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DISCUSSION, REVIEW,
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HISTORY OF PIGMENT CELL RESEARCH IN EUROPE

VIth European Workshop on Melanin Pigmentation (EWMP): The portal to the Foundation of the ESPCR.

By Patrick A. Riley & Jan Borovansky

The VIth EWMP took place in Murcia on September 22-25, 1985. It was organized by A.J. Lozano and A. Manjon and took place in the University of Murcia. It comprised 38 lectures and 68 posters and was divided into 4 sessions of lectures and a poster session:

1. Biosynthesis and properties of melanins (chaired by T. Sarna and G. Prota).
2. Melanocytes: Natural and chemically-induced alterations (chaired by C. Aubert and J.L. Iborra).
3. Kinetic and molecular aspects of tyrosinase (chaired by H. Rorsman and V.J. Hearing).
4. Genetic and biological aspects of melanoma (chaired by N.Cascinelli and J.M. Mascaro).
5. Poster Discussion (chaired by P.A. Riley).

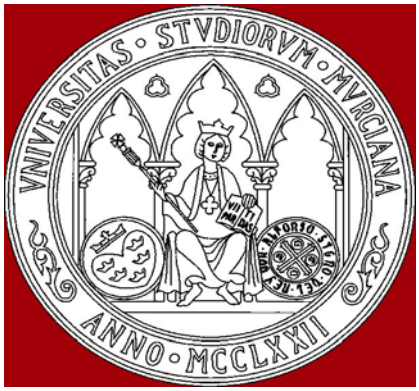

Fig. 1. The first university in Murcia was founded as the Universitas Studiorum Murciana by Alfonso X of Castile around 1272. The current University of Murcia was founded in 1915, making it the tenth oldest university in Spain, but its seal carries the date of the thirteenth century foundation.

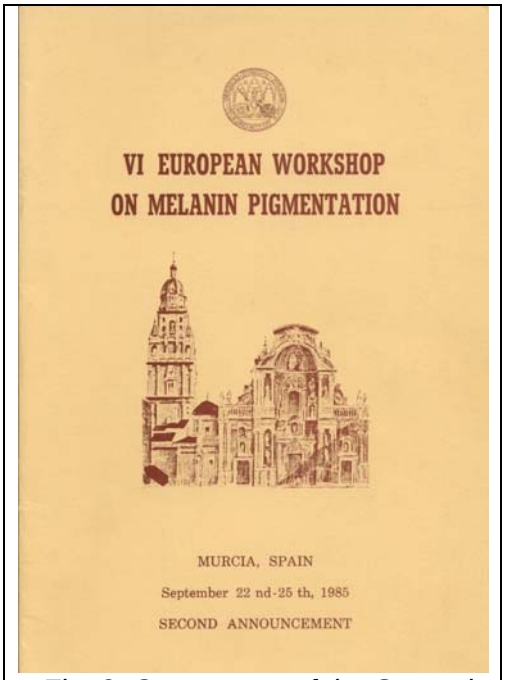

Fig. 2. Cover page of the Second Announcement of the Murcia EWMP.

The meeting was held in the elegant buildings of the University in pleasantly warm weather and included a lively demonstration of Spanish dancing in the cloisters and a highly convivial outing to a local museum of viniculture (Bodegas). The scientific programme included several exceptionally interestig presentations and was held in a very cooperative and relaxed atmosphere. The participants were presented with beautifully decorated Sangria jugs of local manufacture as a memento of a thoroughly enjoyable occasion.

The meeting, organised on a substantial scale, again attracted the attendance of the majority of the major figures in the field wih wide participation from Europe and the USA. As at previous EWMP meetings, and other related events including the ESDR and IPCC, there had been informal discussions in which the establishment of a European society had been mooted. There seemed to be several strands to the argument in favour of such an initiative including the widespread interest in pigment research in Europe, a feeling that it would provide a vehicle for a greater scientific rapprochement with colleagues in the Eastern part of the continent, and, perhaps to some small extent, the sense that the field was dominated by Americans, and in particular the Harvard-Yale axis. In any event, on this occasion José Lozano had scheduled a formal session at which the pros and cons of setting up a European Society were to be discussed. Despite some contrary views, notably expressed by Hans Rorsman, who felt that such a course would undermine the international cooperation embodied by the IPCS, and by Carl Witkop, on the somewhat less serious point that the likely acronym of the proposed society might cause confusion with an organization devoted to the study of extra-sensory perception, when it came to a vote on the issue there was overwhelming support not only from the European contingent but also from the Americans for the formation of a European Society for Pigment Cell Research. Patrick Riley was charged with the task of making the formal arrangements and within a few weeks of the Murcia meeting had produced a draft Constitution. It was agreed that Giuseppe Prota, who had been active in promoting the idea of a European society, should make arrangements for the legal inauguration. Marco d'Ischia was given the unenviable task of translating and adapting the draft Constitution in a format acceptable to Italian law as Giuseppe Prota was to be the "Responsible Person" required in Italian jurisprudence with regard to the formation of a Society. Several lengthy telephone calls were sufficient to iron out the details and the Constitution was duly sworn before a Notary Public in Naples on 11th December 1985. Thus, the auguries for the ESPCR were good as this was the year which saw the most recent return of Halley's comet to our solar system.

For the inauguration Patrick Riley had flown out to Naples on the 10th December and, at dinner with Giuseppe Prota at the aptly-named Hotel Paradiso, a list of candidates for the prospective steering committee was drawn up. In the event, the Articles signed the following day by Giuseppe Prota, Patrick Riley and Pino Cascinelli, who had flown down from Milan for the occasion, required the setting up an Executive Committee pending proper democratic elections. The members were: Fritz Anders, Pino Cascinelli, Ferdy Lejeune, José Lozano, and Hans Rorsman, with Giuseppe Prota as Chairman and Patrick Riley as Secretary/Treasurer.

Fig. 3. (L to R) Richard King, Vincent J Hearing, Giuseppe Prota, José Antonio Lozano.

Fig. 4. (L to R) Arturo Manjón, Patrick A Riley, José Antonio Lozano, Giuseppe Prota, José Luis Iborra.

As elections did not take place until the 1st Scientific Meeting of the ESPCR, held in Sorrento in 1987, this Executive Committee was nominally in charge of the affairs of the Society for two years, although most of the organisation took place in London and in Prota's laboratory in Naples. The arrangements for the first elections were (in retrospect) quite hilarious but finally votes were cast and the first proper Committee was formed, composed largely of elder statesmen of the European pigment cell world. When the results of the election were announced Edgar Frenk was heard to exclaim: " This is not a Committee; it is a Senate!"
Much water has passed under the bridge since then.

J.Borovansky
P.A.Riley

Acknowledgement: We are grateful to Professor Lozano and Professor Garcia-Borron for the photographs – Fig. 3 and 4.

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Pr A. Napolitano)

Melanin structure and properties have been the focus of some interesting papers appeared in this term. In one study (Greco et al, PCMR) on pheomelanin structure by the biomimetic approach based on oxidation of 5-S-cisteinyldopa precursor new dimeric intermediates with a peculiar benzothiazolylthiazinodihydroisoquinoline structure have been identified. Evidence for the presence of such units into the natural pigments has been obtained by chemical degradation of human red hair leading to a thiazolyl pyridine dimeric product, a specific marker of such structural units. A synthetic melanin was obtained from dopa by action of haloperoxidase in the presence of hydrogen peroxide and iodine (V-HPO/I/H2O2,) and investigated by spectroscopic techniques and SEM confirming similarities with other synthetic melanin pigments. (Nicolai et al. Int J. Biochem). A review by the photophysical group at Lund (Huijser et al, Physical Chemistry Chemical Physics) summarizes recent studies on UV-dissipative mechanisms of melanin model and precursors and the implications for the in vivo pigments. Among techniques reported for investigation of melanin properties are an EPR study to analyze binding of dopa melanin to a drug, kanamycin, and copper(II) ions (Najder-Kozdrowska et al, Spectroscopy), and a new interferometric methodology involving exposure of melanin to two coherent beams from a low-power UV laser to follow photodegradation of the pigment at two wavelengths UVA (355 nm) and UVC (244 nm). (Farley et al, Optics letters)
In the melanin based materials section, a most interesting report by the Farinola's group in Bari on the use of a synthetic commercial melanin for implementation of metal–insulator–silicon semiconductor devices (Ambrico et al Advanced Materials). An ultrathin electrochemically formed melanin-iron coating was found to largely improve the catalytic activity of Au nano particles for both hydrogen peroxide electroreduction and hydrogen evolution reaction (Orive et al, Nanoscale).

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2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

Although melanin plays a critical role to protect skin from the harmful effects of solar UV radiation, excess melanin synthesis can lead to hyper-pigmentation disorders and to the subsequent clinical and cosmetic concerns. Therefore, development of melanin synthesis inhibitors is of great interest and increasing efforts have been dedicated to the discovery of depigmenting agents. **Niki *et al*** investigated the mechanism of action of 1-(2,4-Dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylpheny) propane (DP), a compound recently reported as a novel tyrosinase inhibitor. In this study the authors found that DP reduced melanin content in normal human melanocytes and reconstructed skin model without decrease of cell number or cell viability. In human skin equivalent, DP showed higher efficacy on inhibition of eumelanin synthesis than that of kojic acid. Although DP showed a mild effectiveness in inhibiting human tyrosinase, this compound induced a strong reduction of melanin content in human melanocytes, suggesting that its mechanism to reduce melanin synthesis does not involve a simple inhibition of tyrosinase activity. To support this hypothesis, the authors reported that DP treatment reduced tyrosinase protein level, with no effects on tyrosinase mRNA expression, indicating that DP affects tyrosinase at a post-transcription level. All together the results of this study showed that DP acts through dual mechanisms to reduce melanin synthesis: by direct inhibition of tyrosinase activity via an antioxidant effect, and by the acceleration of tyrosinase degradation. **Fujita and co-workers** identified and characterized a novel melanogenesis inhibitor, 16-hydroxy-9-oxo-10*E*,12*E*,14*E*-octadecatrienoic acid, also known as Corchorifatty acid B (CFAB) . The authors reported that CFAB did not affect tyrosinase activity or transcription, but it specifically accelerates the rate of tyrosinase decrease. They showed also that CFAB-induced decrease in tyrosinase took place in the post-Golgi compartments, unexpectedly suggesting that CFAB-induced decrease in tyrosinase did not occur in lysosomes or proteasomes. Two possible mechanisms for CFAB-induced tyrosinase degradation have been proposed: the first is the accumulation of tyrosinase in multivescicular bodies or the accelarate exosome secretion in melanocytes; the second is the shedding at the plasma membrane by an unidentified protease. Although the authors were not able to determine the exact mechanism of CFAB that induces tyrosinase decrease, it is likely that this mechanism would be novel and distinct from that of currently used skin-lightening molecules. Manassantin A from *Saururus chinensis* inhibits melanin production in α-MSH-activated B16 melanoma cells, in which the lignan compound decreases α-MSH-inducible protein levels of tyrosinase in the cells but does not directly affect the catalytic activity of cell-free tyrosinase. **Lee HD *et al*** elucidated molecular basis of the antimelanogenic activity of manassantin A, focusing on cAMP-inducible tyrosinase levels. Manassantin A was found to inhibit the cAMP elevator 3-isobutyl-1-methylxanthine (IBMX)- or dibutyryl cAMP-induced melanin production in B16 cells or in melan-a melanocytes. Regarding the manassantin A molecular mechanism, the authors showed that this molecule directly inhibited the Ser-133 phosphorylation of CREB in the cells, an activation index for MITF induction, which sequentially suppressed the expression of tyrosinase or other melanogenic enzymes for melanin biosynthesis. Curcumin is a plant-derived polyphenol, which has been reported to suppress melanogenesis in B16 melanoma cells. However, little is known about whether curcumin affects melanogenesis in cultured human melanocytes. In addition, the molecular mechanism for the antimelanogenic effects of curcumin remains largely unknown**. Tu and co-workers** elucidated the potential mechanism by which curcumin inhibits melanogenesis in melanocytes. This study also examined the melanogenesis-related proteins including MITF, tyrosinase, TRPs and t he melanogenesis-regulating molecules including PI3K/Akt, GSK3β, ERK and p38 MARK. Curcumin significantly inhibited melanogenesis in normal human melanocytes. Moreover experimental data suggested that curcumin-induced phosphorylation of Akt/GSK3β, ERK and p38 may contribute to the antimelanogenic effects of this compound. **Lee EJ *et al*** elucidated the inhibitory mechanisms of N-(3,5-dimethylphenyl)-3-methoxybenzamide (A(3)B(5)), a biaryl amide derivative, in melanin production. The authors showed that A(3)B(5) had no effect on the production and activity of tyrosinase. However A(3)B(5) treatment downregulated melanin production and melanoma cell growth via proteosomal degradation of TRP-2, suggesting this molecule as a possible therapeutic agent that effectively regulates both hyperpigmentation and melanoma growth in the skin. **Song and co-workers** examined the potential of p-coumaric acid (PCA) and its hydrophobic derivative, methyl *p*-coumarate (MPC), as hypopigmenting agents for topical use. The results of this study showed that topically applied PCA in the form of a cream can attenuate the inflammation and pigmentation due to strong UVB exposure *in vivo* in animal experiments, supporting, together with previous human trials, the uselfuness of PCA to counteract skin hyperpigmentation. In contrast, MPC was found to be less effective than PCA against skin pigmentation *in vivo* although it inhibited melanin synthesis in cultured melanocytes more effectively, probably due to lower efficiency of transdermal delivery. **Nakajima *et al.*** evaluated the effects of an extract of the medical plant *Withania somnifera* on the endothelin 1 (EDN1)-stimulated pigmentation of human epidermal equivalents and melanoma cells. The results showed that *Withania somnifera* exerts a depigmenting effect on EDN1-mediated pigmentation accompanied by a reduction in eumelanin content. The expression of melanocyte-specific genes and proteins including MITF, tyrosinase, Tyrp1, Dct, Pmel17, are significantly decrease in EDN-1 stimulated cells treated with the plant extract compared to the EDN1 stimulated cells. Analysis of the EDN1-triggered signalling pathways, namely the protein kinase C (PKC)/mitogen activated protein kinase (MAPK) pathway demonstrated that *Withania somnifera* inhibits the EDN1-mediated intracellular signalling cascade by preferentially interrupting the activation or activity of PKC. Taken together, these results suggest that *Withania somnifera* may be considered as a therapeutic tool in those hyperpigmentary disorders such as UVB-melanosis, which are known to be associated to EDN1 stimulation.

Despite of the large number of compounds capable of reducing melanin synthesis *in vitro*, current skin whitening agents often have insufficient efficacy and harmful side effects. In this optic the development of novel siRNA-based treatments to reduce pigmentation can be a good strategy to obtain effective and safe reduction of excessive melanin production. **Van Gele and co-workers** developed a physiological relevant 3D pigmented human skin model which could be useful for functional testing of RNAi-induced depigmentation and studying modulation of pigment transfer between melanocytes and keratinocytes. The authors selected tyrosinase, as a target to validate the effect of silencing on pigmentation. Reduced or defective melanin skin pigmentation is associated with an increased risk of skin cancer and development of various pathological states. Currently, there are numerous reports on the development of antimelanogenesis agentts. By comparison, the development of melanogenesis agents for photoprotection and hypopigmentation disorders is not entirely satisfactory. **Kang *et al*** evaluated the effects of 5,7-dimethylflavone (5,7-DMF) on melanin synthesis in B16F10 melanoma cells and determined the role of cAMP-dependent signaling in its biological actions. Melanin content and tyrosinase activity were significantly increased by 5,7-DMF, as were protein levels of tyrosinase, TRP-1, and TRP-2. Moreover 5,7-DMF markedly increased protein levels of MITF and CREB phosphorylation. Results of this study showed that activation of the PKA/CREB cascade and inhibition of Akt/GSK-3β signalling by 5,7-DMF cooperate to strongly induce melanin synthesis. The authors suggested that melanogenesis is induced by 5,7-DMF primarily through elevated intracellular cAMP levels, and consequently, 5,7-DMF may be an effective natural pigmentation stimulator for photoprotection and hypopigmentation disorders. **Ye and co-worker** investigated the involvement of MAPK pathways in the melanogenic effect of apigenin, a flavonoid abundantly present in a variety of fruits and leafy vegetables, in B16 cells. Apigenin treatment induced a dose-dependent increase of expression of tyrosinase, TRP-1, TRP-2 and MITF at 48 h. Furthermore although the phosphorylation of JNK or ERK was not significantly p38 phosphorylation was dose-dependent increased by apigenin at 48h. The application of a selective inhibitor of p38 MAPK decreased, although did not completely abolish, the stimulatory effect of apigenin on tyrosianse activity. These findings indicated that apigenin promotes melanogenesis, at least in part, by activating the p38 MAPK pathway.

Dopamine (DA), a kind of catecholamines, represents an initiator of oxidative stress and elevated levels of DA are found in the serum and urine of vitiligo patients. Since the flavonoid apigenin has been shown to have different biological properties including antioxidant, anti-inflammatory and anti-cancer activities, **Lin *et al.*** studied the antioxidant protective effects of this compound against the DA-mediated apoptosis of melanocytes and its underlying molecular mechanism of action. The results presented in the paper clearly demonstrate that apigenin treatment significantly reduces both DA-induced melanocyte death and ROS production. Moreover, apigenin inhibits DA-mediated activation of the intracellular pathways of JNK, p38, MAPK and Akt suggesting that its biological action may be mediated via inhibition of these oxidative stress related signals. In summary, even if the efficacy and the safety of apigenin and other antioxidants require additional studies, these results provide apigenin as a potential antioxidant agent in the treatment of vitiligo.

Nuclear factor E2-related factor 2 (Nrf2)-antioxidant response element (ARE) has been shown to be one of the major pathway involved in protecting cells from oxidative stress-related damages in many organs and tissues. It also participates in the human skin adaptation pathways to environmental stress. Oxidative stress caused by hydrogen peroxide (H_2O_2) may be involved in the pathogenesis of various skin diseases including vitiligo. **Jian and co-workers** analyzed the anti-oxidative role of the Nrf2-ARE pathway on human melanocytes. The authors hypothesized that this antioxidant pathway may contribute to the protection of melanocytes against H_2O_2 mediated cell death and that its target cytoprotective gene heme oxygenase-1 (HO-1) may act as critical protective factor during the oxidative stress induced by H_2O_2. Using Nrf2 short interfering RNA (siRNA) and pCMV6-XL5-Nrf2 to downregulate or upregulate Nrf2 expression and using specific inhibitors or activators of HO-1, the study demonstrates the crucial involvement of HO-1 in protecting melanocytes against H_2O_2-mediated cell death through a Nrf2-dependent pathway, indicating that this pathway is crucial for melanocytes to deal with oxidative stress. Due to the important role exerted by oxidative stress in vitiligo pathogenesis, the authors suggest that activation of Nrf2 transcription or the increase of HO-1 levels may be an effective approach to reduce melanocyte damage and death in vitiligo lesions.

The study by **Hendi *et al.*** had the aim to define the number and distribution of melanocytes on formalin-fixed, non lesional sun-damaged skin collected from the head or neck of a large series of patients (100 patients, from age 18 to 105 years, who were undergoing Mohs micrographic and reconstructive surgery for basal cell and squamous cell carcinoma of the face and neck) observed at two distinct referral centers in USA (Florida and Minnesota). Moreover, the authors aimed to evaluate and quantify the presence and the extension of features characteristic of melanoma in these non-cancerous samples of sun-damaged skin. The overall goal of the authors was to provide useful information to physicians who use light microscopy to diagnose and treat melanoma to be aware of the various histological features (i-e. high melanocyte density, mild to moderate confluence of melanocytes, focal pagetosis, superficial follicular extension (<1 mm) and mild or moderate cytologic atypia) that may be observed on sun-damaged skin in the absence of a melanocytic neoplasm.

The incidence of melanoma is continuously increasing and there are no effective therapies once the disease becomes metastatic. Even if mutations of the tumour suppressor gene P53 are quite frequent in many types of cancer, mutational inactivation of P53 in melanoma is uncommon and wild-type P53 is expressed at high levels. **Avery-Kiejda and co-workers** analyzed the transcript expression of known P53 target genes in extracts collected from 82 metastatic melanoma and 6 melanoma cell lines and compare to normal cells (human melanocytes and fibroblasts) using whole genome bead arrays. The results demonstrate that many P53 target genes mainly involved in apoptosis were under-expressed in melanoma whereas genes regulating cell cycle appeared over-expressed. In addition, the study shows that this altered expression is independent on P53 status. Similarly, down-regulation of P53 by short-hairpin RNA in melanoma cells resulted in minimal effect on the expression of P53 target genes. On the other hand, P53 inhibition in melanocytes led to

changes in the expression of many P53 taget genes that were characteristic of melanoma cells and resulted in increased growth. On the contrary, the authors demonstrated that knockdown of P53 in melanoma cells resulted in reduction of proliferation. In summary, these results indicate that in melanoma there is a deregulation of the P53 target genes involved in the control of apoptosis and cell cycle and that this altered functional activity of P53 may contribute to the proliferation of melanoma and to the potential failure to elicit effective responses to pro-apoptotic treatment such as chemotherapy.
Eumelanin pigment is formed in melanocytes as a result of the action of several enzymes including dopachrome tautomerase (DCT), which leads to the formation of the intermediate dihydroxyindole-2-carboxylic acid (DHICA). DCT enzyme has been shown to protect cells against radical-mediated damages and apoptosis through DHICA. The melanocortin 1 receptor (MC1R) gene is highly polymorphic and the red hair colour (RHC) variants R151C, R160W and D294H known as "R" alleles are associated to increased risk of developing melanoma. Lower penetrant MC1R alleles V60L, V92M and R163Q had reduced odds ratios respect to the consensus (+) or wild type allele and were labelled as "r" variants. **Ainger *et al.*** using a co-culture system of melanocytes and pooled samples of keratinocytes studied the dendricity and the mRNA and protein expression of the melanogenic enzyme DCT in low penetrant "r" homozygote and "R/+" heterozygote MC1R variant allele expressing cells in comparison to that of wild type (WT) cells.
Cutaneous pigmented lesions, especially those heavily melanized, may be challenging to diagnose. Immunohistochemical stainings such as MART-1, S100, HMB45 are often of help. However, the use of the commonly employed chromogen 3,3'-diaminobenzidine (DAB) may render pigmented lesions difficult to be analyzed due to the resulting brown staining of both melanocytes and pigment. Azure blue counterstaining stains melanin granules blue-green and can therefore distinguish melanocytes immunolabelled with melanoma antigen recognized by T-cells (MART-1) from melanophages. **Hillesheim and co-workers** analyzed whether microphthalmia transcription factor (MiTF) staining may be as effective as MART-1/Azure blue to identify and quantify melanocytes in the context of solar lentigo and melanoma *in situ* (MIS). Twenty cases each of solar lentigo and MIS were evaluated and no difference in the mean melanocytic count between MART-1/Azure blue and MITF staining was observed. Moreover, MITF nuclear staining resulted to facilitate interpretation in comparison to the cytoplasmic localization of MART-1 and required less laboratory preparation. Based on these findings, the authors suggest the use of MiTF as an efficient and alternative immunohistochemical marker to MART-1 with Azure blue counterstaining in heavily pigmented melanocytic lesions.

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Development of a 3D pigmented skin model to evaluate RNAi-induced depigmentation. Exp Dermatol. 2011 Jun 24. [Epub ahead of print]

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Activation of p38 MAPK pathway contributes to the melanogenic property of apigenin in B16 cells. Exp Dermatol. 2011 May 25. [Epub ahead of print].

3. MSH, MCH, other hormones, differentiation

(Pr M. Böhm)

POMC, alpha-MSH and MC1R – what's new ?

Impact of UV wavelength on the expression of the cutaneous analogon of the HPA axis
It is well known that ultraviolet light (UV) irradiation, the most ubiquitous environmental stressor, regulates the expression of various components of the proopiomelanocortin (POMC) system in human skin. This phenomenon has been coined "analogon of the hypothalamic-pituitary-adrenal (HPA) axis" (Slominski et al., Physiol. Rev. 2000). However, which wavelength of the electromagnetic spectrum of the sun light (UVA, UVB or UVC) is most efficient in inducing this cutaneous response was not systematically examined. In an interesting new study using both human skin organ cultures as well as co-cultures of epidermal melanocytes and keratinocytes Skobowiat et al. (J. Physiol. Endocrinol. Metab. 2011, Epub) addressed this important question. The degree of stimulation of several genes of the HPA axis (CRH, POMC, MC1R, MC2R, CYP11A1 and CYP11B1) genes was dependent on UV wavelengths and doses. The highly energetic wavelengths UVC and UVB were most efficient in their inductive effects. At protein level, a marked induction in the expression levels of CRH, POMC, ACTH and CYP11A1 and of cortisol was detected. Increased β-END levels was also seen following UVA treatment. Immunocytochemical investigations localized the above POMC components predominantly in the epidermis with additional accumulation of CRH, β-END and ACTH in the dermis. Interestingly, UVB and UVC resulted also in a decrease in the protein expression of the glucocorticoid receptor. Thus, the induction of natural sun light to induce the cutaneous POMC system is dependent on the most energetic wavelengths of UV while for UVA overlapping or alternative mechanisms appear to exist.

Red hair color variants and their impact on alpha-MSH-mediated induction of dopachrome tautomerase in melanocytes co-cultured with keratinocytes

Only little is known about the impact of the red hair color (RHC) variants R151C, R160W and D294H of the melanocortin 1 receptors (MC1R), known as "R" alleles, on expression of dopachrome tautomerase. Roberts et al. (J. Cell Physiol. 2008) previously reported that the R homozygote variants R151C-/-, R160W-/- and the compound heterozygote R151C/R160W were unable to mount a response to NDP-alpha-MSH although they responded to the artificial cAMP induced forskolin. In a subsequent study Aigner et al. (Exp. Dermatol. 2011; 20; 681-684) investigated the dendricity and dopachrome tautomerase (DCT) responses of low penetrant 'r' homozygote and 'R/+' heterozygote MC1R variant allele expressing melanocytes in a co-culture system with keratinocytes. The V60L-/- homozygote r variant cells exhibited a similar response to ligand as WT MC1R strains. V92M-/- homozygote r variant cells had generally greater dendricity and expressed higher DCT than the WT cells. The R151C+/- heterozygote cells displayed similar responses to WT cells, while the R160W+/- and D294H+/- variant cells were reduced in their responses to NDP-MSH. However, the latter cells still had an active cAMP response after forskolin treatment. The data highlight the dominant negative effect of these alleles on the MC1R WT allele that has previously been demonstrated genetically and biochemically.

Defining the function of MC1R function in the melanocyte – from mice and man

The MC1R with its complex function in the melanocyte can still be considered a chart-breaker in PCMR. In a recent review by Beaumont et al. (Eur. J. Pharmakol. 2011; 660: 103-110) the authors discuss to what extent mouse coat color models, human genetic association studies, and in vitro cell culture studies have been instrumental to understand the molecular mechanisms underlying the association between melanocortin MC1R variant alleles and the red hair colour phenotype. In this paper, some new strategies and models such as 2D and 3D cell culture systems as well as new transgenic models or humanized models are also briefly discussed.

4. Photobiology

(Dr N. Smit)

NOT AVAILABLE

5. Neuromelanins

(Pr M. d'Ischia)

The molecular mechanisms involved in the etiology of Parkinson disease are the focus of continuing interest. A possible origin from a neuroinflammatory state secondary to exposure to an infectious agents was supported by a study (Rohn and Catlin, 2011) showing immunolocalization of the influenza A virus in the substantia nigra pars compacta within neuromelanin granules as well as on tissue macrophages. These results underscore the central role of neuromelanin in the pathogenetic mechanisms of the disease.

The interplay between microglia and human neuromelanin in the degenerating pathways of dopaminergic neurons in the substantia nigra was addressed in a study by Zhang et al (2011). The most significant outcome was the demonstration that extracellular neuromelanin can induce microglia activation with production of nitric oxide, ROS and pro-inflammatory agents, and neurodegeneration in cell cultures. These observations may guide future research aimed at dissecting pathogenic mechanisms and at orienting therapeutic strategies.

Murphy et al. (2011) advanced the hypothesis that a recently identified serotonin-derived melanoid product, a sort of neuromelanin derived from an unconventional non-catecholamine pathway, may mediate oxidative stress and free radical damage to neurons by virtue of its ability to bind Fe2+ readily and induce redox processes.

The mechanisms of aminochrome-induced cell death were investigated in a cell line from rat substantia nigra (Paris et al., 2011). By using an integrated set of experiments, the authors suggested that autophagy is important in aminochrome-induced cell death, and that different mechanisms may operate in the presence and in the absence of DT-diaphorase inhibitors.

Finally, Napolitano et al. (2011) provided an overview of the oxidative pathways of the main catecholamine neurotransmitters, dopamine and norepinephrine, and briefly address the analogies and differences in the reaction pathways in relation to their potential implication for neuronal degeneration.

- Murphy Meghan M., Miller Elizabeth D., Fibuch Eugene E., Seidler Norbert W.
  Redox mechanism of neurotoxicity by a serotonin-acrolein polymeric melanoid. Neurotoxicity Research 19(2), 353-360, 2011.
  Abstract: Postoperative cognitive dysfunction may be assocd. with the toxic products of lipid peroxidn., such as the □,□-unsatd. aldehyde acrolein, which accumulates in aging. We previously identified an acrolein-mediated, serotonin-derived melanoid product, or SDM. This study further characterizes this putative novel neuromelanin, which is not made from catecholamines. In addn. to its strong protein-binding properties, we obsd. that SDM binds Fe2+ readily and exhibits complex redox characteristics. SDM may exist as a two-dimensional network of polymers that coalesce into larger entities exhibiting electroactive properties. These observations suggest that SDM may contribute to the decline in cognition due to focal degeneration from SDM-mediated free-radical prodn. We know that inhalational anesthetics sequester acrolein, which is toxic to neurons, and we propose that the local increase in acrolein depletes serotonin levels and enhances neuronal vulnerability through the prodn. of neuromelanin-like structures, such as SDM.

- Napolitano A., Manini P., d'Ischia M.
  Oxidation chemistry of catecholamines and neuronal degeneration: an update. Current Medicinal Chemistry 18(12), 1832-1845, 2011.
  Abstract: A review. Aberrant oxidative pathways of catecholamine neurotransmitters, i.e. dopamine and norepinephrine, are an important biochem. correlate of catecholaminergic neuron loss in some disabling neurodegenerative diseases of the elderly, notably Parkinson's disease. In an oxidative stress setting, under conditions of elevated lipid peroxidn., iron accumulation, impaired mitochondrial functioning and antioxidant depletion, catecholamines are oxidatively converted to the corresponding o-quinones, which may initiate a cascade of spontaneous reactions, including intramol. cyclization, aminoethyl side chain fission and interaction with mol. targets. The overall outcome of the competing pathways may vary depending on contingent factors and the biochem. environment, and may include formation of nitrated derivs., neuromelanin deposition, generation of chain fission products, conjugation with L-Cys leading eventually to cytotoxic responses and altered cellular function. In addn., catecholamines may interact with products of lipid peroxidn. and other species derived from oxidative breakdown of biomols., notably glyoxal and other aldehydes, leading e.g. to tetrahydroisoquinolines via Pictet-Spengler chem. After a brief introductory remark on oxidative stress biochem., the bulk of this review will deal with an overview of the basic chem. pathways of catecholamine oxidn., with special emphasis on the analogies and differences between the central neurotransmitters dopamine and norepinephrine. This chem. will form the basis for a concise discussion of the latest advances in the mechanisms of catecholamine-assocd. neurotoxicity in neuronal degeneration.

- Paris, Irmgard; Munoz, Patricia; Huenchuguala, Sandro; Couve, Eduardo; Sanders, Laurie H.; Greenamyre, John Timothy; Caviedes, Pablo; Segura-Aguilar, Juan.
  Autophagy Protects Against Aminochrome-Induced Cell Death in Substantia Nigra-Derived Cell Line. Toxicological Sciences 121(2), 376-388, 2011.

Abstract: Aminochrome, the precursor of neuromelanin, has been proposed to be involved in the neurodegeneration neuromelanin-contg. dopaminergic neurons in Parkinson's disease. The authors aimed to study the mechanism of aminochrome-dependent cell death in a cell line derived from rat substantia nigra. The authors found that aminochrome (50 □M), in the presence of NAD(P)H-quinone oxidoreductase, EC 1.6.99.2 (DT)-diaphorase inhibitor dicoumarol (DIC) (100 □M), induces significant cell death (62□3%; $p < 0.01$), increase in caspase-3 activation ($p < 0.001$), release of cytochrome C, disruption of mitochondrial membrane potential ($p < 0.01$), damage of mitochondrial DNA, damage of mitochondria detd. with TEM, a dramatic morphol. change characterized as cell shrinkage, and significant increase in no. of autophagic vacuoles. To det. the role of autophagy on aminochrome-induced cell death, the authors incubated the cells in the presence of vinblastine and rapamycin. Interestingly, 10 □M vinblastine induces a 5.9-fold ($p < 0.001$) and twofold ($p < 0.01$) significant increase in cell death when the cells were incubated with 30 □M aminochrome in the absence and presence of DIC, resp., whereas 10 □M rapamycin preincubated 24 h before addn. of 50 □M aminochrome in the absence and the presence of 100 □M DIC induces a significant decrease ($p < 0.001$) in cell death. In conclusion, autophagy seems to be an important protective mechanism against two different aminochrome-induced cell deaths that initially showed apoptotic features. The cell death induced by aminochrome when DT-diaphorase is inhibited requires activation of mitochondrial pathway, whereas the cell death induced by aminochrome alone requires inhibition of autophagy-dependent degrading of damaged organelles and recycling through lysosomes.

- Rohn, Troy T.; Catlin, Lindsey W.
Immunolocalization of influenza A virus and markers of inflammation in the human Parkinson's disease brain. PLoS One 6(5), e20495, 2011.
Abstract : Although much is known regarding the mol. mechanisms leading to neuronal cell loss in Parkinson's disease (PD), the initiating event has not been identified. Prevailing theories including a chem. insult or infectious agent have been postulated as possible triggers, leading to neuroinflammation. We present immunohistochem. data indicating the presence of influenza A virus within the substantia nigra pars compacta (SNpc) from postmortem PD brain sections. Influenza A virus labeling was identified within neuromelanin granules as well as on tissue macrophages in the SNpc. Further supporting a role for neuroinflammation in PD was the identification of T-lymphocytes that colocalized with an antibody to caspase-cleaved Beclin-1 within the SNpc. The presence of influenza A virus together with macrophages and T-lymphocytes may contribute to the neuroinflammation assocd. with this disease.

- Zhang, Wei; Phillips, Kester; Wielgus, Albert R.; Liu, Jie; Albertini, Alberto; Zucca, Fabio A.; Faust, Rudolph; Qian, Steven Y.; Miller, David S.; Chignell, Colin F.; Wilson, Belinda; Jackson-Lewis, Vernice; Przedborski, Serge; Joset, Danielle; Loike, John; Hong, Jau-Shyong; Sulzer, David; Zecca, Luigi.
Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: implications for progression of Parkinson's disease. Neurotoxicity Research 19(1), 63-72, 2011.
Abstract: In Parkinson's disease (PD), there is a progressive loss of neuromelanin (NM)-contg. dopamine neurons in substantia nigra (SN) which is assocd. with microgliosis and presence of extracellular NM. Herein, we have investigated the interplay between microglia and human NM on the degeneration of SN dopaminergic neurons. Although NM particles are phagocytized and degraded by microglia within minutes in vitro, extracellular NM particles induce microglial activation and ensuing prodn. of superoxide, nitric oxide, hydrogen peroxide (H2O2), and pro-inflammatory factors. Furthermore, NM produces, in a microglia-depended manner, neurodegeneration in primary ventral midbrain cultures. Neurodegeneration was effectively attenuated with microglia derived from mice deficient in macrophage antigen complex-1, a microglial integrin receptor involved in the initiation of phagocytosis. Neuronal loss was also attenuated with microglia derived from mice deficient in phagocytic oxidase, a subunit of NADPH oxidase, that is responsible for superoxide and H2O2 prodn., or apocynin, an NADPH oxidase inhibitor. In vivo, NM injected into rat SN produces microgliosis and a loss of tyrosine hydroxylase neurons. Thus, these results show that extracellular NM can activate microglia, which in turn may induce dopaminergic neurodegeneration in PD. Our study may have far-reaching implications, both pathogenic and therapeutic.

6. Genetics, molecular and developmental biology

(Dr. Lluís Montoliu)

- Agarwal P, Verzi MP, Nguyen T, Hu J, Ehlers ML, McCulley DJ, Xu SM, Dodou E, Anderson JP, Wei ML, Black BL.
The MADS box transcription factor MEF2C regulates melanocyte development and is a direct transcriptional target and partner of SOX10. Development. 138(12):2555-65, 2011.
Waardenburg syndromes are characterized by pigmentation and autosensory hearing defects, and mutations in genes encoding transcription factors that control neural crest specification and differentiation are often associated with Waardenburg and related disorders. For example, mutations in SOX10 result in a severe form of Waardenburg syndrome, Type IV, also known as Waardenburg-Hirschsprung disease, characterized by pigmentation and other neural crest defects, including defective innervation of the gut. SOX10 controls neural crest development through interactions with other transcription factors. The MADS box transcription factor MEF2C is an important regulator of brain, skeleton, lymphocyte and cardiovascular development and is required in the neural crest for craniofacial development. Here, we establish a novel role for MEF2C in melanocyte development. Inactivation of Mef2c in the neural crest of mice results in reduced expression of melanocyte genes during development and a significant loss of pigmentation at birth due to defective differentiation and reduced abundance of melanocytes. We identify a transcriptional enhancer of Mef2c that directs expression to the neural crest and its derivatives, including melanocytes, in transgenic mouse embryos. This novel Mef2c neural crest enhancer contains three functional SOX binding sites and a single essential MEF2 site. We demonstrate that Mef2c is a direct transcriptional target of SOX10 and MEF2 via this evolutionarily conserved enhancer. Furthermore, we show that SOX10 and MEF2C physically interact and function cooperatively to activate the Mef2c gene in a feed-forward transcriptional circuit, suggesting that MEF2C might serve as a potentiator of the transcriptional pathways affected in Waardenburg syndromes.

- Aydin IT, Beermann F.
A mart-1::Cre transgenic line induces recombination in melanocytes and retinal pigment epithelium. Genesis. 49(5):403-9, 2011. doi: 10.1002/dvg.20725.

- Budi EH, Patterson LB, Parichy DM.
Post-embryonic nerve-associated precursors to adult pigment cells: genetic requirements and dynamics of morphogenesis and differentiation. PLoS Genet. 7(5):e1002044, 2011. Epub 2011 May 19.

- Cullinane AR, Curry JA, Carmona-Rivera C, Summers CG, Ciccone C, Cardillo ND, Dorward H, Hess RA, White JG, Adams D, Huizing M, Gahl WA.
A BLOC-1 Mutation Screen Reveals that PLDN Is Mutated in Hermansky-Pudlak Syndrome Type 9. Am J Hum Genet. 88(6):778-87? 2011.

- Cullinane AR, Vilboux T, O'Brien K, Curry JA, Maynard DM, Carlson-Donohoe H, Ciccone C; NISC Comparative Sequencing Program, Markello TC, Gunay-Aygun M, Huizing M, Gahl WA.
Homozygosity Mapping and Whole-Exome Sequencing to Detect SLC45A2 and G6PC3 Mutations in a Single Patient with Oculocutaneous Albinism and Neutropenia. J Invest Dermatol. 2011 Jun 16. doi: 10.1038/jid.2011.157.

- Emi K. Nishimura.
Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. Pigment Cell & Melanoma Research 24 (3): 401–410, 2011.
Most mammals are coated with pigmented hair. Melanocytes in each hair follicle produce melanin pigments for the hair during each hair cycle. The key to understanding the mechanism of cyclic melanin production is the melanocyte stem cell (MelSC) population, previously known as ‘amelanotic melanocytes’. The MelSCs directly adhere to hair follicle stem cells, the niche cells for MelSCs and reside in the hair follicle bulge–subbulge area, the lower permanent portion of the hair follicle, to serve as a melanocyte reservoir for skin and hair pigmentation. MelSCs form a stem cell system within individual hair follicles and provide a ‘hair pigmentary unit’ for each cycle of hair pigmentation. This review focuses on the identification of MelSCs and their characteristics and explains the importance of the MelSC population in the mechanisms of hair pigmentation, hair greying, and skin repigmentation.

- Hirobe T.
How are proliferation and differentiation of melanocytes regulated ? Pigment Cell Melanoma Res. 24(3):462-78, 2011.
Coat colors are determined by melanin (eumelanin and pheomelanin). Melanin is synthesized in melanocytes and accumulates in special organelles, melanosomes, which upon maturation are transferred to keratinocytes. Melanocytes differentiate from undifferentiated precursors, called melanoblasts, which are derived from neural crest cells. Melanoblast/melanocyte proliferation and differentiation are regulated by the tissue environment, especially by keratinocytes, which synthesize endothelins, steel factor, hepatocyte growth factor, leukemia inhibitory factor and

granulocyte-macrophage colony-stimulating factor. Melanocyte differentiation is also stimulated by alpha-melanocyte stimulating hormone; in the mouse, however, this hormone is likely carried through the bloodstream and not produced locally in the skin. Melanoblast migration, proliferation and differentiation are also regulated by many coat color genes otherwise known for their ability to regulate melanosome formation and maturation, pigment type switching and melanosome distribution and transfer. Thus, melanocyte proliferation and differentiation are not only regulated by genes encoding typical growth factors and their receptors but also by genes classically known for their role in pigment formation.

- Iacovelli J, Zhao C, Wolkow N, Veldman P, Gollomp K, Ojha P, Lukinova N, King A, Feiner L, Esumi N, Zack DJ, Pierce EA, Vollrath D, Dunaief JL.
  Generation of Cre transgenic mice with postnatal RPE-specific ocular expression. Invest Ophthalmol Vis Sci. 52(3):1378-83, 2011. Print 2011 Mar.

- Ida-Eto M, Ohgami N, Iida M, Yajima I, Kumasaka MY, Takaiwa K, Kimitsuki T, Sone M, Nakashima T, Tsuzuki T, Komune S, Yanagisawa M, Kato M.
  Partial requirement of endothelin receptor B in spiral ganglion neurons for postnatal development of hearing. J Biol Chem. 2011 Jun 28.

- Jin Y, Birlea SA, Fain PR, Gowan K, Riccardi SL, Holland PJ, Bennett DC, Herbstman DM, Wallace MR, McCormack WT, Kemp EH, Gawkrodger DJ, Weetman AP, Picardo M, Leone G, Taïeb A, Jouary T, Ezzedine K, van Geel N, Lambert J, Overbeck A, Spritz RA.
  Genome-wide analysis identifies a quantitative trait locus in the MHC class II region associated with generalized vitiligo age of onset. J Invest Dermatol. 131(6):1308-12, 2011.

- Lukas Sommer.
  Generation of melanocytes from neural crest cells. Pigment Cell & Melanoma Research 24 (3): 411–421, 2011.
  The neural crest is a transient structure in vertebrate embryos that generates multiple neural and mesenchymal cell types as well as melanocytes. Melanocytes in the skin either derive directly from neural crest cells populating the skin via a dorsolateral migratory pathway or arise by detaching from nerves innervating the skin. Several transcription factors, such as FoxD3, Sox10, Pax3, and Mitf, take part in a genetic network regulating melanocyte formation from the neural crest. The activity of these intrinsic factors is controlled and modulated by extracellular signals including canonical Wnt, Edn, Kitl, and other signals that remain to be identified. Here, we summarize the current view of how melanocytes are specified from the neural crest and put this process into the context of spatiotemporal lineage decisions in neural crest cells.

- Nishikawa-Torikai S, Osawa M, Nishikawa SI.
  Functional Characterization of Melanocyte Stem Cells in Hair Follicles. J Invest Dermatol. 2011 Jul 14. doi: 10.1038/jid.2011.195.

- Picardo M, Cardinali G.
  The genetic determination of skin pigmentation: KITLG and the KITLG/c-Kit pathway as key players in the onset of human familial pigmentary diseases. J Invest Dermatol. 131(6):1182-5, 2011.

- Pośpiech E, Draus-Barini J, Kupiec T, Wojas-Pelc A, Branicki W.
  Gene-gene interactions contribute to eye colour variation in humans. J Hum Genet. 56(6):447-55, 2011. doi: 10.1038/jhg.2011.38.

- Taylor KL, Lister JA, Zeng Z, Ishizaki H, Anderson C, Kelsh RN, Jackson IJ, Patton EE.
  Differentiated melanocyte cell division occurs in vivo and is promoted by mutations in Mitf. Development. 2011 Jul 19.
  Coordination of cell proliferation and differentiation is crucial for tissue formation, repair and regeneration. Some tissues, such as skin and blood, depend on differentiation of a pluripotent stem cell population, whereas others depend on the division of differentiated cells. In development and in the hair follicle, pigmented melanocytes are derived from undifferentiated precursor cells or stem cells. However, differentiated melanocytes may also have proliferative capacity in animals, and the potential for differentiated melanocyte cell division in development and regeneration remains largely unexplored. Here, we use time-lapse imaging of the developing zebrafish to show that while most melanocytes arise from undifferentiated precursor cells, an unexpected subpopulation of differentiated melanocytes arises by cell division. Depletion of the overall melanocyte population triggers a regeneration phase in which differentiated melanocyte division is significantly enhanced, particularly in young differentiated melanocytes. Additionally, we find reduced levels of Mitf activity using an mitfa temperature-sensitive line results in a dramatic increase in differentiated melanocyte cell division. This supports models that in addition to promoting differentiation, Mitf also promotes withdrawal from the cell cycle. We suggest differentiated cell division is relevant to melanoma progression because the human melanoma mutation MITF(4TΔ2B) promotes increased and serial differentiated melanocyte division in zebrafish. These results reveal a novel pathway of differentiated melanocyte division in vivo, and that Mitf activity is essential for maintaining cell cycle arrest in differentiated melanocytes.

- Tryon RC, Higdon CW, Johnson SL.
Lineage relationship of direct-developing melanocytes and melanocyte stem cells in the zebrafish. PLoS One. 2011;6(6):e21010. Epub 2011 Jun 16.

- Van Gele M, Geusens B, Speeckaert R, Dynoodt P, Vanhoecke B, Van Den Bossche K, Lambert J.
Development of a 3D pigmented skin model to evaluate RNAi-induced depigmentation. Exp Dermatol. 2011 Jun 24. doi: 10.1111/j.1600-0625.2011.01319.x.

7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borron)

- Cioaca D, Ghenea S, Spiridon LN, Marin M, Petrescu AJ, Petrescu SM.
  C-terminus glycans with critical functional role in the maturation of secretory glycoproteins. PLoS One. 6(5):e19979, 2011.
  The N-glycans of membrane glycoproteins are mainly exposed to the extracellular space. Human tyrosinase is a transmembrane glycoprotein with six or seven bulky N-glycans exposed towards the lumen of subcellular organelles. The central active site region of human tyrosinase is modeled here within less than 2.5 Å accuracy starting from Streptomyces castaneoglobisporus tyrosinase. The model accounts for the last five C-terminus glycosylation sites of which four are occupied and indicates that these cluster in two pairs - one in close vicinity to the active site and the other on the opposite side. We have analyzed and compared the roles of all tyrosinase N-glycans during tyrosinase processing with a special focus on the proximal to the active site N-glycans, s6:N337 and s7:N371, versus s3:N161 and s4:N230 which decorate the opposite side of the domain. To this end, we have constructed mutants of human tyrosinase in which its seven N-glycosylation sites were deleted. Ablation of the s6:N337 and s7:N371 sites arrests the post-translational productive folding process resulting in terminally misfolded mutants subjected to degradation through the mannosidase driven ERAD pathway. In contrast, single mutants of the other five N-glycans located either opposite to the active site or into the N-terminus Cys1 extension of tyrosinase are temperature-sensitive mutants and recover enzymatic activity at the permissive temperature of 31°C. Sites s3 and s4 display selective calreticulin binding properties. The C-terminus sites s7 and s6 are critical for the endoplasmic reticulum retention and intracellular disposal. Results herein suggest that individual N-glycan location is critical for the stability, regional folding control and secretion of human tyrosinase and explains some tyrosinase gene missense mutations associated with oculocutaneous albinism type I.

- Fairhead M, Thöny-Meyer L.
  Bacterial tyrosinases: old enzymes with new relevance to biotechnology. N Biotechnol. 2011 May 31.
  Tyrosinases are copper-containing dioxygen activating enzymes found in many species of bacteria and are usually associated with melanin production. These proteins have a strong preference for phenolic and diphenolic substrates and are somewhat limited in their reaction scope, always producing an activated quinone as product. Despite this fact they have potential in several biotechnological applications, including the production of novel mixed melanins, protein cross-linking, phenolic biosensors, production of l-DOPA, phenol and dye removal and biocatalysis. Although most studies have used Streptomyces sp. enzymes, there are several other examples of these proteins that are also of potential interest. For instance a solvent tolerant enzyme has been described, as well as an enzyme with both tyrosinase and laccase activities, enzymes with altered substrate preferences, an enzyme produced as an inactive zymogen as well as examples which do not require auxiliary proteins for copper insertion (unlike the Streptomyces sp. enzymes which do require such a protein). This article will summarise the reports on the biotechnological applications of bacterial tyrosinases as well as the current information available on the different types of this enzyme.

- Fujieda N, Ikeda T, Murata M, Yanagisawa S, Aono S, Ohkubo K, Nagao S, Ogura T, Hirota S, Fukuzumi S, Nakamura Y, Hata Y, Itoh S.
  Post-translational His-Cys cross-linkage formation in tyrosinase induced by copper(II)-peroxo species. J Am Chem Soc. 133(5):1180-3, 2011.
  Autocatalytic formation of His-Cys cross-linkage in the enzyme active site of tyrosinase from Aspergillus oryzae has been demonstrated to proceed by the treatment of apoenzyme with Cu(II) under aerobic conditions, where a (μ-η(2):η(2)-peroxo)dicopper(II) species has been suggested to be involved as a key reactive intermediate.

- Ghanem G, Fabrice J.
  Tyrosinase related protein 1 (TYRP1/gp75) in human cutaneous melanoma. Mol Oncol. 5(2):150-5, 2011.
  Melanoma prognosis is based on specific pathological features at the primary lesion. In metastatic patients, the extent of lymph node involvement is also an important prognosis indicator. Many progression markers both in tissues and serum, including circulating tumor cells, have been studied and new molecular markers are awaited from high-throughput screenings to discriminate between clinical stages and predict disease progression. The present review focuses on human tyrosinase related protein 1 also known as gp75 glycoprotein (Tyrp1/gp75), a melanosomal protein involved in the pigmentary machinery of the melanocyte and often used as differentiation marker, with a special emphasis on its emerging roles in the malignant melanocyte and melanoma progression.

- Ha YM, Kim JA, Park YJ, Park D, Kim JM, Chung KW, Lee EK, Park JY, Lee JY, Lee HJ, Yoon JH, Moon HR, Chung HY.
  Analogs of 5-(substituted benzylidene)hydantoin as inhibitors of tyrosinase and melanin formation. Biochim Biophys Acta. 1810(6):612-9, 2011.

BACKGROUND: Many tyrosinase inhibitors find application in cosmetics and pharmaceutical products for the prevention of the overproduction of melanin in the epidermis. A series of 5-(substituted benzylidene)hydantoin derivatives 2a-2k were prepared, and their inhibitory activities toward tyrosinase and melanin formation were evaluated. METHODS: The structures of the compounds were established using (1)H and (13)C NMR spectroscopy and mass spectral analyses. All the synthesized compounds were evaluated for their mushroom tyrosinase inhibition activity. RESULTS: The best results were obtained for compound 2e which possessed hydroxyl group at R(2) and methoxy group at R(3), respectively. We predicted the tertiary structure of tyrosinase, simulated its docking with compound 2e and confirmed that this compound interacts strongly with mushroom tyrosinase residues as a competitive tyrosinase inhibitor. In addition, we found that 2e inhibited melanin production and tyrosinase activity in B16 cells. CONCLUSIONS: Compound 2e could be considered as a promising candidate for preclinical drug development in skin hyperpigmentation applications. GENERAL SIGNIFICANCE: This study will enhance understanding of the mechanism of tyrosinase inhibition and will contribute to the development of effective drugs for use hyperpigmentation.

- Ha YM, Park JY, Park YJ, Park D, Choi YJ, Kim JM, Lee EK, Han YK, Kim JA, Lee JY, Moon HR, Chung HY.
Synthesis and biological activity of hydroxy substituted phenyl-benzo[d]thiazole analogues for antityrosinase activity in B16 cells. Bioorg Med Chem Lett. 21(8):2445-9, 2011.
In this study, we synthesized hydroxy and/or alkoxy substituted phenyl-benzo[d]thiazole derivatives using substituted benzaldehydes and 2-aminothiophenol in MeOH. The structures of these compounds were established by (1)H and (13)CNMR and mass spectral analyzes. All synthesized compounds were evaluated for their mushroom tyrosinase inhibition activity. Out the 12 generated compounds, 2a and 2d exhibited much higher tyrosinase inhibition activity (45.36-73.07% and 49.94-94.17% at 0.01-20 μM, respectively) than kojic acid (9.29-50.80% at 1.25-20 μM), a positive control. The cytotoxicity of 2a and 2d was evaluated using B16 cells and the compounds were found to be nontoxic. Compounds 2a and 2d were also demonstrated to be potent mushroom tyrosinase inhibitors, displaying IC(50) values of 1.14±0.48 and 0.01±0.0002 μM, respectively, compared with kojic acid, which has an IC(50) value of 18.45±0.17 μM. We also predicted the tertiary structure of tyrosinase, simulated the docking with compounds 2a and 2d and confirmed that the compounds strongly interact with mushroom tyrosinase residues. Kinetic plots showed that 2a and 2d are competitive tyrosinase inhibitors. Substitutions with a hydroxy group at R(3) or both R(3) and R(1) of the phenyl ring indicated that these groups play a major role in the high binding affinity to tyrosinase. We further found that compounds 2a and 2d inhibit melanin production and tyrosinase activity in B16 cells. These results may assist in the development of new potent tyrosinase inhibitors against hyperpigmentation.

- Hida T, Sohma H, Kokai Y, Kawakami A, Hirosaki K, Okura M, Tosa N, Yamashita T, Jimbow K.
Rab7 is a critical mediator in vesicular transport of tyrosinase-related protein 1 in melanocytes. J Dermatol. 38(5):432-41, 2011.
How melanosomal proteins such as enzymic proteins (tyrosinase and tyrosinase-related proteins, Tyrps) and structural protein (gp100) are transported from Golgi to melanosomal compartments is not yet fully understood. A number of small GTPases have been found to be associated with melanosomes and we have identified one of them, Rab7, a regulator of vesicular transport, organelle motility, phospholipid signaling and cytosolic degradative machinery, as being involved in the transport of Tyrp1 from Golgi to stage I melanosomes. This study further characterizes the role of Rab7 as a regulator of differential sorting of melanosomal proteins in this process. Murine melanocytes were transiently transfected with a plasmid encoding either wild-type (Rab7WT), constitutively active (Rab7Q67L) or dominant-negative (Rab7N125I and Rab7T22N) Rab7. Through immunocytostaining and confocal laser scanning microscopy, we quantitatively compared the bio-distribution of melanosomal proteins between Rab7WT-expressing cells and mutant Rab7-expressing cells. We also characterized their differential elimination from melanosomal compartments by Rab7 by utilizing a proteasome inhibitor, MG132. Our findings indicate that Rab7 plays an important role in differential sorting of tyrosinase, Tyrp1 and gp100 in early melanogenesis cascade, and that it is more specifically involved with Tyrp1 than tyrosinase and gp100 in the trafficking from Golgi to melanosomes and the specific exit from the degradative process.

- Ismaya WT, Rozeboom HJ, Schurink M, Boeriu CG, Wichers H, Dijkstra BW.
Crystallization and preliminary X-ray crystallographic analysis of tyrosinase from the mushroom Agaricus bisporus. Acta Crystallogr Sect F Struct Biol Cryst Commun. 67(Pt 5):575-8, 2011.
Tyrosinase catalyzes the conversion of tyrosine to dihydroxyphenylalanine quinone, which is the main precursor for the biosynthesis of melanin. The enzyme from Agaricus bisporus, the common button mushroom, was purified and crystallized in two different space groups. Crystals belonging to space group P2(1) (unit-cell parameters a = 104.2, b = 105.0, c = 119.1 Å, β = 110.6°, four molecules per asymmetric unit) diffracted to 3.0 Å resolution. Crystals belonging to space group P2(1)2(1)2 (unit-cell parameters a = 104.0, b = 104.5, c = 108.4 Å, two molecules per asymmetric unit) diffracted to 2.6 Å resolution. It was essential to include 5 mM HoCl(3) in all crystallization conditions in order to obtain well diffracting crystals.

- Ismaya WT, Rozeboom HJ, Weijn A, Mes JJ, Fusetti F, Wichers HJ, Dijkstra BW.
Crystal Structure of Agaricus bisporus Mushroom Tyrosinase: Identity of the Tetramer Subunits and Interaction with Tropolone. Biochemistry. 50(24):5477-5486, 2011.

Tyrosinase catalyzes the conversion of phenolic compounds into their quinone derivatives, which are precursors for the formation of melanin, a ubiquitous pigment in living organisms. Because of its importance for browning reactions in the food industry, the tyrosinase from the mushroom Agaricus bisporus has been investigated in depth. In previous studies the tyrosinase enzyme complex was shown to be a H(2)L(2) tetramer, but no clues were obtained of the identities of the subunits, their mode of association, and the 3D structure of the complex. Here we unravel this tetramer at the molecular level. Its 2.3 Å resolution crystal structure is the first structure of the full fungal tyrosinase complex. The complex comprises two H subunits of 392 residues and two L subunits of 150 residues. The H subunit originates from the ppo3 gene and has a fold similar to other tyrosinases, but it is 100 residues larger. The L subunit appeared to be the product of orf239342 and has a lectin-like fold. The H subunit contains a binuclear copper-binding site in the deoxy-state, in which three histidine residues coordinate each copper ion. The side chains of these histidines have their orientation fixed by hydrogen bonds or, in the case of His85, by a thioether bridge with the side chain of Cys83. The specific tyrosinase inhibitor tropolone forms a pre-Michaelis complex with the enzyme. It binds near the binuclear copper site without directly coordinating the copper ions. The function of the ORF239342 subunits is not known. Carbohydrate binding sites identified in other lectins are not conserved in ORF239342, and the subunits are over 25 Å away from the active site, making a role in activity unlikely. The structures explain how calcium ions stabilize the tetrameric state of the enzyme.

- Jana S, Sinha M, Chanda D, Roy T, Banerjee K, Munshi S, Patro BS, Chakrabarti S.
Mitochondrial dysfunction mediated by quinone oxidation products of dopamine: Implications in dopamine cytotoxicity and pathogenesis of Parkinson's disease. Biochim Biophys Acta. 1812(6):663-73, 2011.
The study has demonstrated that dopamine induces membrane depolarization and a loss of phosphorylation capacity in dose-dependent manner in isolated rat brain mitochondria during extended in vitro incubation and the phenomena are not prevented by oxyradical scavengers or metal chelators. Dopamine effects on brain mitochondria are, however, markedly prevented by reduced glutathione and N-acetyl cysteine and promoted by tyrosinase present in the incubation medium. The results imply that quinone oxidation products of dopamine are involved in mitochondrial damage under this condition. When PC12 cells are exposed to dopamine in varying concentrations (100-400μM) for up to 24h, a pronounced impairment of mitochondrial bio-energetic functions at several levels is observed along with a significant (nearly 40%) loss of cell viability with features of apoptotic nuclear changes and increased activities of caspase 3 and caspase 9 and all these effects of dopamine are remarkably prevented by N-acetyl cysteine. N-acetyl cysteine also blocks nearly completely the dopamine induced increase in reactive oxygen species production and the formation of quinoprotein adducts in mitochondrial fraction within PC12 cells and also the accumulation of quinone products in the culture medium. Clorgyline, an inhibitor of MAO-A, markedly decreases the formation of reactive oxygen species in PC12 cells upon dopamine exposure but has only mild protective actions against quinoprotein adduct formation, mitochondrial dysfunctions, cell death and caspase activation induced by dopamine. The results have indicated that quinone oxidation products and not reactive oxygen species are primarily involved in cytotoxic effects of dopamine and the mitochondrial impairment plays a central role in the latter process. The data have clear implications in the pathogenesis of Parkinson's disease.

- Kaljunen H, Gasparetti C, Kruus K, Rouvinen J, Hakulinen N.
Crystallization and preliminary X-ray analysis of Aspergillus oryzae catechol oxidase. Acta Crystallogr Sect F Struct Biol Cryst Commun. 67(Pt 6):672-4, 2011.
Catechol oxidase is an enzyme that catalyzes the oxidation of o-diphenols to the corresponding o-quinones. It is a copper-containing enzyme with a binuclear copper active site. Here, the crystallization and multiple-wavelength anomalous dispersion data collection of catechol oxidase from the mould fungus Aspergillus oryzae are described. During the purification, three forms of the enzyme (39.3, 40.5 and 44.3 kDa) were obtained. A mixture of these three forms was initially crystallized and gave crystals that diffracted to 2.5 Å resolution and belonged to space group P3(2)21, with unit-cell parameters a = b = 118.9, c = 84.5 Å, α = β = 90, γ = 120°. A preparation containing only the shorter form (39.3 kDa) produced crystals that diffracted to 2.9 Å resolution and belonged to space group P2(1)2(1)2(1), with unit-cell parameters a = 51.8, b = 95.3, c = 139.5 Å, α = β = γ = 90°.

- Kang HY, Suzuki I, Lee DJ, Ha J, Reiniche P, Aubert J, Deret S, Zugaj D, Voegel JJ, Ortonne JP.
Transcriptional profiling shows altered expression of wnt pathway- and lipid metabolism-related genes as well as melanogenesis-related genes in melasma. J Invest Dermatol. 131(8):1692-700, 2011.
Melasma is a commonly acquired hyperpigmentary disorder of the face, but its pathogenesis is poorly understood and its treatment remains challenging. We conducted a comparative histological study on lesional and perilesional normal skin to clarify the histological nature of melasma. Significantly, higher amounts of melanin and of melanogenesis-associated proteins were observed in the epidermis of lesional skin, and the mRNA level of tyrosinase-related protein 1 was higher in lesional skin, indicating regulation at the mRNA level. However, melanocyte numbers were comparable between lesional and perilesional skin. A transcriptomic study was undertaken to identify genes involved in the pathology of melasma. A total of 279 genes were found to be differentially expressed in lesional and perilesional skin. As was expected, the mRNA levels of a number of known melanogenesis-associated genes, such as tyrosinase, were found to be elevated in lesional skin. Bioinformatics analysis revealed that the most lipid metabolism-associated genes were downregulated in lesional skin, and this finding was supported by an impaired barrier function in melasma.

Interestingly, a subset of Wnt signaling modulators, including Wnt inhibitory factor 1, secreted frizzled-related protein 2, and Wnt5a, were also found to be upregulated in lesional skin. Immunohistochemistry confirmed the higher expression of these factors in melasma lesions.

- Kim SY, Hahn HG, Nam KD, Park KC, Yun HY, Baek KJ, Kwon NS, Kim DS.
A derivative of 2-aminothiazole inhibits melanogenesis in B16 mouse melanoma cells via glycogen synthase kinase 3β phosphorylation. J Pharm Pharmacol. 63(8):1031-6, 2011.
We have investigated whether KHG25855 Objectives (2-cyclohexylamino-1,3-thiazole hydrochloride) affected melanogenesis in B16 Melanin content and mouse melanoma cells, and the mechanisms involved. Methods tyrosinase activity were measured using an ELISA reader after cells were treated with KHG25855. KHG25855-induced signalling pathways were examined using KHG25855 decreased melanin production in a Western blot analysis. Key findings dose-dependent fashion, but KHG25855 did not directly inhibit tyrosinase, the rate-limiting melanogenic enzyme. The expression of microphthalmia-associated transcription factor, tyrosinase, and the related signal transduction pathways were also investigated. The effects of KHG25855 on the extracellular signal-regulated kinase and cAMP response element binding protein signalling pathways were determined, and KHG25855 was shown to have no effect on these signalling pathways. The Wnt signalling pathway is also deeply involved in melanogenesis, and so glycogen synthase kinase 3β (GSK3β) phosphorylation was assessed after KHG25855 treatment; KHG25855 caused GSK3β phosphorylation (inactivation), but the level of β-catenin was not changed by KHG25855. Furthermore, α-melanocyte stimulating hormone-induced tyrosinase We expression was downregulated by KHG25855. Conclusions propose that KHG25855 showed hypopigmentary activity through tyrosinase downregulation via GSK3β phosphorylation.

- Lee EJ, Lee YS, Hwang S, Kim S, Hwang JS, Kim TY.
N-(3,5-Dimethylphenyl)-3-Methoxybenzamide (A(3)B(5)) Targets TRP-2 and Inhibits Melanogenesis and Melanoma Growth. J Invest Dermatol. 131(8):1701-9, 2011.
Melanin protects the skin from harmful environmental factors such as UV light. However, excessive melanin production induces hyperpigmentation. Previously, N-(3,5-dimethylphenyl)-3-methoxybenzamide (A(3)B(5)), a biaryl amide derivative, was identified for its ability to inhibit melanin production. However, its detailed mechanism of action has not been investigated. We elucidated the inhibitory mechanisms of A(3)B(5) in melanin production. Our results showed that A(3)B(5) had no effect on the production and activity of tyrosinase, an enzyme involved in melanogenesis. However, A(3)B(5) markedly decreased both constitutively expressed and UVB-induced tyrosinase-related protein 2 (TRP-2), which plays an important role along with tyrosinase in melanogenesis. The TRP-2 downregulation caused by A(3)B(5) may occur through proteasomal degradation because the A(3)B(5)-induced TRP-2 downregulation was inhibited by the ubiquitination inhibitor, MG-132. In addition, A(3)B(5) inhibited the proliferation of melanocytes and melanoma cells by arresting cells in the G1 stage of the cell cycle and moderately suppressed tumor growth in vivo. Taken together, our results indicate that A(3)B(5) downregulates melanin production and melanoma cell growth via proteosomal degradation of TRP-2 and suggest that A(3)B(5) can be a possible therapeutic agent that effectively regulates both hyperpigmentation and melanoma growth in the skin.

- Lee HD, Lee WH, Roh E, Seo CS, Son JK, Lee SH, Hwang BY, Jung SH, Han SB, Kim Y.
Manassantin A inhibits cAMP-induced melanin production by down-regulating the gene expressions of MITF and tyrosinase in melanocytes. Exp Dermatol. 2011 May 16. doi: 10.1111/j.1600-0625.2011.01296.x.]
Microphthalmia-associated transcription factor (MITF) is inducible in response to cAMP through the cAMP-responsive element-binding protein (CREB) and plays a pivotal role in the melanocyte-specific expression of tyrosinase or tyrosinase-related proteins (TRPs) for melanin biosynthesis. Manassantin A from Saururus chinensis inhibits cAMP-induced melanin production in B16 melanoma cells. Here, we focused on molecular basis of the antimelanogenic activity. Manassantin A consistently inhibited the cAMP elevator 3-isobutyl-1-methylxanthine (IBMX)- or dibutyryl cAMP-induced melanin production in B16 cells or in melan-a melanocytes by down-regulating the expression of tyrosinase or TRP1 gene. Moreover, manassantin A suppressed MITF induction through IBMX-activated CREB pathway, directly inhibiting the Ser-133 phosphorylation of CREB. However, manassantin A did not affect IBMX-increased cAMP levels in these cells but also other cAMP-dependent melanogenic pathways through post-translational modifications of MITF. This putative molecular mechanism of manassantin A in the inhibition of melanin production suggests its pharmacological potential in skin hyperpigmentation.

- Lee SA, Son YO, Kook SH, Choi KC, Lee JC.
Ascorbic acid increases the activity and synthesis of tyrosinase in B16F10 cells through activation of p38 mitogen-activated protein kinase. Arch Dermatol Res. 2011 Jun 11.
Ascorbic acid, a potential antioxidant, is known to inhibit melanogenesis. However, there are conflicting findings that ascorbic acid has very low stability and acts as a pro-oxidant, eventually increasing proliferation and melanin content in melanoma cells. In the present study, we explored the effects of ascorbic acid on the activity and expression of tyrosinase and melanin pigmentation in the presence and absence of α-melanocyte-stimulating hormone (α-MSH) using B16F10 melanoma cells. The mechanism by which ascorbic acid stimulated the expression of tyrosinase was also investigated. No inhibitory effect on melanin content was observed in ascorbic acid-treated cells, regardless of the presence of α-MSH. Ascorbic acid stimulated the activity and expression of tyrosinase and increased the expression of

melanogenic regulatory factors, such as tyrosinase-related protein-1 (TRP-1), dihydroxyphenylalaminechrome tautomerase (TRP-2), and microphthalmia-associated transcription factor (MITF). Ascorbic acid also induced phosphorylation of p38 mitogen-activated protein kinase (MAPK). The inhibition of p38 MAPK pathway by SB203580 led to the suppression of tyrosinase, TRP-1, and TRP-2 expression in cells treated with ascorbic acid. Combined treatment with N-acetyl-L: -cysteine and/or desferrioxamine mesylate attenuated the stimulating effect of ascorbic acid on tyrosinase activation in the cells. Collectively, ascorbic acid stimulates tyrosinase activity and expression in B16F10 cells via activation of p38 MAPK signaling and subsequent up-regulation of MITF, tyrosinase, and TRP expression.

- Liang CH.
Ov-16 [4-(3,4-dihydroxybenzoyloxymethyl)phenyl-O-β-D-glucopyranoside] inhibits melanin synthesis by regulating expressions of melanogenesis-regulated gene and protein. Exp Dermatol. 2011 Jun 14. doi: 10.1111/j.1600-0625.2011.01311.
Ov-16 (4-(3,4-dihydroxybenzoyloxymethyl)phenyl-O-β-D-glucopyranoside), a polyphenolic glycoside that is isolated from oregano (Origanum vulgare L.), can scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. This investigation is the first to study in detail the hypopigmentary properties of Ov-16. It μg/ml Ov-16 inhibits the activity of mushroom demonstrates that 0-1000 tyrosinase (Tyr) in a concentration-dependent manner. The inhibitionary Tyr kinetics of Ov-16 towards the oxidation of L-DOPA was found to be uncompetitive. Following the treatment of human skin premalignant kerationcyte HaCaT cells, human skin fibroblast Hs68 cells and mice melanoma B16 cells with μg/ml), cell viability was Ov-16 (0-100 >98%, suggesting that Ov-16 is non-toxic. Ov-16 can reduce cellular Tyr activity, DOPA oxidase activity and melanin synthesis in B16 cells that are stimulated by the α-melanocyte-stimulating hormone (α-MSH). Moreover, Ov-16 inhibited the production of melanin in Streptomyces bikiniensis without affecting the growth of the microorganism. The treatment of B16 cells with Ov-16 considerably reduced the gene expressions of melanocortin-1 receptor (Mc1r), microphthalmia-associated transcription factor (Mitf), Tyr, tyrosinase-related proteins-2 (Trp-2) and Trp-1, as determined by RT-PCR. The expressions of Mc1r, Mift, Tyr, Trp-2 and TrpP-1 protein in Ov-16-treated B16 cells were also significantly reduced, as determined by western blotting and fluorescent staining analysis. These results suggest that Ov-16 exhibits hypopigmentary performance.

- Matoba Y, Bando N, Oda K, Noda M, Higashikawa F, Kumagai T, Sugiyama M.
A molecular mechanism for copper transportation to tyrosinase that is assisted by a metallochaperone, Caddie. J Biol Chem. 2011 Jul 5.
The Cu(II)-soaked crystal structure of tyrosinase that is present in a complex with a protein, designated "caddie," which we previously determined, possesses two copper ions at its catalytic center. We had identified two copper-binding sites in the caddie protein and speculated that copper bound to caddie may be transported to the tyrosinase catalytic center. In our present study, at a 1.16 to 1.58 Å resolution, we determined the crystal structures of tyrosinase complexed with a caddie prepared by altering the soaking time of the copper ion, and the structures of tyrosinase complexed with different caddie mutants that displays little or no capacity to activate tyrosinase. Based on these structures, we propose a molecular mechanism by which two copper ions are transported to the tyrosinase catalytic center with the assistance of caddie acting as a metallochaperone.

- Niki Y, Yoshida M, Ando H, Wakamatsu K, Ito S, Harada N, Matsui M, Yarosh DB, Ichihashi M.
1-(2,4-Dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylpheny)propane inhibits melanin synthesis by dual mechanisms. J Dermatol Sci. 63(2):115-21, 2011.
BACKGROUND: 1-(2,4-Dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylpheny)propane (DP) was reported as a novel tyrosinase inhibitor by Nesterov et al. In previous study, we showed that DP is an antioxidant and accelerates the fading of UVB-induced tan in human skin but details of inhibiting mechanism of DP in melanogenesis remain incomplete. OBJECTIVE: To clarify additional mechanisms of DP inhibition of melanogenesis, we studied the effect of DP on tyrosinase processing and degradation. METHODS: Tyrosinase inhibition was assessed using mushroom and human tyrosinase. The effect of DP on mRNA and protein levels as well as glycosylation and degradation of tyrosinase was examined using normal human epidermal melanocytes (NHEM). RESULTS: DP was 200 times more potent than that of kojic acid in inhibiting mushroom tyrosinase activity. In contrast, DP (IC(50)=200μM) was significantly less effective at inhibiting tyrosinase from NHEM. DP decreased melanin content in cultured NHEM after 7th day (IC(50)=10μM). The IC(50) for DP against human tyrosinase activity was found to be at least 20 times higher than that of melanin synthesis. At a non-cytotoxic concentration DP did not decrease tyrosinase mRNA however protein level decreased by 46% after 48h treatment. DP did not alter the ratio of mature and immature tyrosinase assayed by endo H cleavage. Tyrosinase degradation assays revealed that DP accelerated tyrosinase degradation in NHEM. CONCLUSIONS: We found that DP acts through dual mechanisms to reduce melanin synthesis; by inhibition of tyrosinase activity via an anti-oxidant effect, and, more importantly, by the acceleration of tyrosinase degradation.

- Panich U, Tangsupa-A-Nan V, Onkoksoong T, Kongtaphan K, Kasetsinsombat K, Akarasereenont P, Wongkajornsilp A.
Inhibition of UVA-mediated melanogenesis by ascorbic acid through modulation of antioxidant defense and nitric oxide system. Arch Pharm Res. 34(5):811-20, 2011.

Ascorbic acid (AA) has been well known as a skin whitening agent, although attempts have been made to evaluate its protective role against ultraviolet (UV)-induced skin hyperpigmentation or increased melanin production. While melanogenesis is a defense mechanism of the skin against UV irradiation, melanin overproduction may also contribute to melanoma initiation. UVA might play a role in melanogenesis through promoting oxidative stress, which occurs as the result of increased formation of oxidants and/or reactive nitrogen species (RNS) including nitric oxide (NO). Therefore, we investigated the antimelanogenic effect of AA (7.5-120 μM) in association with its inhibitory effect on UVA-induced oxidant formation, NO production through endothelial and inducible NO synthases (eNOS and iNOS) activation and impairment of antioxidant defense using G361 human melanoma cells. Our study demonstrated a comparable ability of AA with that of kojic acid, a well-known tyrosinase inhibitor in inhibiting mushroom tyrosinase. Melanin content was reduced by AA, but neither tyrosinase activity nor mRNA levels were reduced by AA at non-cytotoxic concentrations in UVA-irradiated G361 cells. AA was shown to inhibit UVA-mediated catalase (CAT) inactivation, glutathione (GSH) depletion, oxidant formation and NO production through suppression of eNOS and iNOS mRNA. We report herein that AA can protect against UVA-dependent melanogenesis possibly through the improvement of antioxidant defense capacity and inhibition of NO production through down-regulation of eNOS and iNOS mRNA.

- Park SY, Jin ML, Kim YH, Kim Y, Lee SJ.
Aromatic-turmerone inhibits α-MSH and IBMX-induced melanogenesis by inactivating CREB and MITF signaling pathways. Arch Dermatol Res. 2011 Jun 10.
This study investigated the anti-melanogenic effect of aromatic (ar)-turmerone on alpha-melanocyte stimulating hormone (α-MSH) and 3-isobuty-1-methxlzanthine (IBMX)-induced tyrosinase (Tyr), tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2) expression in B16F10 melanoma cells. We demonstrated that ar-turmerone inhibits α-MSH and IBMX-induced melanin synthesis and tyrosinase activity. Data also showed that ar-turmerone inhibits the expression of tyrosinase, TRP-1, and TRP-2 in α-MSH- and IBMX-stimulated B16F10 cells. In addition, ar-turmerone exhibits stronger anti-melanogenic effects than curcumin. Furthermore, ar-turmerone strongly inhibited α-MSH- and IBMX-induced microphthalmia-associated transcription factor by suppressing the activity of cyclic adenosine monophosphate (cAMP)-responsive element binding protein in α-MSH-stimulated B16F10 cells. Our data revealed that ar-turmerone is a novel, effective, anti-melanogenic agent that functions by downregulating tyrosinase, Trp-1, and Trp-2 gene expression. Therefore, ar-turmerone may be a useful therapeutic agent for treating hyperpigmentation disorders, such as freckles and melasma, and as a beneficial additive in whitening cosmetics.

- Rescigno A, Bruyneel F, Padiglia A, Sollai F, Salis A, Marchand-Brynaert J, Sanjust E.
Structure-activity relationships of various amino-hydroxy-benzenesulfonic acids and sulfonamides as tyrosinase substrates. Biochim Biophys Acta. 1810(8):799-807, 2011.
BACKGROUND: o-Aminophenols have been long recognised as tyrosinase substrates. However their exact mode of interaction with the enzyme's active site is unclear. Properly vic-substituted o-aminophenols could help gain some insight into tyrosinase catalytic mechanism. METHODS: Eight vic-substituted o-aminophenols belonging to two isomeric series were systematically evaluated as tyrosinase substrates and/or activators and/or inhibitors, by means of spectrophotometric techniques and HPLC-MS analysis. Some relevant kinetic parameters have also been obtained. RESULTS: Four o-aminophenolic compounds derived from 3-hydroxyorthanilic acid (2-amino-3-hydroxybenzenesulfonic acid) and their four counterparts derived from the isomeric 2-hydroxymetanilic acid (3-amino-2-hydroxybenzenesulfonic acid) were synthesised and tested as putative substrates for mushroom tyrosinase. While the hydroxyorthanilic derivatives were quite inactive as both substrates and inhibitors, the hydroxymetanilic compounds on the contrary all acted as substrates for the enzyme, which oxidised them to the corresponding phenoxazinone derivatives. GENERAL SIGNIFICANCE: Based on the available structures of the active sites of tyrosinases, the different affinities of the four metanilic derivatives for the enzyme, and their oxidation rates, we propose a new hypothesis regarding the interaction between o-aminophenols and the active site of tyrosinase that is in agreement with the obtained experimental results.

- Rolff M, Schottenheim J, Decker H, Tuczek F.
Copper-O(2) reactivity of tyrosinase models towards external monophenolic substrates: molecular mechanism and comparison with the enzyme. Chem Soc Rev. 40(7):4077-98, 2011.
The critical review describes the known dicopper systems mediating the aromatic hydroxylation of monophenolic substrates. Such systems are of interest as structural and functional models of the type 3 copper enzyme tyrosinase, which catalyzes the ortho-hydroxylation of tyrosine to DOPA and the subsequent two-electron oxidation to dopaquinone. Small-molecule systems involving μ-η^2:η^2 peroxo, bis-μ-oxo and trans-μ-1,2 peroxo dicopper cores are considered separately. These tyrosinase models are contrasted to copper-dioxygen systems inducing radical reactions, and the different mechanistic pathways are discussed. In addition to considering the stoichiometric conversion of phenolic substrates, the available catalytic systems are described. The second part of the review deals with tyrosinase. After an introduction on the occurrence and function of tyrosinases, several aspects of the chemical reactivity of this class of enzymes are described. The analogies between the small-molecule and the enzymatic system are considered, and the implications for the reaction pathway of tyrosinase are discussed (140 references).

- Sato K, Toriyama M.
The inhibitory effect of non-steroidal anti-inflammatory drugs (NSAIDs) on the monophenolase and diphenolase activities of mushroom tyrosinase. Int J Mol Sci. 12(6):3998-4008, 2011.
In the present work, we investigated the effect of non-steroidal anti-inflammatory drugs (NSAIDs) on the monophenolase and diphenolase activity of mushroom tyrosinase. The results showed that diflunisal and indomethacin inhibited both monophenolase and diphenolase activity. For monophenolase activity, the lag time was extended in the presence of diflunisal. In the presence of indomethacin, the lag time did not change. IC(50) values of monophenolase activity were estimated to be 0.112 mM (diflunisal) and 1.78 mM (indomethacin). Kinetic studies of monophenolase activity revealed that both diflunisal and indomethacin were non-competitive inhibitors. For diphenolase activity, IC(50) values were estimated to be 0.197 mM (diflunisal) and 0.509 mM (indomethacin). Diflunisal and indomethacin were also found to be non-competitive diphenolase inhibitors.

- Siegbahn PE, Borowski T.
Comparison of QM-only and QM/MM models for the mechanism of tyrosinase. Faraday Discuss. 148:109-17; discussion 207-28, 2011.
Two previous studies on the mechanism of tyrosinase have given quite conflicting results. In a QM-only study using a rather small model, a mechanism was suggested in which the tyrosine proton is removed before catalysis. This was followed by catalytic cycles where a superoxo ligand attacks the phenolate ring. In another, more recent study, at the QM/MM level including the entire protein in the model, a quite different mechanism was instead advocated where a bridging O2H ligand was homolytically cleaved. That mechanism was rejected in the earlier QM-only study as having a prohibitively large barrier for O-O bond cleavage. In the present study, this discrepancy between the previous studies is investigated by new QM-only and QM/MM calculations.

- Song K, An SM, Kim M, Koh JS, Boo YC.
Comparison of the antimelanogenic effects of p-coumaric acid and its methyl ester and their skin permeabilities. J Dermatol Sci. 63(1):17-22, 2011.
BACKGROUND: p-Coumaric acid (PCA) inhibits human tyrosinase (TYR) activity and melanin synthesis in human epidermal melanocytes. OBJECTIVE: The purpose of the current study was to examine the potential of PCA and its hydrophobic derivative, methyl p-coumarate (MPC), as hypopigmenting agents for topical use. METHODS: PCA and MPC were comparatively tested against in vitro human TYR enzyme activity and cellular melanin synthesis in human epidermal melanocytes. Permeation studies were undertaken using an artificial lipophilic membrane and an excised porcine skin. In vivo hypopigmenting efficacy was assessed on the skin of melanin-possessing hairless mice exposed to UVB. RESULTS: Although PCA was a stronger inhibitor than MPC against TYR activity in vitro, the former inhibited cellular melanin synthesis less effectively than the latter. A non-cell based permeability assay indicated that PCA was practically impermeable through the lipophilic barrier while MPC was highly permeable. In contrast, an ex vivo skin permeation study demonstrated that topically applied PCA in the form of a cream can diffuse into the aqueous medium underneath the skin. No MPC was released from a MPC cream but PCA was released instead as a bio-converted product. Topical application of PCA cream attenuated the UVB-induced erythema formation and pigmentation in mice models, more effectively compared with MPC cream. CONCLUSION: PCA may be useful as an active ingredient for topical applications for a hypopigmenting effect. MPC has potential as a hypopigmenting agent but requires rather invasive methods for its delivery to the target cells.

- Tajima R, Oozeki H, Muraoka S, Tanaka S, Motegi Y, Nihei H, Yamada Y, Masuoka N, Nihei K.
Synthesis and evaluation of bibenzyl glycosides as potent tyrosinase inhibitors. Eur J Med Chem. 46(4):1374-81, 2011.
Bibenzyl glycosides 1-6 were synthesized from 2,4-dihydoxybenzaldehyde and xylose, glucose, cellobiose or maltose. The key steps in the synthesis were the Wittig reaction and trichloroacetimidate glycosylation. Tests for tyrosinase inhibitory activity showed that all were significantly active, indicating that they are unique hydrophilic tyrosinase inhibitors. Bibenzyl xyloside 2 is a particularly potent inhibitor (IC(50) = 0.43 μM, 17 times higher than that of kojic acid). These results suggest that the hydrophilic cavity of tyrosinase might accommodate the bulky carbohydrate on the bibenzyl scaffold.

- Wan P, Hu Y, He L.
Regulation of melanocyte pivotal transcription factor MITF by some other transcription factors. Mol Cell Biochem. 354(1-2):241-6, 2011.
The microphthalmia transcription factor (MITF) is discussed as the master gene for melanocytic survival and a key transcription factor regulating the expression of tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1), and tyrosinase-related protein-2 (TRP-2). MITF is influenced in a complex manner by a large number of different extracellular and intracellular proteins. Many transcription factors are able to modulate the expression and/or transcriptional activity of MITF in vivo. In this review, we summarize these transcription factors that regulate MITF and their interactions. The Sry-related HMG box 10 (SOX10) can directly transactivate the MITF gene and cooperate with MITF to activate TRP-2 expression. The Paired box 3 (PAX3) can increase MITF expression by binding to its promoter and simultaneously prevent MITF from activating downstream genes by competition for enhancer occupancy. Activated signal transducer

and activator of transcription 3 (STAT3) and protein inhibitor of activated STAT3 (PIAS3) are able to regulate transcriptional activity of MITF through their interaction. Activated cAMP response element binding protein (CREB) can bind the cAMP response element to increase the MITF gene expression. MITF expression can also be initiated by lymphoid-enhancing factor-1 (LEF-1) and be temporally facilitated by the synergy of LEF-1 and MITF. Both immunoglobulin transcription factor-2 (ITF2) and forkhead-box transcription factor D3 (FOXD3) can negatively regulate MITF expression. All the above-mentioned transcription factors constitute a regulatory network that precisely modulates the MITF.

- Ye Y, Wang H, Chu JH, Chou GX, Yu ZL.
Activation of p38 MAPK pathway contributes to the melanogenic property of apigenin in B16 cells. Exp Dermatol. 2011 May 25. doi: 10.1111/j.1600-0625.2011.01297.x.
We investigated the involvement of MAPK pathways in the melanogenic effect μm)-dependently h dose (5-20 of apigenin in B16 cells. Apigenin treatment for 48 up-regulated protein expression levels of microphthalmia-associated transcription factor (MITF) and melanogenic enzymes including tyrosinase, tyrosinase-related protein-1 (TRP-1) and TRP-2 and enhanced the phosphorylation of p38 MAPK, without affecting the phosphorylation of JNK or ERK h)-dependently elevated the μm apigenin time (6-48 MAPK. Treatment with 10 protein expressions of p-p38, MITF and melanogenic enzymes. Moreover, PD169316, a selective inhibitor of p38 kinase, suppressed the stimulatory effects of apigenin on tyrosinase activity and melanin synthesis, which were accompanied by decreased MITF protein expression. In conclusion, apigenin increased melanogenesis in B16 cells, at least in part, by activating the p38 MAPK pathway. The novel findings of this study shed light on the molecular mechanisms underlying the melanogenic activity of apigenin and suggest that apigenin/its derivatives may be potentially used for treating hypopigmentation disorders.

8. Melanosomes

(Pr J. Borovansky)

Reviews are dominant this time. In addition to the review of *Kondo & Hearing*, and to the that of *van Boorn et al.* offering an integrated view of monobenzone evoked immunity against pigmented cells and their constituents /see also *van Boorn et al /J. Invest Dermatol. 131(6): 1240-1251, 2011/* , a new book *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions* (Wiley/Blackwell 2011, ISBN: 978-3-527328925; http://eu.wiley.com/WileyCDA/WileyTitle/productCd-3527328920.html) has just appeared. It is a compendium of 13 reviews encompassing the present knowledge on melanins and melanosomes. As for melanosomes, the book covers the history of melanosome research (*J.Borovanský*), properties and functions of ocular melanosomes (*M.Rozanowska*). neuromelanin granules (*K.C. Double et al.)*, biogenesis of melanosomes (*C. Delevoye et al*.), transport and distribution of melanosomes (*M.Van Gele & J. Lamber, Colombo et al.t),* genetics of the melanosome structure and function (*V.J. Hearing*), physiological and pathological functions of melanosomes (*J. Borovansky & P. A. Riley*) and melanosomes in dysplastic naevi(*S. Pavel et al*.).

Melanosome transfer was studied by *Ando et al*. and *Choi et al*; intracellular traffic by *Bruno et al*.
(Bio)chemical composition of melanosomes. *Biesemeier et a*l compared the chemical composition of melanosomes, lipofuscin and melanolipofuscin granules in human RPE. *Wogelius et al*. demonstrated that in fossils metal zoning pattern may be exploited to map chemical residues of **the** melanin pigment after **the** melanosome structure had been destroyed.
Melanosome-specific protein MART-1 was studied by *Wang et al.*
Histopathological case reports on melanocytic pilomatrix carcinoma (*Soler et al.)* and on metastatic balloon cell melanoma (*Lee et al*.) touched melanosomes only superficially.
Other topics: *Chou et al*. have characterized a new tool how to predict the subcellular localization of proteins with melanosomes as one of 22 locations.

- Ando H, Niki Y, Yoshida M, Ito M, Akiyama K, Kim JH, Yoon TJ, Matsui MS, Yarosh DB, Ichihashi M. **Involvement of pigment globules containing multiple melanosomes in the transfer of melanosomes from melanocytes to keratinocytes.** Cell Logistics 1(1):12-20, 2011.
  The authors developed a new melanocyte-keratinocyte co-culture system using a microporous membrane filter that allows frequent and reproducible melanosome transfer and can isolate keratinocytes reliably. Using that method, a new mechanism for the transfer of melanosomes was identified where pigment globules containing multiple melanosomes are released into the extracellular space by melanocytes and are then ingested by keratinocytes.

- Biesemeier A, Schraermeyer U, Eibl O.
  Chemical composition of melanosomes, lipofuscin and melanolipofuscin granules of human RPE tissues. Exp. Eye Res 93(1): 29-39, 2011.
  Biesemeier et al. performed a thorough comparative study of the chemical composition of melanosomes, lipofuscin and melanolipofuscin granules in the RPE tissues of two donor eyes. Quantitative analytical electron microscopy techniques enabled them to compare the composition of the 3 kinds of granules in situ, i.e. without the need of prior granule isolation from the tissue, which is the strategy, as for the melanosome, introduced by *Pohla et al./Mikroskopie(Wien) 40, 1983, 273-284/* and specifically used for human RPE by *Ulshafer et al. /Arch. Ophthalmol. 108, 1990, 113-117/.* They conclude that the chemical composition of melanosomes and lipofuscin granules is different and, as for melanolysosome granules they confirmed the existence of melanin and lipofuscin moieties in them.

- Borovanský J.
  History of Melanosome Research. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions,* 1st Ed.. Ed. by J. Borovanský and P. A. Riley.
  Wiley-VCH Verlag GmbH & Co. Weinheim., pp. 1-19 , 2011.

- Borovanský J, Riley PA.
  Physiological and Pathological Functions of Melanosomes. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions,* 1st Ed.. Ed. by J. Borovanský and P. A. Riley,Wiley-VCH Verlag GmbH & CoWeinheim., pp. 343-381 , 2011.

- Bruno L, Salierno M, Wetzler DR, Despósito MA, Levi V.
  Mechanical Properties of Organelles Driven by Microtubule-Dependent Molecular Motors in Living Cells.
  PLOS One 6(4): e18332, 2011.
  Bruno et al used single particle tracking approach to obtain trajectories of the melanosomes in the processive transport along microtubules with high temporal and spatial resolutions in immortalized Xenopus laevis melanophores. The dynamics of melanosomes perpendicular to the main transport direction was quantitatively analyzed. The data interpreted

according to a model based on Langevin equation showed that the stiffness for both the kinesin and dynein transported organelles is by one order smaller than that measured for molecular motors *in vitro*. The analysis of the melanosome motion in the cells in which dynactin was disrupted revealed that the stiffness of the motor linker depends on the dynactin integrity.

- Choi EJ, Kang YG, Kim J, Hwang JK.
Macelignan Inhibits Melanosome Transfer Mediated by Protease-Activated Receptor-2 in Keratinocytes
Biol. Pharm. Bull. 34(5) 748-754, 2011.
Macelignan, isolated from *Myristica fragrans* HOUTT (nutmeg) , inhibits melanosome transfer by downregulating PAR-2, thereby reducing keratinocyte phagocytosis and PGE2 secretion, which in turn inhibits dendrite formation in B16F10 melanoma cells.

- Chou KC, Wu ZC, Xiao X.
iLoc-Euk: A Multi-Label Classifier for Predicting theSubcellular Localization of Singleplex and Multiplex Eukaryotic Proteins. PLoS ONE 6(3): e18258. doi:10.1371/journal.pone.0018258.
Predicting protein subcellular localization is an important and difficult problem, particularly when query proteins may have the multiplex character, i.e., simultaneously exist at, or move between, two or more different subcellular location sites. Most of the existing protein subcellular location predictor can only be used to deal with the single-location or ''singleplex''proteins. Actually, multiple-location or ''multiplex'' proteins should not be ignored because they usually possess some unique biological functions worthy of our special notice. By introducing the ''multi-labeled learning'' and ''accumulationlayerscale'', a new predictor, called iLoc-Euk, has been developed that can be used to deal with the systems containing both singleplex and multiplex proteins. Melanosome compartment represents one of 22 location sites detectable by means of the new predictor.

- Colombo S, Berlin I, Delmas V, Larue J.
Classical and Nonclassical Melanocytes in Vertebrates. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions.* 1st Ed.. Ed. by J. Borovanský and P. A. Riley,Wiley-VCH Verlag GmbH & CoWeinheim., pp.21-61 , 2011.

- Delevoye C, Giordano F, Marks MS, Raposo G.
Biogenesis of Melanosomes. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions,* 1st Ed.. Ed. by J. Borovanský and P. A. Riley,Wiley-VCH Verlag GmbH & CoWeinheim., pp. 247-294 , 2011.

- Double KL., Maruyama W, Naoi M, Gerlach M, Riederer P.
Biological Role of Neuromelanin in the Human Brain and Its Importance in Parkinson´s Disease. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions,* 1st Ed.. Ed. by J. Borovanský and P. A. Riley,Wiley-VCH Verlag GmbH & Co,Weinheim., pp.225-246 , 2011.

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Genetics of Melanosome Structure and Function. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis,Physiological, and Pathological Functions,* 1st Ed.. Ed. by J. Borovanský and P. A. Riley,Wiley-VCH Verlag GmbH & Co,Weinheim., pp. 323-341 , 2011.

- Kondo T, Hearing, VJ.
Update on the regulation of mammalian melanocyte function and skin pigmentation. Expert Rev. Dermatol. 6(1): 97-108, 2011.
A review, among other, devoted to the biogenesis of melanosomes, intramelanosomal pH, transport and traffic of melanosomes, and to pigmentary disorders related to the dysfunction of melanosome- related proteins. Some citations are briefly commented.

- Lee L, Zhou F, Simms A, Wieczorek R, Fang Y, Subietas-Mayol A, Wang B, Heller P, Huang H, Pei Z, Osman I, Meehan S, Lee P.
Metastatic balloon cell malignant melanoma: a case report and literature review. Int J Clin Exp Pathol.. 4(3):315-321, 2011.
A neck lymph node metastasis showed **a** complete replacement of the lymph node by large, foamy cells. Though the tumor was amelanocytic and Fontana-Masson stain failed to reveal melanin, it stained positively for S-100, HMB-45, and Melan-A. Ultrastructurally, the foamy cells were characterized by cytoplasmic vacuolization and a lack of melanosomes.

- Pavel S, Smit NPM, Pizinger K.
Dysplastic Nevi as Precursor Melanoma Lesions. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions,*1st Ed.. Ed. by J. Borovanský and P. A. Riley. Wiley-VCH Verlag GmbH & Co. Weinheim., pp. 383-393 , 2011.

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Properties and Functions of Ocular Melanins and Melanosomes. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions,* 1st Ed.. Ed. by J. Borovanský and P. A. Riley. Wiley-VCH Verlag GmbH & Co. Weinheim., pp.187-224 , 2011.

- Soler AP, Kindel SE. McCloskey G., Burchette JL.
Cell-cell adhesion proteins in melanocytic pilomatrix carcinoma. Rare Tumors 2(3): e43, 2010.
Histopathological study of a case of melanocytic pilomatrix carcinoma. Melanocytic pilomatrix carcinoma is composed of highly atypical epithelial matrix cells exhibiting atypical mitoses admixed with benign dendritic melanocytes. Pigmented dendritic melanocytes highlighted by immunohistochemical positivity with melanocyte markers S-100 , Melan-A, and HMB45 were studied with light microscopy only, hence the information on melanosomes is lacking.

- van den Boorn JG, Melief CJ, Luiten RM.
Monobenzone-induced depigmentation: from enzymatic blockade to autoimmunity. Pigment Cell Melanoma Res. Jun 20. doi: 10.1111/j.1755-148X.2011.00878.x. [Epub ahead of print], 2011.

- Van Gele M., Lambert J.
Transport and Distribution of Melanosomes. In: *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions,* 1st Ed.. Ed. by J. Borovanský and P. A. Riley. Wiley-VCH Verlag GmbH & Co. Weinheim., pp.295-322 , 2011.

- Wang J, Jia R, Yao Y, Cun B, Huang X, Gu P, Ge S, Fan X.
Differential expression of Mart-1 in human uveal melanoma cells. Mol Med Report. 4(5):799-803, 2011.
The melanoma antigen recognized by T cell-1 (Mart-1), one of the melanosome-specific proteins, has been widely studied as a marker recognized by cytotoxicity T lymphocytes In this study, the differential expression of Mart-1 was investigated in four human UM cells (SP6.5, VUP, OCM-1 and OM431) on three levels of analysis: messenger ribonucleic acid (mRNA), protein and, eventually, morphology. The results suggest its potential use in uveal melanoma therapy.

- Wogelius RA, Manning PL, Barden HE, Edwards NP, Webb SM, Sellers WI, Taylor KG, Larson PL, Dodson P, You H, Da-Qing L, Bergmann U.
Trace Metals as Biomarkers for Eumelanin Pigment in the Fossil Record. Science. 2011 Jun 30. [Epub ahead of print]
Synchrotron x-ray techniques were applied to several fossil and extant organisms, including Confuciusornis sanctus, to map and characterize possible chemical residues of melanin pigments. The results show that trace metals, such as copper, are present in fossils as organometallic compounds most likely derived from original eumelanin. The distribution of these compounds provides a long-lived biomarker of the melanin presence and density within a range of fossilized organisms. Metal zoning patterns may have been preserved long after melanosome structures had been destroyed.

9. Melanoma experimental, cell culture

(Dr R. Morandini)

Conventional chemotherapy against Melanoma is not very successful. One of the reasons is the increase of expression of DNA repair genes that contributes to the extreme resistance shown by melanoma to DNA-damaging drugs. One of the proteins involved in the DNA repair mechanism is ERCC1 that is important for both melanoma growth and resistance to cisplatin as demonstrated in a mouse xenograft model (Song *et al*. 2011). Interestingly, in mice with ERCC1-deficient melanoma grafts, cisplatin administration resulted in a complete regression of tumors after two cycles of treatments. This raises the possibility that an ERCC1 inhibitor in combination with cisplatin could be effective against melanoma.
Some tumor-derived soluble factors can contribute to immune escape and the aggressiveness of melanoma. One of them seems to be soluble HLA-E. Allard *et al*. have developed a specific ELISA to detect sHLA-E in blood to be used as a serum marker in patients.

The role of autophagy in determining the aggressiveness and therapeutic resistance of melanoma has not yet been fully appreciated. Ma *et al*. has demonstrated that patients having tumors with a high autophagic index were less likely to respond to treatment and had a shorter survival compared with those with a low autophagic index. In conclusions, autophagy seems to be a potential prognostic factor and therapeutic target in melanoma. On the other hand, autophagy can be easily studied in-vitro in three dimensional cell cultures.

MSX2 is a transcription factor acting as a key regulator in embryonic development and has been described to have a role in breast and pancreatic cancers. Gremel *et al.* have explored the functional and clinical relevance of MSX2 in malignant melanoma. Ectopic expression of MSX2 in melanoma cell lines led to the induction of apoptosis and inhibited cell invasion in a three-dimensional spheroid assay. It seems than MSX2 may be an important regulator of melanoma cell invasion and survival; furthermore, the authors identified the cytoplasmic expression of the protein as a biomarker for good prognosis in malignant melanoma patients.

Another study showing a difference expression of BRN2 and MITF transcription factors between 2D and 3D cell cultures has been recently published by Thurber *et al.* The expression is higher in spheroid than in 2D culture.

A potentially new drug, biflorin, (isolated from Capraria biflora) against melanoma has been studied by *Vasconcellos et al*. Flow cytometry analysis showed that biflorin may lead to an apoptotic death in melanoma cells, inducing DNA fragmentation and mitochondria depolarization, without affecting membrane integrity. In B16 melanoma-bearing mice, administration of biflorin increased the mean survival rate.

A. Signal transduction and cell culture

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  Functional and prognostic relevance of the homeobox protein MSX2 in malignant melanoma. Br J Cancer. 2011 Jul 5.

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  Interleukin-1{beta} Increases Baseline Expression and Secretion of Interleukin-6 by Human Uveal Melanocytes In Vitro via the p38 MAPK/NF-{kappa}B Pathway. Invest Ophthalmol Vis Sci. 52(6):3767-74, 2011.

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B. Melanin and cell culture

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Anti-inflammatory and anti-melanogenic steroidal saponin glycosides from Fenugreek (Trigonella foenum-graecum L.) seeds. Planta Med. 77(7):705-10, 2011.

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Interactions between GIPC-APPL and GIPC-TRP1 regulate melanosomal protein trafficking and melanogenesis in human melanocytes. Arch Biochem Biophys. 508(2):227-33, 2011.

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Depigmenting mechanism of NSAIDs on B16F1 melanoma cells. Arch Dermatol Res. 303(3):171-80, 2011.

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C. 3D cell culture and/or skin reconstitution

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Functional Characterization of Melanocyte Stem Cells in Hair Follicles. J Invest Dermatol. doi: 10.1038/jid.2011.195, 2011.

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D. Other tools and cell culture

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E. Melanoma Experimental

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CTLA-4 blockade increases antigen-specific CD8(+) T cells in prevaccinated patients with melanoma: three cases. Cancer Immunol Immunother. 2011 Apr 5.

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

Calendar of Events

2011 41st Annual ESDR Meeting
September, 7-10 Barcelona, Spain
Contact: Web: www.esdr.org

2011 Perspectives in Melanoma XV
September, 16-17 New York, USA
New York Marriott at Brooklyn Bridge
Chairs: JM Kirkwood and AMM Eggermont
Contact: Web: imedex.com/AppWeb/announcements/a260-01.asp

2011 XXIth IPCC
September, 21-24, Bordeaux, France
Contact: Pr Alain TAÏEB
Web: www.ipcc2011.org

2011 20th European Academy of Dermatology and Venereology Congress (EADV)
October 20-24, Lisbon, Portugal
Contact: Web: http://eadvlisbon2011.org/event/eadv-2011/

2012 XVIIth Meeting of the ESPCR
September, Geneva, Switzerland
Contact: Dr Bernhard WEHRLE-HALLER

2012 42nd Annual ESDR Meeting
September 19-22, Venice, Italy
Contact: Web: www.esdr.org

2012 5th Asian Society for Pigment Cell Research
November 3-4, New Delhi, India
Contact: Web: http://www.aspcr.org/

2013 International Investigative Dermatology
May 8-11, Edinburgh, Scotland
Contact: Web: www.esdr.org

2013 8th World Congress of Melanoma

July 18–20, Hamburg, Germany

Contact: 8th World Congress of Melanoma
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