: Hair graying is the most obvious sign of aging in humans, yet its mechanism is largely unknown. Here, we used melanocyte-tagged transgenic mice and aging human hair follicles to demonstrate that hair graying is caused by defective self-maintenance of melanocyte stem cells. This process is accelerated dramatically with Bcl2 deficiency, which causes selective apoptosis of melanocyte stem cells, but not of differentiated melanocytes, within the niche at their entry into the dormant state. Furthermore, physiologic aging of melanocyte stem cells was associated with ectopic pigmentation or differentiation within the niche, a process accelerated by mutation of the melanocyte master transcriptional regulator Mitf.

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Genetic disorders of pigmentation. Clin Dermatol 23(1):56-67, 2005.

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Melanocytes derived from patients with Hermansky-Pudlak Syndrome types 1, 2, and 3 have distinct defects in cargo trafficking. J Invest Dermatol 124(2):420-427, 2005.

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Human placental lipid induces melanogenesis through p38 MAPK in B16F10 mouse melanoma. Pigment Cell Res 18(2):113-121, 2005.

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Preservation of eumelanin hair pigmentation in proopiomelanocortin-deficient mice on a nonagouti (a/a) genetic background. Endocrinology 146(3):1245-1253, 2005.

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Interplay between MITF, PIAS3, and STAT3 in mast cells and melanocytes. Mol Cell Biol 24(24):10584-10592, 2004.

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The evolution of genes for pigmentation in African cichlid fishes. Gene 343(2):337-346, 2004.

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FGF-2 blocks TGF-beta1-mediated suppression of Bcl-2 in normal melanocytes. Exp Dermatol 14(3):202-208, 2005.

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Secrets to a healthy Sox life: lessons for melanocytes. Pigment Cell Res 18(2):74-85, 2005.

7. Tyrosinase, TRPs, other enzymes

(Prof. J.C. Garcia-Borron)

- Blaszczyk WM, Arning L, Hoffmann KP, Epplen JT.
A Tyrosinase missense mutation causes albinism in the Wistar rat. Pigment Cell Res. 2005 Apr;18(2):144-5.
Abstract: Tyrosinase serves as a key enzyme in the synthesis of melanin. In humans mutations in the TYR gene are associated with type 1 oculocutaneous albinism (OCA1) that leads to reduced or absent pigmentation of skin, hair and eye. Various mutations causing OCA in man, mouse, rabbit and cattle have been identified throughout the Tyrosinase gene including nonsense, missense, frameshift and splice site alterations. Here we report a missense substitution at codon R299H in exon 2 of the Tyr gene in the albino Wistar rat. As this very exchange has already been described in OCA patients, our findings reinforce the significance of this region for normal catalytic activity of tyrosinase protein.

- Boissy RE, Richmond B, Huizing M, Helip-Wooley A, Zhao Y, Koshoffer A, Gahl WA.
Melanocyte-specific proteins are aberrantly trafficked in melanocytes of Hermansky-Pudlak syndrome-type 3. Am J Pathol. 166(1):231-40, 2005.
Abstract: Hermansky-Pudlak Syndrome-type 3 (HPS-3) is a relatively mild subtype of HPS with minimal cutaneous and ocular depigmentation. The HPS-3 gene encodes a novel protein of unknown function with a predicted molecular weight of 114 kd. To assess the role of the HPS3 protein in melanization, cultured melanocytes developed from HPS-3 patients were evaluated biochemically and histologically for activity and localization of melanocyte-specific proteins. Endogenous tyrosinase activity of HPS-3 melanocytes was substantial, but tyrosinase activity and melanin synthesis was suppressed in intact melanocytes. However, the level of suppression, as well as extent to which up-regulation by isobutylmethylxanthine and cholera toxin was muted, was less that in HPS-1 melanocytes. Ultrastructurally, HPS-3 melanocytes contained morphologically normal melanosomes, predominantly of stage I and II with minimal stage III and few stage IV melanosomes. Dihydroxyphenylalanine (DOPA) histochemistry demonstrated an increase in melanization of melanosomes. Unique to HPS-3 melanocytes were numerous DOPA-positive 50-nm vesicles and tubular elements present throughout the cell body and dendrites. Tyrosinase, tyrosinase-related protein-1 (Tyrp1), dopachrome tautomerase (Dct), and LAMP1 and 3 localization in HPS-3 melanocytes, as evaluated by immunocytochemistry and confocal microscopy, demonstrated a fine, floccular distribution in contrast to the coarse, granular distribution characteristic of control melanocytes. The localization profile of other proteins expressed by melanocytes (ie, Silver/Pmel17, Melan-A/MART-1, LAMP2, Rab 27, transferrin, c-kit, adaptin-3, and the HPS1 protein) appeared normal. These results suggest that a specific subset of melanocyte proteins are aberrantly trafficked throughout the HPS-3 melanocyte and may be responsible for the reduction in melanin synthesis.

- da Silva GF, Tay WM, Ming LJ.
Catechol oxidase-like oxidation chemistry of the 1-20 and 1-16 fragments of Alzheimer's disease-related beta -amyloid peptide: Their structure-activity correlation and the fate of hydrogen peroxide. J Biol Chem. 2005 Feb 7; [Epub ahead of print]
Abstract: The Cu(+2) complexes of the 1-16 and the 1-20 fragments of the Alzheimer's disease-related ss-amyloid peptide (CuAss) show significant oxidative activities toward a catechol-like substrate trihydroxylbenzene and plasmid DNA cleavage. The latter reflects possible oxidative stress to biological macromolecules, yielding supporting data to the pathological role of these soluble Ass fragments. The former exhibits enzyme-like kinetics and is dependent on [H(2)O(2)], exhibiting k(cat) of 0.066 s(-1) (6000 folds higher than the reaction without CuAss) and k(cat)/K(m) of 37.2 M(-1)s(-1) under saturating [H(2)O(2)] of ~0.24%. This kinetic profile is consistent with metal-centered redox chemistry for the action of CuAss. A mechanism is proposed by the use of the catalytic cycle of dinuclear catechol oxidase as a working model. Trihydroxylbenzene is also oxidized by CuAss aerobically without H(2)O(2), affording rate constants of 6.50 x 10(-3)s(-1) and 3.25 M(-1)s(-1). This activity is also consistent with catechol oxidase action in the absence of H(2)O(2), wherein the substrate binds and reduces the Cu(2+) center first, followed by O(2) binding to afford the micro-(2):(2)-peroxo intermediate which oxidizes a second substrate to complete the catalytic cycle. A tetragonally distorted octahedral metal coordination sphere with three coordinated His side chains and some specific H-bonding interactions is concluded from the electronic spectrum of CuAss, hyperfine-shifted (1)H NMR spectrum of CoAss, and molecular mechanics calculations. The results presented here are expected to add further insight into the chemistry of metallo-Ass which may assist better understanding of the neuropathology of Alzheimer's disease.

- Fujiwara Y, Sahashi Y, Aritro M, Hasegawa S, Akimoto K, Ninomiya S, Sakaguchi Y, Seyama Y.
Effect of simultaneous administration of vitamin C, L-cysteine and vitamin E on the melanogenesis. Biofactors. 21(1-4):415-8, 2004.
Abstract: The effect of simultaneous administration of vitamin C (ascorbic acid), L-cystein (Cys) and vitamin E (tocopherol) on the melanogenesis in vivo and in vitro was studied. Forty-eight brownish guinea pigs were divided into 4 groups as follows: VC group, VC+Cys group, VC+Cys+VE group and control group. They were given these vitamins by oral administration every day. UV-B exposure (0.384 J/cm2) on their depleted back skin was

done at the day 8, 10, 12, 15 17 and 19. After UV-B irradiation, vitamins were administrated further 3 weeks. The luminosity score was measured using a Color Reader CR-11 (Minolta, Co) and the numbers of DOPA-positive melanocytes of their back skin were counted. B16 melanoma cells were incubated with VC, N-acetyl cystein (NAC) and VE. After 4 days of incubation, cells were harvested. The melanin contents and the tyrosinase activities in cells were measured. The luminosity score in the VC+VE+Cys group was higher than those in the other groups. The numbers of DOPA-positive melanocytes of guinea pigs treated with VC, VE and Cys were significantly decreased compared with those in VC group. In B16 melanoma cells, simultaneous treatment of VC, VE and NAC was the most effective to decrease the melanin contents and to inhibit tyrosinase activity.

- Graells J, Vinyals A, Figueras A, Llorens A, Moreno A, Marcoval J, Gonzalez FJ, Fabra A.
Overproduction of VEGF concomitantly expressed with its receptors promotes growth and survival of melanoma cells through MAPK and PI3K signaling. J Invest Dermatol. 123(6):1151-61, 2004.
Abstract: Vascular endothelial growth factor (VEGF) is an important mediator of tumor-associated angiogenesis, and consequently it has been associated with metastasis. We report here that the overexpression of VEGF(165) in melanoma xenografts promotes an acceleration of tumor growth and an increase in angiogenesis as well as the spontaneous metastasis formation. In addition, VEGF receptors (VEGFR)1, VEGFR2 and neurophilin-1 are expressed in A375 melanoma cells. Forced overexpression of VEGF in these cells induces cell growth and triggers survival activity in serum-starved cultures, by a mechanism dependent on the mitogen-activating protein kinase signaling pathway. Furthermore, these effects are dependent MEK 1/2 activity. Kinase domain region-specific tyrosine kinase inhibitors dramatically reduced DNA synthesis to 20% with respect to the controls, although they did not completely suppress either the p44 or p42-phosphorylated forms of extracellular signal-regulated protein kinase. These inhibitors also provoked a decrease in Akt phosphorylation. We observed a dramatic reduction in survival after treatment with phosphatidylinositol 3'-kinase (PI3K)-specific inhibitor in the presence of specific tyrosinase inhibitors. We suggest that the overproduction of VEGF(165) concomitantly expressed with its receptors favors cell growth and survival of melanoma cells through MAPK and PI3K signaling pathways. These data support the involvement in melanoma growth and survival of a VEGF-dependent internal autocrine loop mechanism, at least in vitro.

- Greggio E, Bergantino E, Carter D, Ahmad R, Costin GE, Hearing VJ, Clarimon J,Singleton A, Eerola J, Hellstrom O, Tienari PJ, Miller DW, Beilina A, Bubacco L, Cookson MR.
Tyrosinase exacerbates dopamine toxicity but is not genetically associated with Parkinson's disease. J Neurochem. 93(1):246-56, 2005.
Abstract: Tyrosinase is a key enzyme in the synthesis of melanin in skin and hair and has also been proposed to contribute to the formation of neuromelanin (NM). The presence of NM, which is biochemically similar to melanin in peripheral tissues, identifies groups of neurons susceptible in Parkinson's disease (PD). Whether tyrosinase is beneficial or detrimental to neurons is unclear; whilst the enzyme activity of tyrosinase generates dopamine-quinones and other oxidizing compounds, NM may form a sink for such radical species. In the present study, we demonstrated that tyrosinase is expressed at low levels in the human brain. We found that mRNA, protein and enzyme activity are all present but at barely detectable levels. In cell culture systems, expression of tyrosinase increases neuronal susceptibility to oxidizing conditions, including dopamine itself. We related these in vitro observations to the human disease by assessing whether there was any genetic association between the gene encoding tyrosinase and idiopathic PD. We found neither genotypic or haplotypic association with three polymorphic markers of the gene. This argues against a strong genetic association between tyrosinase and PD, although the observed contribution to cellular toxicity suggests that a biochemical association is likely.

- Hachiya A, Kobayashi A, Yoshida Y, Kitahara T, Takema Y, Imokawa G.
Biphasic expression of two paracrine melanogenic cytokines, stem cell factor and endothelin-1, in ultraviolet B-induced human melanogenesis. Am J Pathol. 165(6):2099-109, 2004.
Abstract: Stem cell factor (SCF) and endothelin-1 (ET-1) have been reported to be up-regulated at the protein and gene levels in human epidermis after ultraviolet B (UVB) irradiation and to play central roles in UVB-induced pigmentation. However, little is known about the time sequence of SCF and ET-1 expression in UVB-exposed human epidermis and the coordination of their roles during epidermal pigmentation. To clarify such parameters in UVB-exposed human skin, we measured the expression patterns of SCF and ET-1 (as well as of their corresponding receptors) at the gene level at various times during UVB-induced human pigmentation. When human forearm skin was exposed to UVB radiation at two minimal erythemal doses, the expression of SCF mRNA transcripts was significantly enhanced at 3 days after irradiation with an early decrease and subsequently constant expression of SCF receptor (c-KIT) mRNA transcripts. In contrast, up-regulation of ET-1 and endothelin B receptor (ET(B)R) mRNA expression was synchronized at 5 to 10 days after irradiation in concert with an increased expression of tyrosinase mRNA transcripts and the increase in pigmentation. In parallel the expression of tyrosinase and ET(B)R proteins as well as ET-1 was up-regulated at 7 to 10 days after irradiation, whereas KIT protein decreased at 3 days after irradiation and returned to the nonirradiated control level at 5 days after irradiation. When cultured human melanocytes were treated with human recombinant SCF, ET(B)R protein expression and the binding of (125)I-labeled ET-1 to the ET(B)R were significantly increased, further suggesting the preferential and coordinated role of early expression of SCF in UVB-induced melanogenesis. These findings

suggest that SCF/KIT signaling is predominantly involved in the early phase of UVB-induced human pigmentation during which it stimulates the ET-1/ET(B)R linkage that is associated with the later phase of UVB-induced melanogenesis.

- Hall AM, Orlow SJ.
Degradation of tyrosinase induced by phenylthiourea occurs following Golgi maturation. Pigment Cell Res. 2005 Apr;18(2):122-9.
Abstract: Tyrosinase, the rate-limiting enzyme of melanin synthesis, is a di-copper metalloprotein that catalyzes the conversion of l-tyrosine to l-DOPAquinone. Phenylthiourea (PTU) is a well-known inhibitor of tyrosinase and melanin synthesis and is known to interact with sweet potato catechol oxidase, an enzyme possessing copper binding domain homology to tyrosinase. While PTU is frequently used to induce hypopigmentation in biological systems, little is known about its effects on tyrosinase and other melanogenic proteins. We have found that PTU induces degradation of tyrosinase but not of other melanogenic proteins including the tyrosinase-related metalloproteins tyrosinase-related protein (Tyrp)1 and Tyrp2. Using pulse-chase analysis coupled with glycosidase digestion, we observed that tyrosinase degradation occurs following complete maturation of the protein and that degradation was reversed by cysteine protease inhibitor E64 but not proteasome inhibitor N-acetyl-l-leucinyl-l-leucinyl-l-norleucinal. We conclude that PTU specifically induces tyrosinse degradation following Golgi maturation. Our data suggest that in addition to well-known ER-directed quality control, tyrosinase is also subject to post-Golgi quality control.

- Hirobe T.
Role of keratinocyte-derived factors involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes. Pigment Cell Res. 2005 Feb;18(1):2-12.
Abstract: Melanocytes characterized by the activities of tyrosinase, tyrosinase-related protein (TRP)-1 and TRP-2 as well as by melanosomes and dendrites are located mainly in the epidermis, dermis and hair bulb of the mammalian skin. Melanocytes differentiate from melanoblasts, undifferentiated precursors, derived from embryonic neural crest cells. Because hair bulb melanocytes are derived from epidermal melanoblasts and melanocytes, the mechanism of the regulation of the proliferation and differentiation of epidermal melanocytes should be clarified. The regulation by the tissue environment, especially by keratinocytes is indispensable in addition to the regulation by genetic factors in melanocytes. Recent advances in the techniques of tissue culture and biochemistry have enabled us to clarify factors derived from keratinocytes.
Alpha-melanocyte-stimulating hormone, adrenocorticotrophic hormone, basic fibroblast growth factor, nerve growth factor, endothelins, granulocyte-macrophage colony-stimulating factor, steel factor, leukaemia inhibitory factor and hepatocyte growth factor have been suggested to be the keratinocyte-derived factors and to regulate the proliferation and/or differentiation of mammalian epidermal melanocytes. Numerous factors may be produced in and released from keratinocytes and be involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes through receptor-mediated signaling pathways.

- Hoashi T, Watabe H, Muller J, Yamaguchi Y, Vieira WD, Hearing VJ.
Mart-1 is required for the function of melanosomal matrix protein PMEL17/GP100 and the maturation of melanosomes. J Biol Chem. 2005 Jan 28; [Epub ahead of print]
Abstract: More than 125 genes that regulate pigmentation have been identified to date. Of those, MART-1 has been widely studied as a melanoma-specific antigen and as a melanosome-specific marker. While the functions of other melanosomal proteins, such as tyrosinase, tyrosinase related protein-1, dopachrome tautomerase and Pmel17, are known, the function of MART-1 in melanogenesis, is unclear. A role for MART-1 in pigmentation is expected since its expression pattern and subcellular distribution is quite similar to the other melanosomal proteins and usually correlates with melanin content. We investigated the function of MART-1 using a multidisciplinary approach, including the use of siRNA to inhibit MART-1function and the use of transfection to re-express MART-1 in MART-1 negative cells. We show that MART-1 forms a complex with Pmel17 and affects its expression, stability, trafficking and the processing which is required for melanosome structure and maturation. We conclude that MART-1 is indispensable for Pmel17 function and thus plays an important role in regulating mammalian pigmentation.

- Lavado A, Olivares C, Garcia-Borron JC, Montoliu L.
Molecular basis of the extreme dilution mottled mouse mutation: a combination of coding and noncoding genomic alterations. J Biol Chem. 280(6):4817-24, 2005. Epub 2004 Nov 30.
Abstract: Tyrosinase is the rate-limiting enzyme in melanin biosynthesis. It is an N-glycosylated, copper-containing transmembrane protein, whose post-translational processing involves intracytoplasmic movement from the endoplasmic reticulum to the Golgi and, eventually, to the melanosome. The expression of the tyrosinase (Tyr) gene is controlled by several regulatory regions including a locus control region (LCR) located 15 kb upstream from the promoter region. The extreme dilution mottled mutant mice (Tyrc-em) arose spontaneously at the MRC Institute in Harwell (United Kingdom) from a chinchilla-mottled mutant (Tyrc-m) stock, whose molecular basis corresponds to a rearrangement of 5'-upstream regulatory sequences including the LCR of the Tyr gene. Tyrc-em mice display a variegated pigmentation pattern in coat and eyes, in agreement with the LCR translocation, but also

show a generalized hypopigmented phenotype, not seen in Tyrc-m mice. Genomic analyses of Tyrc-em mice showed a C1220T nucleotide substitution within the Tyr encoding region, resulting in a T373I amino acid change, which abolishes an N-glycosylation sequon located in the second metal ion binding site of the enzyme. Tyrosinase from Tyrc-em displayed a reduced enzymatic activity in vivo and in vitro, compared with wild-type enzyme. Deglycosylation studies showed that the mutant protein has an abnormal glycosylation pattern and is partially retained in the endoplasmic reticulum. We conclude that the phenotype of the extreme dilution mottled mouse mutant is caused by a combination of coding and noncoding genomic alterations resulting in several abnormalities that include suboptimal gene expression, abnormal protein processing, and reduced enzymatic activity.

- Lee J, Jung E, Park J, Jung K, Park E, Kim J, Hong S, Park J, Park S, Lee S, Park D.
Glycyrrhizin induces melanogenesis by elevating a cAMP level in b16 melanoma cells. J Invest Dermatol. 124(2):405-11, 2005.
Abstract: In mammalian melanocytes, melanin synthesis is controlled by tyrosinase, the critical enzyme in the melanogenic pathway. A recent report showed that the stimulation of melanogenesis by glycyrrhizin (GR) is because of an increased tyrosinase expression at mRNA and protein levels. But, the molecular events of melanogenesis induced by GR remain to be elucidated. In this study, using B16 melanoma cells, we showed that GR activated activator protein-1 (AP-1) and cyclic response filament "CRE" promoters, but not the nuclear factor-kappaB promoter. In addition, although GR stimulated mitogen-activated protein (MAP)
kinase, p42/44(mapk), consistent with GR-induced AP-1 promoter activation, GR-induced melanogenesis was not blocked by PD98059, an MEK1 inhibitor, suggesting that MAPkinase induced by GR does not have a direct effect on the level of melanin content. But, GR-induced melanogenesis was inhibited by an inhibitor of protein kinase A (H-89). This result was further confirmed by the fact that GR induced the phosphorylation of CRE binding protein (CREB) and inhibition of glycogen synthase kinase 3beta phosphorylation as well as the production of cAMP, indicating that GR induces melanogenesis through cAMP signaling. In addition, the fact that GR-induced CRE activation was blocked by H-89 but GR-induced increase of cAMP production was not suggests that GR operates upstream of protein kinase A.

- Lyons LA, Imes DL, Rah HC, Grahn RA.
Tyrosinase mutations associated with Siamese and Burmese patterns in the domestic cat (Felis catus). Anim Genet. 36(2):119-26, 2005.
Abstract: The Siamese cat has a highly recognized coat colour phenotype that expresses pigment at the extremities of the body, such as the ears, tail and paws. This temperature-sensitive colouration causes a 'mask' on the face and the phenotype is commonly referred to as 'pointed'. Burmese is an allelic variant that is less temperature-sensitive, producing more pigment throughout the torso than Siamese. Tyrosinase (TYR) mutations have been suspected to cause these phenotypes because mutations in TYR are associated with similar phenotypes in other species. Linkage and synteny mapping in the cat has indirectly supported TYR as the causative gene for these feline phenotypes. TYR mutations associated with Siamese and Burmese phenotypes are described herein. Over 200 cats were analysed, representing 12 breeds as well as randomly bred cats. The SNP associated with the Siamese phenotype is an exon 2 G > A transition changing glycine to arginine (G302R). The SNP associated with the Burmese phenotype is an exon 1 G > T transversion changing glycine to tryptophan (G227W). The G302R mutation segregated concordantly within a pedigree of Himalayan (pointed) Persians. All cats that had 'pointed' or the Burmese coat colour phenotype were homozygous for the corresponding mutations, respectively, suggesting that these phenotypes are a result of the identified mutations or unidentified mutations that are in linkage disequilibrium. Because the same mutations were identified in different breeds with similar phenotypes, the mutations are likely to be identical by descent rather than multiple mutation events occurring at the same site.

- Mallick S, Singh SK, Sarkar C, Saha B, Bhadra R.
Human placental lipid induces melanogenesis by increasing the expression of tyrosinase and its related proteins in vitro. Pigment Cell Res. 18(1):25-33, 2005.
Abstract: Lipids, particularly sphingolipids, are emerging as novel regulators of cellular activity. A placental total lipid fraction (PTLF), the total lipid prepared from an hydroalcoholic extract of fresh term human placenta, was previously shown to have a pigment-inducing activity in an animal model. The PTLF contains sphingolipids which stimulate DNA synthesis and melanin formation with marked morphological changes in B16F10 melanoma cells. In order to identify the mechanism underlying the increased melanin synthesis, B16F10 cells were treated with PTLF to assess the catalytic activities of tyrosinase (i.e. tyrosine hydroxylase and DOPA oxidase), the key regulatory enzyme of melanin synthesis. Tyrosine hydroxylase (estimated by the release of (3)H(2)O) as well as DOPA oxidase (measured spectrophotometrically and also in non-denaturing gels), was stimulated significantly by PTLF. Western blot analysis demonstrated an increase in the expression of tyrosinase, tyrosinase related proteins 1 and 2 (TRP1 and TRP2) at the protein level and RT-PCR analysis revealed stimulated transcription of tyrosinase, TRP1 and TRP2 mRNAs in PTLF-treated B16F10 cells. Actinomycin D and cycloheximide, inhibitors of transcription and translation, respectively, inhibited PTLF-induction of tyrosinase activity with a corresponding decrease in melanogenesis. In all cases, the response to PTLF was similar to that induced by alpha-melanocyte stimulating hormone, a well-known stimulator of melanogenesis. Thus, these results provide the basis of action of

PTLF stimulated melanogenesis in B16F10 cells showing that this placental extract is a strong inducer of pigmentation at the transcriptional and translational levels.

- Masuda T, Yamashita D, Takeda Y, Yonemori S.
Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from Garcinia subelliptica. Biosci Biotechnol Biochem. 69(1):197-201, 2005.
Abstract: The tyrosinase inhibitory activity of methanol extracts of the leaves of 39 plant species growing on the seashore of Iriomote island (Okinawa, Japan) was investigated. The extracts of Hibiscus tiliaceus, Carex pumila, and Garcinia subelliptica showed potent activity among them. The inhibitors in the extract of Garcinia subelliptica were purified by assay-guided fractionation to give two biflavonoids. These were known compounds (2R,3S-5,7,4',5",7",3"',4"'-heptahydroxy flavanone[3-8"] flavone and 5,7,4',5",7",3"',4"'-heptahydroxy[3-8"] biflavanone), although their strong inhibitory activity toward tyrosinase is revealed for the first time in this work. One of these biflavonoids (2R,3S-5,7,4',5",7",3"',4"'-heptahydroxy flavanone[3-8"] flavone) showed much stronger activity (IC50 2.5 microM) than that of kojic acid (IC50 9.1 microM) when L-tyrosine was used as the substrate.

- Merkel M, Moller N, Piacenza M, Grimme S, Rompel A, Krebs B.
Less symmetrical dicopper(II) complexes as catechol oxidase models--an adjacent thioether group increases catecholase activity. Chemistry. 11(4):1201-9, 2005.
Abstract: Three new unsymmetrical compartmental dinucleating ligands, 4-bromo-2-(4-methylpiperazin-1-ylmethyl)-6-[{2-(1-piperidyl)ethyl}aminomethyl]phenol (HL1), 4-bromo-2-(4-methylpiperazin-1-ylmethyl)-6-[{2-(morpholin-4-yl)ethyl}aminomethyl]phenol (HL2), and 4-bromo-2-(4-methylpiperazin-1-ylmethyl)-6-[{2-(thiomorpholin-4-yl)ethyl}aminome thyl]phenol (HL3), have been synthesized in order to model the active site of type 3 copper proteins. The dicopper(II) complexes of these ligands give first hints about the influence of a thioether group close to the metal site. The bromophenol-based ligands have one piperazine arm and one other bidentate arm in positions 2 and 6 of the phenolic ring, respectively. With each ligand a dinuclear copper(II) complex was prepared and structurally characterized. The copper ions were found to have square pyramidal environments and a mixture of endogenous phenoxo and exogenous acetate bridging. The influence of a heteroatom in one arm of the ligand on catecholase activity and speciation in solution was studied by UV/Vis spectroscopy, ESI-MS experiments and, DFT calculations.

- Negroiu G, Dwek RA, Petrescu SM.
Tyrosinase-related protein-2 and -1 are trafficked on distinct routes in B16 melanoma cells. Biochem Biophys Res Commun. 328(4):914-21, 2005.
Abstract: Tyrosinase related protein (TRP)-1 and -2 regulate the main steps in melanin synthesis and are immune targets in skin cancer or autoimmune pigmentary disorders. We found that ionophore monensin (Mon) and the quaternary amine chloroquine (CQ) discriminate between the traffic routes of TRP-2 and TRP-1. TRP-2 N-glycan processing is interrupted by Mon between ER and trans-Golgi, whereas this process continues for TRP-1. Mature TRP-2 is diverted by CQ treatment to a degradation pathway which depends on functional vacuolar ATPases. Conversely, the subcellular distribution and stability of TRP-1 were not affected by CQ. We propose that TRP-2 is sorted and trafficked in the early secretory pathway with a cargo which does not include TRP-1; post Golgi, TRP-2 intersects the endocytic pathway following a route via early endosomes, possibly by rapid recycling from the plasma membrane. These data show that highly structural homologous glycoproteins use distinct trafficking pathways in the same cell.

- Oetting WS, Garrett SS, Brott M, King RA.
P gene mutations associated with oculocutaneous albinism type II (OCA2). Hum Mutat. 25(3):323, 2005.
Abstract: Oculocutaneous albinism type II (OCA2) is the most common form of albinism in humans. OCA2 has been previously associated with mutations of the P gene, the human homologue to the murine pink-eyed dilution gene. The P gene encodes a 110 kDa protein containing 12 potential membrane spanning domains and is associated with melanosomal membranes. The specific function of the P protein is currently unknown but is thought to be involved in tyrosinase processing and transport. We report nine novel mutations in the P gene associated with OCA2. These include two missense mutations, c.1938A>C (p.Ile646Val) and c.1556T>C (p.Val519Ala); one nonsense mutation c.612G>A (p.Trp204X); five frameshift mutations: c.2372underscore;2373delTC, c.1555delG, c.1938underscore;1939insC, c.2050delT, and c.1045underscore;1046delAT; and a splice site mutation c.1951+1G>A. We also report 12 novel polymorphisms including one amino acid substitution, c.2365underscore;2366GC>CA (p.Ala789Glu). At present, there is no functional assay to determine if a mutation is truly pathogenic. The presence of numerous polymorphisms of the P gene in the coding region, several of which result in amino acid substitutions, makes molecular diagnosis problematic. To ensure accurate molecular diagnosis, further mutational analysis will be necessary to produce a comprehensive list of mutations associated with OCA2. This information will also help define the critical functional domains of the P protein. Mutations associated with OCA2 can be found in the Albinism Database (http://albinismdb.med.umn.edu).

- Ostankovitch M, Robila V, Engelhard VH.

Regulated folding of tyrosinase in the endoplasmic reticulum demonstrates that misfolded full-length proteins are efficient substrates for class I processing and presentation. J Immunol. 174(5):2544-51, 2005.
Abstract: Short-lived protein translation products have been proposed to be the principal substrates that enter the class I MHC processing and presentation pathway. However, the biochemical nature of these substrates is poorly defined. Whether the major processing substrates are misfolded full-length proteins, or alternatively, aberrantly initiated or truncated polypeptides still remains to be addressed. To examine this, we used melanoma in which one-third of wild-type tyrosinase molecules were correctly folded and localized beyond the Golgi, while the remainder were present in the endoplasmic reticulum in an unfolded/misfolded state. Increasing the efficiency of tyrosinase folding using chemical chaperones led to a reduction in the level of substrate available to the proteasome and decreased the expression of a tyrosinase-derived epitope. Conversely, in transfectants expressing tyrosinase mutants that are completely misfolded, both proteasome substrate and epitope presentation were significantly enhanced. Proteasome substrate availability was a consequence of misfolding and not simply due to retention in the endoplasmic reticulum. Thus, the extent of folding/misfolding of a full-length protein is an important determinant of the level of epitope presentation.

- Popescu CI, Paduraru C, Dwek RA, Petrescu SM.
Soluble tyrosinase is an ER- associated degradation substrate retained in the ER by calreticulin and BIP and not calnexin. J Biol Chem. 2005 Jan 27; [Epub ahead of print]
Abstract: Tyrosinase is a type I membrane protein regulating the pigmentation process in humans. Mutations of the human tyrosinase gene cause the tyrosinase negative type I oculocutaneous albinism (OCAI). Some OCA I mutations were shown to delete the transmembrane domain or to affect its hydrophobic properties resulting in soluble tyrosinase mutants that are retained in the endoplasmic reticulum(ER). To understand the specific mechanisms involved in the ER retention of soluble tyrosinase we have constructed a tyrosinase mutant truncated at its C-terminal end and investigated its maturation process. The mutant is retained in the ER and it is degraded through the proteasomal pathway. We determined that the mannose trimming and lectin interactions are required for an efficient degradation process. Moreover, this soluble ER- associated degradation (ERAD) substrate is stopped at the ER quality control check point with no requirements for an ER-Golgi recycling pathway. Co-immmunoprecipitation experiments show that soluble tyrosinase interacts with calreticulin and BiP and not calnexin during its ER transit. Expression of soluble tyrosinase in calreticulin deficient cells results in the export of soluble tyrosinase out of the ER, indicating the calreticulin role in ER retention. Taken together these data show that OCAI soluble tyrosinase is an ERAD substrate that unlike other albino tyrosinases associates with calreticulin and BiP. The lack of specificity for calnexin interaction reveals a novel role for calreticulin in OCA I albinism.

- Richmond B, Huizing M, Knapp J, Koshoffer A, Zhao Y, Gahl WA, Boissy RE.
Melanocytes derived from patients with Hermansky-Pudlak Syndrome types 1, 2, and 3 have distinct defects in cargo trafficking. J Invest Dermatol. 124(2):420-7, 2005.
Abstract: Hermansky-Pudlak Syndrome (HPS) is a genetically heterogeneous disorder in which mutations in one of several genes interrupts biogenesis of melanosomes, platelet dense bodies, and lysosomes. Affected patients have oculocutaneous albinism, a bleeding diathesis, and sometimes develop granulomatous colitis or pulmonary fibrosis. In order to assess the role of HPS genes in melanosome biogenesis, melanocytes cultured from patients with HPS subtypes 1, 2, or 3 were assessed for the localization of various melanocyte proteins. Tyrosinase, Tyrp1, and Dct/Tyrp2 were atypically and distinctly expressed in HPS-1 and HPS-3 melanocytes, whereas only tyrosinase showed an atypical distribution in HPS-2 melanocytes. The HPS1 and AP3B1 (i.e., HPS-2) gene products showed no expression in HPS-1 and HPS-2 melanocytes, respectively, whereas HPS-3 melanocytes exhibited normal expression for both proteins. In normal human melanocytes, the HPS1 protein was expressed as an approximately 80 kDa molecule with both granular and reticular intracellular profiles. In HPS-1, lysosome associated membrane protein 1 (LAMP1), and LAMP3 were localized to abnormal large granules; in HPS-2, all LAMPs exhibited a normal granular expression; and in HPS-3, LAMP1, and LAMP3 exhibited a distinct less granular and more floccular pattern. In contrast, the expressions of Rab 27, transferrin, and cKit were unaffected in all three HPS genotypes. These data demonstrate that the three initially identified subtypes of human HPS exhibit distinct defects in the trafficking of various melanocyte-specific proteins.

- Rotolo S, Diotti R, Gordon RE, Qiao RF, Yao Z, Phelps RG, Dong J.
Effects on proliferation and melanogenesis by inhibition of mutant BRAF and expression of wild-type INK4A in melanoma cells. Int J Cancer. 2005 Jan 18; [Epub ahead of print]
Abstract: Activating BRAF mutations and loss of wild-type INK4A expression both occur at high frequencies in melanomas. Here, we present evidence that BRAF and INK4A have different effects on melanogenesis, a marker of melanocytic differentiation. Human melanoma cell line 624Mel harbors mutations in both BRAF and INK4A. The in vitro and in vivo growth of these cells was inhibited by either reduced expression of mutant BRAF using stable retroviral RNA interference (RNAi) or retrovirus-mediated stable expression of wild-type INK4A cDNA. Consistent with the observed growth inhibition, phosphorylation of S780 and S795 in pRB, both CDK4/6 targets, was suppressed in cells expressing either mutant BRAF RNAi or wild-type INK4A. Interestingly, melanoma cells expressing mutant BRAF RNAi had increased pigmentation, produced more mature melanosomes and melanin and expressed higher levels of tyrosinase and tyrosinase-related protein-1, whereas melanogenesis was not induced

by wild-type INK4A. We found that the melanocyte lineage-specific master control protein microphthalmia-associated transcription factor was upregulated by inhibition of mutant BRAF, which may be the cause for the melanogenic effect of BRAF RNAi. The results suggest that, although both BRAF and INK4A lesions promote cell growth and tumor formation, mutant BRAF may also induce dedifferentiation in melanoma cells.

- Singh SK, Sarkar C, Mallick S, Saha B, Bera R, Bhadra R.
Human placental lipid induces melanogenesis through p38 MAPK in B16F10 mouse melanoma. Pigment Cell Res. 18(2):113-21, 2005.
Abstract: Melanogenesis is one of the characteristic functional activities of melanocyte/melanoma and is regulated via mitogen-activated protein kinase (MAPK) and Akt/protein kinase B (PKB) pathways. Placental total lipid fraction (PTLF), prepared from a hydroalcoholic extract of fresh term human placenta contains sphingolipids and was recently shown to stimulate melanogenesis via up-regulation of the key enzyme tyrosinase in B16F10 mouse melanoma cells. How such lipids mediate their effects on pigmentation and tyrosinase expression is a particularly important aspect of melanogenesis. To study the signaling that leads to tyrosinase expression, we have investigated the roles of the MAPK and Akt/PKB pathways in B16F10 melanoma cells in melanogenesis in response to PTLF. Treatment of cells with PTLF led to the time dependent phosphorylation of p38 MAPK. SB203580, a p38 MAPK inhibitor, completely blocked the PTLF-induced melanogenesis by inhibiting promoter activity and subsequent expression of tyrosinase. Phosphatidylinositol 3-kinase (PI3K) inhibitor, LY294002 a blocker of the Akt signaling pathway, or an inhibitor of MEK (MAPK/ERK Kinase), PD98059 when included along with PTLF was found to potentiate PTLF-induced phosphorylation of p38 MAPK together with tyrosinase expression and melanogenesis. The results suggest that the activation of p38 MAPK plays a crucial role in PTLF-induced B16F10 melanogenesis by up-regulating tyrosinase expression.

- Tatzel J, Poser I, Schroeder J, Bosserhoff AK.
Inhibition of melanoma inhibitory activity (MIA) expression in melanoma cells leads to molecular and phenotypic changes. Pigment Cell Res. 18(2):92-101, 2005.
Abstract: The secreted protein melanoma inhibitory activity (MIA) is highly expressed in malignant melanoma but not in melanocytes and is associated with tumor progression in vivo. Here, we further investigated the functional role of MIA by inhibiting MIA expression of the human melanoma cell line HMB2 via stable antisense MIA cDNA transfection, and subsequent analysis of the cell clones. MIA-deficient cell clones showed several changes in cell morphology and growth pattern. In monolayer and three-dimensional culture enhanced cell-cell contacts were formed. Furthermore, a re-induction of pigment synthesis in comparison with the amelanotic parental cell line HMB2 was observed. Molecular analyses revealed a re-expression of tyrosinase-related protein 1 (Trp-1) and tyrosinase in the MIA-deficient cell clones necessary for melanin synthesis. In accordance, re-expression of MIA in the MIA-deficient melanoma cell clones resulted in downregulation of Trp-1. To identify the molecular mechanisms of MIA regulating pigmentation, MITF and PAX3, two positive regulators of Trp-1 and tyrosinase transcription, and PIAS3, a negative regulator of MITF activity, were analyzed. Only in MIA-deficient cells, expression of PAX3 mRNA and MITF protein was found. In contrast, strong expression of PIAS3 was detected in HMB2 but not in the MIA-deficient cells. To our knowledge this is the first report demonstrating a correlation between MIA expression and pigmentation and morphology of melanocytic cells.

- Tepper AW, Bubacco L, Canters GW.
Interaction between the type-3 copper protein tyrosinase and the substrate analogue p-nitrophenol studied by NMR. J Am Chem Soc. 127(2):567-75, 2005.
Abstract: The interaction of the monooxygenating type-3 copper enzyme Tyrosinase (Ty) from Streptomyces antibioticus with its inhibitor p-nitrophenol (pnp) was studied by paramagnetic NMR methods. The pnp binds to oxidized Ty (Ty(met)) and its halide (F(-), Cl(-)) bound derivatives with a dissociation constant in the mM range. The Cu(2) bridging halide ion is not displaced upon the binding of pnp showing that the pnp does not occupy the Cu(2) bridging position. The binding of pnp to Ty(met) or Ty(met)Cl leads to localized changes in the type-3 (Cu-His(3))(2) coordination geometry reflecting a change in the coordination of a single His residue that, still, remains coordinated to Cu. The binding of pnp to Ty(met)Cl causes a decrease in the Cu(2) magnetic exchange parameter -2J from 200 cm(-)(1) in the absence to 150 +/- 10 cm(-)(1) in the presence of pnp. From the (1)H and (2)D NMR relaxation parameters of pnp bound to Ty(met), a structural model of pnp coordination to the Ty type-3 center could be derived. The model explains the absence of hydroxylase activity in the closely related type-3 copper protein catechol oxidase. The relevance of the experimental findings toward the Ty catalytic mechanism is discussed.

- Wojtasek H.
Regulation of tyrosinase by tetrahydropteridines-What is real? A comment on the work published by Wood et al. on December 24, 2004. Biochem Biophys Res Commun. 329(3):801-3, 2005.

- Wood JM, Chavan B, Hafeez I, Schallreuter KU.
Regulation of tyrosinase by tetrahydropteridines and H2O2. Biochem Biophys Res Commun. 325(4):1412-7, 2004.

Abstract: Recently two alternative mechanisms have been put forward for the inhibition of tyrosinase by 6R-l-erythro 5,6,7,8-tetrahydrobiopterin (6BH(4)). Initially allosteric uncompetitive inhibition was demonstrated due to 1:1 binding of 10(-6)M 6BH(4) to a specific domain 28 amino acids away from the Cu(A) active site of the enzyme. Alternatively it was then shown that 10(-3)M 6BH(4) inhibit the reaction by the reduction of the product dopaquinone back to l-dopa. In the study presented herein we have used two structural analogues of 6BH(4) (i.e., 6,7-(R,S)-dimethyl tetrahydrobiopterin and 6-(R,S)-tetrahydromonapterin) confirming classical uncompetitive inhibition due to specific binding of the pyrimidine ring of the pterin moiety to the regulatory domain on tyrosinase. Under these conditions there was no reduction of l-dopaquinone back to l-dopa by both cofactor analogues. Inhibition of tyrosinase by 6BH(4) occurs in the concentration range of 10(-6)M after preactivation with l-tyrosine and this mechanism uncouples the enzyme reaction producing H(2)O(2) from O(2). Moreover, a direct oxidation of 6BH(4) to 7,8-dihydrobiopterin by tyrosinase in the absence of the substrate l-tyrosine was demonstrated. The enzyme was activated by low concentrations of H(2)O(2) (<0.3 x 10(-3)M), but deactivated at concentrations in the range 0.5-5.0 x 10(-3)M. In summary, our results confirm a major role for 6BH(4) in the regulation of human pigmentation.

8. Melanosomes

(Prof. J. Borovansky)

Biogenesis of melanosomes was reviewed by *Hearing* and studied in various subtypes of Hermansky-Pudlak syndrome (*Richmond et al*). Melanosome distribution was investigated by *Kuroda & Fukuda* and specifically in the RPE by *Gibbs et al.* Ubiquitylation of Melan-A/MART-1 was shown to be important for its sorting to lysosomes , where it is predominantly degraded (*Lévy et al*). Prof Simon´s group has continued to introduce modern techniques into melanosome research, focused this time on comparing melanosomes from black and red hair and from various ocular pigment tissues in relation to their age, respectively (2 papers by *Liu et al*). *Liu & Simon* raised an interesting question whether metal ions (characteristically present in melanosomes) could influence melanin structural organization and at which point(s) in the structural assembly the associated forces were important. *Masters et al* demonstrated cytotoxic potential of melanosomes due to their ability to absorb and convert light energy.

- Gibbs D, Azarian SM, Lillo C, Kitamoto J, Klomp AE, Steel KP, Libby RT, Williams DS.
  Role of myosin VIa and Rab27a in the motility and localization of RPE melanosomes. J Cell Sci 117(26): 6473-6483, 2004.
  Comments: Many proteins in mouse RPE cells that could potentially participate in melanosome transport were detected, but of those tested, only myosin VIIa and Rab27a were found to be required for normal distribution. Two other expressed proteins, melanophilin and myosin Va, both of which are required for normal melanosome distribution in melanocytes, were not required in RPE, despite the association of myosin Va with the RPE melanosome fraction.

- Hearing VJ.
  Biogenesis of pigment granules: a sensitive way to regulate melanocyte function. J Dermatol Sci 37(1): 3-14, 2005.
  Comments: Review article based on author´s Tanioku Kihei Memorial Lecture. It deals with the biogenesis of the melanosome as a unique organelle (giving details on its enzymatic, structural and other protein components), with sorting of melanosomal proteins to melanosomes and with transport of melanosomes to dendrites and transfer to keratinocytes. Inherited pigmentary diseases related to dysfunction of melanosomal proteins are briefly summarized. The article contains several beautiful colour illustrations.

- Kuroda TS, Fukuda M.
  Rab27A-binding protein Slp2-a is required for peripheral melanosome distribution and elongated cell shape in melanocytes. Nature Cell Biol 6(12): 1195-1203, 2004.
  Comments: Synaptogamin-like protein /Slp/ family is implicated in regulating Rab27A-mediated transport. Slp2-a, so far uncharacterized Rab27A binding protein , was shown to colocalize with Rab27A on melanosomes and to be the most abundantly expressed member of the Slp family in melanocytes. Knockdown of endogenous Slp2-a protein markedly reduced the number of melanosomes in the cell periphery of mouse melanocytes and induced also a change of melanocyte morphology from their usually elongated to a more rounded shape.

- Lévy F, Muehlethaler K, Salvi S, Peitrequin AL, Lindholm CK, Cerottini JC, Rimoldi D.
  Ubiquitylation of a melanosomal protein by HECT-E3 ligases serves as sorting signal for lysosomal degradation. Mol Biol Cell 2005 /Epub ahead of print/
  Comments: The authors have provided evidence that Melan-A/MART-1, melanocytic transmembrane protein, which accumulates in vesicles at the trans side of Golgi apparatus and in melanosomes, can interact with E3 type of ubiquitin ligases to become ubiquitylated and subsequently sorted to lysosomes and degraded. A mutant Melan-A lacking ubiquitin-acceptor residues displayed increased half life and accumulated in melanosomes. Proteasome enzymes also appear to participate in Melan-A degradation, but the lysosomal pathway in pigment cells was predominant.

- Liu Y, Hong L, Wakamatsu K, Ito S, Adhyaru BB, Cheng CY, Bowers CR, Simon JD.
  Comparisons of the structural and chemical properties of melanosomes isolated from retinal pigment epithelium, iris and choroid of newborn and mature bovine eyes. Photochem Photobiol 2005 /Epub ahead of print/
  Comments: Structural (size and shape) and chemical properties (total content of amino acids, eu- and phaeomelanin concentrations) of melanosomes isolated from RPE, iris and choroid of both mature and newborn bovine eyes were compared. The phaeomelanin content was found to be low in all 3 types of melanosomes studied regardless of their different shape and size, which suggests that the type of melanin cannot be simply determined from the shape of the melanosome /which is in exact accord with our earlier conclusions that structural protein(s) but not melanin decide about the melanosome shape and ultrastructure –/ *cf. Hach et al/ Sborník lék 94, 1993, 113; Borovanský et al/ Arch Dermatol Res 289, 1997, 145/.*

- Liu Y, Hong L, Wakamatsu K, Ito S, Adhyaru BB, Cheng CY, Bowers CR, Simon JD.
  Comparison of structural and chemical properties of black and red human hair melanosomes. Photochem Photobiol 81(1): 135-144, 2005.

Comments: Melanosomes from black and red hair were isolated and characterized by various chemical and physical techniques. The black hair melanosomes had circa 15% amino acid content, whereas those of red hair origin had more than 44% amino acid content. Atomic force microscopy confirmed that eumelanosomes and phaeomelanosomes might have ellipsoidal and spherical shapes, respectively /*see also Jimbow&Takeuchi/Pigment Cell 4, 1979, 308/* . Metal analysis performed by means of modern inductively coupled plasma mass spectroscopy confirmed the presence of Cu^{2+} and Zn^{2+} /*cf. Horcicko et al/Hoppe Seyler´s Z Physiol Chem 354,1973, 203/*, of Ca^{2+} /*cf. Szekeres/Arch Derm Forsch 252, 1975, 297*/ in melanosomes and an increased iron concentration in phaeomelanosomes /*see Rothman & Flesh/Proc Soc Exptl Biol Med 53,1943, 134 and Flesh/Arch Dermatol 101, 1970, 482/.* Magnetic resonance spectra and infrared spectra are shown to be powerful techniques for discerning differences in the amino acid content, 5,6-DHI-2C/5,6-DHI ratio and the degree of crosslinking in the pigment.

- Liu Y, Simon JD.
Metal-ion interactions and the structural organization of *Sepia* eumelanin. Pigment Cell Res 18(1): 42-48, 2005.
Comments: Natural morphology of Sepia melanin granules was not changed when different metal ions were associated with the melanin which means that once assembled the granule is able to absorb or release metal ions without significant structural change. However, when the melanin granules are subjected to mechanical disturbance, the soluble mass fraction of the melanin form different structures depending on the ions present. The authors conclude that metal ions play a fundamental role organizing melanin morphology at the 10-20 nm substructural level. /*See also Chodurek et al/ J Anal Appl Pyrolysis 70, 2003, 43/.*

- Masters BR, So PTC, Buehler C, Barry N, Sutin JD, Matulin WW, Gratton E.
Mitigating thermal mechanical damage potential during two-photon dermal imaging. J Biomed Optics 9(6): 1265-1270, 2004.
Comments: Two-photon excitation fluorescence microscopy allows *in vivo* high resolution imaging of human skin structure but the skin imaging can be associated with the formation of cavitation at the epidermal-dermal junction, which results in thermal mechanical damage of the tissue. The thermal damage mechanism was shown to be associated with one-proton absorption of infrared excitation light by melanosomes present in the epidermal-dermal junction. /*See also Thompson et al/Bull Mathem Biol 58, 1996, 513/.*

- Richmond B, Huizing M, Knapp J, Koshoffer A, Zhao Y, Gahl WA, Boissy RE.
Melanocytes derived from patients with Hermansky-Pudlak syndrome types 1,2 and 3 have distinct defects in cargo trafficking. J Invest Dermatol 124(2): 420-425, 2005.
Comments: In order to assess the role of Hermansky-Pudlak syndrome (HPS) genes in melanosome biogenesis, melanocytes cultured from patients with HPS subtypes 1,2,3 and from control unaffected individual were studied as for the localization of various melanocyte proteins – tyrosinase gene family proteins, HPS gene family proteins and other melanocyte-expressed proteins (LAMP 1-3, Rab 27, tansferrin, cKit). The comparative analysis of the patterns of melanocyte protein distribution in HPS 1,2,3 provided a glimpse into the elaborate mechanism responsible for targetting cargo from the Golgi apparatus to melanosomes (summarized in a beautiful colour figure No.6).

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2005 10th World Congress on Cancers of the Skin

May 13-17, Vienna, Austria
Contact: Elfriede Pomp
Dept of Dermatology, Vienna General Hospital
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Währinger Gürtel 18-20
A - 1090 Vienna
Tel: +00431 40400 7707
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E-mail: info@wccs.at
Web: www.wccs.at

2005 4th International EUROSKIN Conference

May 18-20
Contact: E-mail l: euroskin@t-online.de
Web: www.euroskin.info

2005 3rd EADV Spring Symposium

May 19, Sofia, Bulgaria
Contact: Tel : 359-2-9230-493
Fax: 359-2-952-0241
E-mail: eadvsofia2005@mail.bg
Web: www.eadv.org/sofia2005

2005 29th annual meeting of Israel Society of Dermatology and Venereology

June 6, Petah-Tiqva, Israel
Contact: Tel : 972-3-9376654
Fax: 972-3-9223353
E-mail: mdavid@clalit.org.il

2005 Melanoma Study Group (UK) meeting

June 24, Oxford
Contact: E-mail: admin@melanomastudygroup.co.uk
Web: www.melanomastudygroup.co.uk

2005 IVth IACD World Congress - International Academy of Cosmetic Dermatology

July 3-5 Palais des Congrès de Paris 75017 Paris (France)
Contact: MCI France / IACD 2005
11, rue de Solférino

F - 75007 Paris
Tel.: 33 (0)1 53 85 82 51 - Fax.: 33 (0)1 53 85 82 83
E.mail : iacd2005@mci-group.com
Web: www.iacd-paris2005.com

2005 British Association of Dermatologists 85th Annual Meeting

July 5-8, Glasgow, Scotland
Contact: Conference Manager, British Association of Dermatologists
19 Fitzroy Square
W1T 6EH London
UK
Tel: 44-20-7383-0266
Fax: 44-20-7388-5263
E-mail: admin@bad.org.uk

2005 11th European Hair Research Society Annual Meeting

July 7-9, Lucerne, Switzerland
Contact: Convention Team Lucerne AG
P.O. Box 2552
CH - Lucerne
Tel: 41-41-371-1860
Fax: 41-41-371-1861
E-mail: ctlag@bluewin.ch

2005 XIVth International Pigment Cell Conference (IPCC)

September 18-23, Reston, Virginia, USA
Contact: Dr. V. HEARING
E-mail: hearingv@nih.gov
Web: www.ipcc.info
Satellite Meetings : Friday, September 23, 2005
- "**Melanoma**", coordinated by Meenhard Herlyn
  (co-sponsored by the Society for Melanoma Research)
- "**Photobiology**", co-chaired by Frances Noonan and Sharon Miller
  (co-sponsored by the American Society for Photobiology)
- "**Vitiligo**", co-chaired by Mauro Picardo and Alain Taieb
  (co-sponsored by the IFPCS Vitiligo Special Interest Group)

2005 35th Annual ESDR Meeting

September 22-24, Tübingen, Germany
Contact: E-mail: office@esdr.org
Web: www.esdr.ch

2005 14th Congress of the European Academy of Dermatology and Venereology

October 12-16, London, United Kingdom
Contact: CTS
Data House
Curriers Close, Tile Hill
UK - Coventry CV4 8AW
Tel: +44 (0)870 429 4612 Fax: +44 (0)870 429 4613
Email: eadv@ctsnet.co.uk
Web: www.eadv2005.com

2005 Perspectives in Melanoma IX

November 17-18, Tampa, Florida — Tampa Marriott Waterside
Chairmen: John M. Kirkwood, MD and Alexander M.M. Eggermont, MD, PhD
Contact: Coleson Chase
Tel: +1 (770) 751 7332
Email: meetings@imedex.com
Web: www.imedex.com

2005 19th Annual Meeting of the Japanese Society for Pigment Cell Research (JSPCR)

December 3-4, Yokohama City, Japan
Chair: Prof. Toyoko Akiyama, Keio University
Contact:
E-mail: hakiyama@hc.cc.keio.ac.jp

2006 36th Annual ESDR Meeting

September 7-9, Paris, France
Contact: E-mail: office@esdr.org
Web: www.esdr.ch

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/

2007 37th Annual ESDR Meeting

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

2007 21st World Congress of Dermatology

October 1-5
Contact: E-mail: info@dermato2007.org
Web: www.dermato2007.org

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

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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TRAVEL AWARDS TO ATTEND THE IPCC

The 19th International Pigment Cell Conference (September 18-22, 2005, Reston, Virginia, USA) encourages new and youthful participation in this meeting. A limited number of travel stipend awards ($800 for ESPCR members) are available to society members in good standing who are young investigators working in the field of pigment cell biology. The stipend can be used for the airfare, hotel, and/or meeting registration.

Please note that APPLICATIONS MUST BE SUBMITTED ONLINE FROM THE IPCC WEB PAGE (http://www.palladianpartners.com/IPCC05), although they will be assessed by the ESPCR Travel Awards Committee. A letter from the Faculty mentor or Department Chairman must be sent to Dr. Vince Hearing (by email to hearingv@nih.gov or by FAX to +1 301 402-8787) stating that the applicant is a Predoctoral student, a Postdoctoral fellow or a Junior Faculty member in good standing in that Department, noting your position there. Applications are not complete until that letter is received.

Deadline for Application: May 1, 2005 (supporting letters must also be received by this date)

Notification of Award: June 30, 2005 (or soon thereafter, by email)"