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HAPPY NEW YEAR 2012

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

IPCC Meeting Report
September, 20-24, 2011 – Bordeaux, France

CS1: Developmental biology
Chairs: D. Bennett, T. Kunisada – B. Werhle-Haller
Contributed by all Chairs

The session was preceded by an excellent plenary guest lecture from Tatjana Sauka-Spengler (University of Oxford and previously California Institute of Technology), on gene networks regulating the neural crest (from which melanocytes develop), especially in the chick. DNA pulldown experiments were used to identify transcription factors binding to the promoter of SOX10, itself a known neural crest regulator. These included MYB, ETS1 and SOX9. The role of FOXD3 was investigated - a negative regulator of MITF and melanocytes (see also below). Reporter assays were used to identify several potential regulators of FOXD3 including ETS1, MSX1 and PAX7. Striking time-lapse videos of embryo slice cultures, using fluorescent reporters, showed falling FOXD3 promoter activity and rising SOX10 activity as neural crest cells migrated on the dorsolateral pathway. Apparent negative autoregulation by FOXD3 was also reported, with evidence for this being mediated by the polycomb repressive complex 1 (PRC1).

In the first presentation of session CS1, Wehrle-Haller (University of Geneva) reviewed recent advances, starting with lineage-tracing experiments suggesting that different neural crest cell lineages are already specified before emigration from the neural tube. Furthermore, evidence has emerged that melanocytes can migrate to their epidermal niches via the medial as well as the dorsolateral pathway. He discussed transcriptional controls in the decision of melanocytic stem cells to differentiate, and then focussed on the identification of the intra-epithelial melanocytic niche, by a specific growth-factor and cytokine signature. Analysis of this signature demonstrates a critical role for membrane-anchored Kit-ligand, which appears to go beyond the standard growth and survival promoting function.

Kunisada and colleagues (Gifu University) then reported on studies of the developmental origin of melanocytes using a Sox1-cre/YFP cell-lineage tracking system. They found that a significant fraction of skin melanocytes were derived from Sox1-cre/YFP negative cells, and therefore not from neural crest cells which are from the SOX1-expressing neural tube. Although the exact origin of these Sox1-cre/YFP negative melanocytes is obscure, the specific distribution of these cells in the head and tail skin may be a significant clue to their biological identity and origin.

Saldana-Caboverde (Florida International University) discussed the role of transcription factor ETS1 in melanocyte development. Ets1-deficient mice are hypopigmented and the embryos showed fewer melanoblasts than normal, yet no significant difference in either their proliferation or survival rates around embryonic day 11.5, suggesting that ETS1 acts before this. The pigmentation defect was

aggravated in double Ets1-Sox10 heterozygotes, suggesting that ETS1 and SOX10 synergize during early melanocyte development.

Takahashi (Nara Institute) described melanosome transfer in organ-cultured embryonic chicken skin. Electroporation of a GFP-marker enabled the analysis of intraepithelial movement of melanocytes and their melanosome-filled dendrites with high resolution. The data showed a change from rapidly migrating melanocytes to immobilized melanocytes with dendritic blebbing. Occasionally, these blebs detached and became internalized by neighbouring keratinocytes, showing for the first time melanosome transfer within skin and supporting exosomes as the mode of transfer.

Reyes-Gomez et al. (INRA-ENVA) reported on the function of the Strawberry Notch homolog 2 (Sbno2) gene in melanocyte development. They overexpressed SBNO2 in melanocyte precursors, from the Dct promoter, and found drastically reduced numbers of melanoblasts in the early embryo. Instead, numbers of DCT+ neurons in the dorsal-root and cranial ganglia were increased. They suggested that SBNO2 upregulates FOXD3, known to downregulate MITF, thus steering pluripotent neural crest cells towards neural/glial cell fates.

Transcription factors TFAP2A and TFAP2E are indispensable for melanocyte development in zebrafish. Van Otterloo et al. (University of Iowa) successfully identified SOX10 as a transcriptional target of TFAP2. TFAP2A/TFAP2C-depleted embryos lacked melanoblasts, but developed into pigmented fish upon forced SOX10 expression. This and ChIP data from human melanocytes and melanoma cells provided good evidence that TFAP2 directly regulates SOX10. They also reported direct binding of SOX10 and TFAP2A to the DCT promoter region, suggesting a possible regulatory pathway parallel to MITF.

CS2: Chemistry and biophysics of melanins
Chairs: A Napolitano - J. Menter - P Riley
Contributed by all Chairs

IL3 Physicochemical changes of retinal pigment epithelium melanin with aging and photoaging monitored by advanced EPR techniques.

The initial talk, given by Professor Tadeusz Sarna, reported a series of experiments from his Department that explored changes in the properties – especially metal-binding capacity – of retinal melanin as a function of ageing. On the basis that the major factor in the 'ageing' process was exposure to light, photoageing was investigated using appropriate in vitro model systems. The physical changes induced by light exposure were studied by advanced EPR techniques including 95 GHz continuous wave and X-band saturation recovery electron paramagnetic resonance methods. The data showed that, to a considerable extent, the age-dependent changes in certain key physicochemical properties of RPE melanin are mimicked by photo-bleaching.

IL4 Chemistry and biophysics of melanin.

Neuromelanin (NM) is a brown, insoluble pigment abundant in catecholaminergic neurons in the substantia nigra and locus caeruleus that has been implicated in the etiology of Parkinson's Disease. The biosynthesis and biodegradation of NM has not been elucidated, and there is at least one school of thought that suggests that it may be formed in a random manner via autoxidation of dopamine (DA). The work reported by Professor Wakamatsu described attempts to elucidate these pathways by synthesizing four standard compounds thought to be internal intermediates in NM

synthesis: 5-cysteinyldopamine (CDA), dihydrobenzothiazine – 1 (DHBT – 1), 3-oxodihydrobenzothiazine–1 (ODHBT – 1), and benzothiazoleamine – 1 (BZ - 1). Biodegradation was followed by various heating conditions (100o for 24 hr, 60o for 24 days, 37o for 120 days). Classical, albeit harsh, chemical degradation methods (alkaline H2O2 oxidation, reductive HI hydrolysis) were applied to further elucidate the synthesis and aging process. Results suggested that the benzothiazine moiety is converted to benzothiazole. Comparison of the ratios of degradation markers showed that natural NM is closest to that prepared by heating a synthetic NM – Cys 2:1 molar ratio at either 100o for 8 hr, 60o for 8 days, or 37o for 120 days. The authors concluded that by mimicking this process, it has now become possible to prepare synthetic NM with structural features of natural DM.

C5 Discovery of isoquinoline-containing dimers as the fundamental building blocks of the human red hair pheomelanin.

Dr. Panzella from the Naples' group proposed isoquinoline-containing dimers as fundamental building blocks of natural pheomelanins from human red hair. Originally put forward in the sixties by Professor Minale in Naples, this hypothesis was neglected for many years. Now isolation of benzothiazolylthiazinoisoquinoline dimers from biomimetic oxidation of the pheomelanin precursor, 5-S-cysteinyldopa, coupled with identification of a highly specific structural marker of these units has allowed the original proposal to be put on a firm experimental basis. Interestingly, the isoquinoline dimers showed the same spectral features in the visible region as red hair pheomelanin.

C6 Probing the melanosome surface using molecular rulers.

Dr. Glass, from Professor John Simon's laboratory, described a very ingenious method of measuring the distance between carboxylic acid functions of melanins using isothermal titration calorimetry on Sepia and choroid melanosomes. This method employed diamines with a range of linear interionic distances. Under carefully controlled deprotonated conditions it was possible to estimate the distance between the nearest carboxylic acid groups as approximately 38 nm.

C7 Is melanin a semiconductor: the mysteries of electrical conduction and melanin biosynthesis?

Professor Meredith presented work that reinvestigates the question as to whether the standard model of solid-state melanin as an amorphous semi-conductor is correct. This model does not explain a number of phenomena such as apparent ambipolar behavior and humidity-dependent electrical conductivity. These workers used muon spin relaxation, EPR, and conductivity measurements. They found that melanin has the characteristics of a hybrid proton-electron conductor whose electronic biophysics is dominated by ionic behavior originating from a "comproportionation equilibrium" whereby protons are released in the hydroquinone-quinone reaction. Although the problem is complicated, as the presenter asserted (e.g. how "dry" is humid melanin?), this group believe that a more complete understanding of ion to electron transduction is a key element in bioelectronic interfacing.

C8 Rare melanoma cell detection by thermal emission.

Dr. Amblard concluded the session with a remarkable presentation in which he demonstrated a novel melanoma detecting methodology based on very rapid transient heating of blood in vivo. This procedure was able to heat melanosomes to a sufficiently high temperature to cause thermoemission by melanin detectable in the visible or near intfrared range. This process was apparently non-damaging to blood cells and was an extremely sensitive method of detecting single circulating melanocytes. There was considerable discussion regarding the details of heat

conduction and the intrinsic clinical safety of the procedure.

CS4: Mouse models: pigment cell biology & melanoma
Chairs: L. Montoliu – W. Pavan – E. Nishimura

Contributed by L. Montoliu

This session started with a review presented by Lluis Montoliu (Spain) on mouse models for studying pigment cell biology and pigmentary diseases, where he summarized most of the work done in the field, illustrating the several sources of information currently available, focusing in the IKMC (International KnockOut Mouse Consortium) initiative, and related platforms, aiming to produce and distribute mouse models carrying mutations on every gene of the mouse genome. Bill Pavan (USA) continued with a talk on his innovative and very productive genetic approach to identify new Sox10 modifier loci, using a sensitized ENU mutagenesis screen. The talk included the preliminary description of new mouse mutants that will appear eventually published. These two invited lectures were followed by four short oral presentations. First of them corresponded to Alain Eychène (France), who presented his work on mouse models for understanding Raf signaling in melanocyte and melanoma development, where single or compound ablation of BRAF and CRAF is achieved upon conditional Cre expression in the melanocyte lineage. Second short talk was by Y. Kotobuki (Japan), on their studies on periostin, a new protein found associated to extracellular matrix that promotes tumor growth and progression in cutaneous malignant melanoma. Third short talk was delivered by Eirikur Steingrimsson (Iceland), who summarized his work with an induced suppressor mutation at the microphthalmia locus, resulting in novel insights into bHLHZip transcription factor function. The data he presented indicated that the carboxyl-end of MITF is not essential for normal function in melanocytes and appears to have negative function in the wild type context. Finally, the session concluded with a presentation by Y. Funasaka (Japan) where results shown supported the notion that ultraviolet B, but not ultraviolet A, initiates and promotes melanoma formation in metabotropic glutamate receptor 1 transgenic mouse.

CS5: Chemistry of melanins: standardization workshop roundtable
Chairs: M d'Ischia – S. Ito – JC. Garcia Borron – J. Simon

Contributed by M. d'Ischia, S. Ito and J-C. Garcia Borron

This session was entitled "Methods in Melanin Research" and was formatted as a round table centered around the launching project of the EuMelanet group, that is, the definition of a set of recommended protocols and procedures to be followed for studies of melanins and melanogenesis.
Marco d'Ischia opened the session with a brief summary of the main aims and scope of the EuMelaNet group, and gave an overview of the round table programme.
Jose-Carlos Garcia-Borron raised important and relevant issues concerning enzyme sources and sample preparation for studies of melanogenesis: Are crude melanoma extracts with defined tyrosinase activity suitable enzyme preparations for studies of melanogenesis ? Is the fungal enzyme suitable for studies of melanogenesis ?

Shosuke Ito illustrated the method developed by his group for analysis of tissue melanins based on alkaline hydrogen peroxide degradation. He recommended TTCA (thiazole tricarboxylic acid) as a useful marker of pheomelanins allowing together with PTCA (pyrroletricarboxylic acid) quantitation, the simultaneous determination of eumelanins and pheomelanins.
In the following presentation he went on to review convenient methods for preparation of 5,6-dihydroxyindoles and of cysteinyldopas by the classical dopa/tyrosinase based protocols with modifications. He also addressed the issue of the preparation of analytical markers, namely pyrrolecarboxylic acids (PTCA and PDCA), thiazolecarboxylic acids (TTCA and TDCA), AHP and synthetic eumelanins.
In the subsequent presentation Alessandra Napolitano advocated the value of the benzothiazole BTCA as a more selective pheomelanin marker, easily available by a one pot biomimetic procedure and highly pigment-specific. She also showed the most convenient approach to BTCA synthesis and the alternate methods for gram-scale synthesis of cysteinyldopas. A lively debate followed in which the various methods were critically examined by the Japanese and Neapolitan groups.
Alessandro Pezzella talked about synthetic eumelanins to show that substrates, reaction conditions and isolation/storage protocols may affect significantly the overall structure and properties of synthetic eumelanins. To this aim, the potential of mass spectrometric analysis to distinguish synthetic eumelanins was discussed. It was concluded that preparation protocols may vary depending on the purpose and research goal (structural/biomimetic study or material synthesis).
Paul Meredith contributed with a presentation recapitulating the basics of the chemical disorder model and arguing against major differences between various synthetic eumelanins as judged by morphological analysis, in which stacked architectures were invariably detected. He emphasized that, from the materials science perspective, cost effectiveness and substrate availability are more critical parameters than close control of structural features.
Finally a presentation by Hidekazu Okuda underlined the potential of the theoretical approach to investigate and predict melanin structure besides the classical biosynthetic and degradative methodologies.
Overall, the meeting featured a series of well tuned presentations from different viewpoints which stimulated lively and constructive discussions. Eventually it was agreed that the presentations debated at CS5 were worthy of further assessment and elaboration by the group to generate a consensus paper.

CS8: Update on physiology of cutaneous pigmentation
Chairs: Z. Abdel-Malek – S. Moretti – G. Imokawa

Contributed by Z. Abdel-Malek

IL12 by Zalfa Abdel-Malek: The presentation was a review of the paracrine factors synthesized by keratinocytes and fibroblasts and autocrine factors that regulate human melanocytes. During the past few years, it became evident that some of these factors participate in the response of melanocytes to UV radiation. The primary cytokines interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α) are increased in expression upon UV irradiation of keratinocytes, and in turn IL-1 increases the expression of endothelin-1 (END 1) and α-MSH by these cells. Endothelin-1 and α-MSH interact synergistically to stimulate human melanocyte proliferation and melanogenesis. More recently it was found that these two factors reduce UV-induced

apoptosis and generation of reactive oxygen species (ROS), and enhance repair of DNA photoproducts. The effects of α-MSH are mediated by binding the melanocortin 1 receptor (MC1R), and this results in reducing ROS generation, increasing catalase activity and protein levels, and activating the transcription factors ATF2, Nrf2 and p53 in UV-irradiated melanocytes. Endothelin1 binds the endothelin B receptor on melanocytes, and interestingly, this increases the expression of the MC1R gene. The keratinocyte-derived factor human beta-defensin 3 (HBD3) is a newly identified paracrine factor for melanocytes. HBD3 functions as an antagonist for the human MC1R, inhibiting the α-MSH-induced activation of adenylate cyclase. Nerve growth factor is another keratinocyte-derived factor that inhibits UV-induced apoptosis. An interesting and newly identified fibroblast- and keratinocyte-paracrine factor for melanocytes is neuregulin 1, which is expressed at higher levels in fibroblasts derived from dark skin than in fibroblasts from light colored skin, suggesting a role in determining constitutive pigmentation.

IL13 Genji Imokawa: The role of END 1 and stem cell factor (SCF) in stimulating pigmentation and the mechanisms by which they induce this effect were discussed. The synthesis of both END1 and SCF by keratinocytes is induced upon UV exposure. The crosstalk of the signaling pathways that are activated by these two factors (PKC, tyrosine kinase and Ca+2 mobilization) results in stimulation of the MAPK pathway, and RSK, which in turn phsophorylate CREB and hence MITF. Blocking any of the signaling pathways induced by END1 or SCF inhibited activation of Mitf and expression of its target genes. Using a reconstructed human epidermal equivalent, the stimulatory effect of END 1 and SCF on pigmentation was demonstrated. This experimental model was also used to test the effects of melanogenic inhibitors and herbal extracts on the hyperpigmentary effect of END 1 and SCF. Treatment of the reconstructed human epidermal equivalents with the MEK inhibitor PD98059 inhibited the stimulation of pigmentation by both END1 and SCF, while treatment with the PKC inhibitor Go6983 only inhibited the effect of END, since the effect of SCF is known to be mediated by activating the tyrosine kinase receptor c-kit, not PKC.

C28 D. Kovacs, from Mauro Picardo's group reported on novel effects of DHICA on primary human keratinocyte cultures. Treatment of keratinocytes with micromolar concentrations of DHICA reduced their proliferation, enhanced their differentiation, increased the expression of superoxide dismutase and catalase, and inhibited apoptosis following UVA exposure.

C29 S.G. Coelho from Vincent Hearing's group reported on the use of antibodies that they raised against pheomelanin (DOPA + Cys-melanin), DHI-melanin, DHICA-melanin, and DHI/DHICA melanin. Immunostaining of cultured human melanocytes and skin followed by confocal microscopy proved that these antibodies are valuable for investigating the distribution of eumelanin and pheomelanin in melanocytes and skin with different color. The use of these antibodies was found to be superior to the tranditional Fontana-Mason stain, which is commonly use to visualize melanin.

C30 M. Cario-Andre from Alain Taieb's group described the impact of fibroblasts on pigmentation of human epidermal reconstructs (HER) xenografted onto nude mice. The extent of pigmentation induced upon grafting of the HER correlated with the density of fibroblasts. This effect was reproduced in vitro, and it was fond that hyperpigmentation was associated with an increase in FGF2, which stimulated melanin secretion, and cytokeratin 5 that increased melanosome transfer. Upon comparison of lesional and paralesional skin of a patient with erythema multiforme that resulted in pronounced hypopigmentation, increased FGF2 was observed in lesional skin where melanocytes were either absent or non-functional. The role of

FGF2 in modulating pigmentation is being further examined in HER containing fibroblasts over expressing FGF2.
C31 C. Duval from L'Oreal investigated the effects of fibroblasts on the pigmentation of 3-D skin model composed of fully stratified and pigmented epidermis onto a fibroblast-containing dermal compartment. In the absence of fibroblasts, the 3-D skin model was hyperpigmented. When fibroblasts from donors with different skin type, age, and sun-exposed versus sun-protected sites were included in the 3-D skin model, the extent of pigmentation correlated with the characteristic of the fibroblasts included. Treatment of fibroblast-containing 3-D skin model with Pro-Xylane, a modifier of the extracellular matrix, resulted in hypopigmentation. These results underscore the role of the dermal compartment in modulating skin pigmentation.

Plenary session IV: Photoprotection and beyond: from melanosomes to melanins.

Chairs: M. d'Ischia – K. Wakamatsu – T. Passeron

Contributed by all Chairs

The session started with an invited lecture by **Villy Sundstrom** from Lund University who provided an insightful and inspiring overview of the photochemistry and excited state dynamics of eumelanin building blocks. After an initial description of the basic principles and applications of femtosecond pump probe spectroscopy, he went on to illustrate the approach selected for investigations of eumelanin photoprotective role and mechanisms, showing the potential of a bottom up strategy addressing in sequence the basic photophysical behaviour of fundamental bulding blocks from the monomer to the oligomer stage. The different excited state deactivation mechanisms of DHI and DHICA were highlighted in terms of energy dissipation and free radical formation, the role of excited state intramolecular proton transfer processes was addressed and the perspectives in the field were delineated.

The next invited lecture by **E. Sprecher** from Tel Aviv Sourasky Medical Center is an overview of keratin disorders associated with abnormal pigmentation. He introduced that Epidermolysis with Mottled Pigmentation are caused by mutations in keratin genes, and the identification of various genes has revealed the role of keratin in epidermis which extends to various and critical non-mechanical functions.

A contribution by **John D. Simon and D. N. Peles**, from Duke University, dealing with "Ultraviolet Absorption Properties of Melanosomes Measured by Photoemission Electron Microscopy" was presented by **Dr Glass** from the same laboratory. She provided an interesting and stimulating overview of the potential of the PEEM technique to inquire into the absorption cross section of melanosomes and to elucidate the contribution of the monomer precursors. The general absorption features of melanosomes were discussed in the light of the monomer composition of the melanin components.

Alessandra Napolitano and co-workers, from University of Naples Federico II, reported new data in support of the marked pro-oxidant properties of synthetic pheomelanins in the absence of light. These properties were suggestive of a possible non-enzymatic casing mechanism for the assembly of natural melanins based on the deposition of a eumelanin shell by oxidation of dopa or a suitable precursor on a redox active pheomelanin core.

G. Barsh and coworkers from Stanford University presented their exciting and interesting works regarding the analysis of stripes and spots in mammals by using

genetic approach. Using this approach they found that at least one variation in pattern type is caused by recessive inherited loss-of-function in a gene conserved among all vertebrates. They also introduced a highly sensitive and robust methodology-EcoP151-tagged Detection of Gene Expression among tissues from animals including dogs, cheetahs, zebra.

T. Wiesner and coworkers from Memorial Sloan-Kettering Cancer Center reported a new autosomal dominant syndrome characterized by multiple skin-colored, elevated melanocytic tumors. Segregating with the phenotype of uveal and cutaneous melanomas, they found inactivating germline mutations of the BAP1 gene. Their findings are that BAP1 is a novel susceptible gene for melanocytic neoplasia and a form of epithelioid melanocytic nevus.

Overall, the session was very stimulating and raised the interests of the audience with several questions to the speakers and lively discussions.

CS11: Vitiligo: basic science & medical, clinically-oriented
Chairs: S. K. Hann – T. Anbar – P. Manga

Contributed by T. Anbar

Prof. Tag Anbar, M.D. co-Chaired the session with Dr. P. Manga and Dr. SK Hann.
It was an afternoon session started at 2:30pm by a presentation by Dr. Silvia Moretti (Italy) who talked about the Vitiligo 2011 Update summarizing the recommendations of the VETF. The presentation was interesting to most of the audiences.
The second presentation was by Dr. R Attili about Acrofacial and genital depigmentation is a new pattern disease with features of both vitiligo and vitiligoid lichen sclerosus- a clinical and hispopathological review of 58 cases which was a prospective study analyzing the cases presented by genital and facial depigmented lesions and showing features of lichen sclerosus on histopathological examination. (Confidential comment: discussions were raised reflecting the feeling that some of the audiences were not totally convinced by the presentation and I am afraid this was my feeling as well).
The third presentation was done by Dr. SK Attili on: Histopathological staging of vitiligo lesions and its implications for treatment. Vitiligo lesions were classified into 3 types histopathologically; early with inflammatory infiltrate which responds well to steroids or immuno-suppressants, stable with no inflammatory changes or melanocytes which responds to treatments enhancing melanocytes' migration and long standing cases which benefit mainly of surgical therapies. The presentation was good and interesting.
The fourth presentation was by Dr. J Mosenson on the use of mutant HSP70i to treat vitiligo. It was an animal study supporting the role of this heat shock protein in prophylaxis against depigmentation and highlighting its potential role in treatment of vitiligo.
The last presentation in this session was done by Dr. R Kumar on LXR-a as molecular switch that initiate from vitiligo lesional skin to re-pigmented
skin which highlighted the role of LXRs in the skin in decreasing the melanocyte adhesion and melanocytorrhagy and showed a possible role of targeting this factor when treating vitiligo.
As far as I can remember, Dr. R Yu did not show for his presentation.
The session as a whole was successful and the audiences showed a great interest for what was presented.

CS14: Vitiligo: surgical-instrumental, clinically-oriented
Chairs: N. Rabobee – S. Mulekar – E. Lan

Contributed by Cheng-Che Lan

This session started of with recent advances on use of monochromatic light for treating vitiligo by Dr. Lan from Taiwan. In the presentation, it was shown that monochromatic excimer light is able to induce vitiligo repigmentation more rapidly due to its higher irradiance. In addition, visible light emitted by Helium-Neon laser was able to induce vitiligo repigmentation via stimulation of mitochondria. Since this device is relatively safe, it can be used for pediatric patients and for lesions requiring special consideration such as for lesions around the periocular lesion. The second lecture was presented by Dr. Mulekar from Saudi Arabia. He described his experience of surgical procedures in childhood vitiligo. He stressed the importance of cell transplantation for stable vitiligo, even for pediatric population. Some concerns from the audience include the safety issue for anesthesia for young children. Dr. Araujo from Brazil presented her work using needling technique for treating vitiligo. Many physicians have shown interest in this needling technique after viewer her impressive video, and it is certain that more reports commenting on this technique will soon appear. Finally, Dr. Ghia from India demonstrated that standard technique using trypsin for preparation of cellular grafting is more effective as compared to non-trypsized technique.

Plenary session V. Fundamental aspects of the initiation and progression of melanoma.
Chairs: R. Luiten – N. Hayward – R. Ballotti

Contributed by all Chairs

The guest lecture was given by the director of the RIKEN Omics Science Center, Dr. Yoshihide Hayashizaki who initiated the FANTOM (Functional Annotation of Mammalian Genome) consortium. This consortium aims to identify the transcriptional regulatory networks that define every human cell type. Using the huge deep-sequencing capacity at Riken institute and systematic siRNA knockdown approach they have been able to define complex networks that allow the maintenance of differentiated or proliferative cellular states, as well as the switch from proliferation to differentiation. Focusing on monoblast to monocyte differentiation, they identified aprroximately 30,000 promoters activated during this process. This knowledge opens the way to regenerative medicine applications to generate all types of human tissue or cell types from undifferentiated precursors.

An invited lecture was given by Nick Hayward who reported the discovery of a germ line MITF mutation that predisposes to melanoma both in a familial and sporadic context. Individuals with multiple primary melanomas and a family history of melanoma have more than 8 times the likelihood of being carriers than unaffected controls. This mutation leads to the replacement of glutamic acid at position 318 by a lysine (E318K) within a MITF SUMOylation site and impairs severely MITF SUMOylation. The E318K MITF has altered transcriptional activity towards some of its target genes that could favour melanoma development.

Four short papers were presented in the session. Robert Ballotti also presented results on the MITF E318K mutant. The French study found this mutation to be associated both with melanoma and kidney cancer. In addition to the modification of the target gene repertoire, MITF E318K was shown to increase melanoma and kidney cancer cell migration and invasiveness. A special focus was made on HIF1A which is strongly up-regulated by MITF E318K compared to wild type MITF.
A paper from Dr Arup Kumar Indra's group presented a new mouse model with selective ablation of RXRa and RXRb in melanocytes. They observed increased apoptosis in dermal cells upon UV treatment, while melanocytes appeared to be more resistant to UV radiation.
Dr Frances Noonan presented provocative data. Using albino and pigmented HGF/SF transgenic mice that have a "humanized skin" with extrafollicular melanocytes, Dr Noonan showed that melanin favoured melanoma development after UVA exposure, but not after UVB, providing experimental support for increased melanoma risk in UVA tanning bed users.
Finally a paper from Erikur Steingrimsson's group presented X-ray crystallography and classical DNA binding studies that help to understand the DNA binding specificity of MITF and the dimerization process with different partners. This approach will provide new insights into the functional duality of MITF acting either as an oncogene or as an anti-oncogene in melanoma.

CS17: New pathomechanisms in melanoma
Chairs: F. Meyskens, M. Soengas, N. Basset-Seguin

Contributed by F. Meyskens

Five areas of newly described molecular pathologic mechanisms underlying the process of melanomagenesis were presented by groups from four different countries, (USA, Spain, UK, and France). These include:

a. The neural nitric oxide synthase/nitric oxide pathway intrinsic to melanocytes that becomes aberrantly regulated.
b. The RAB GT Pases that regulate late-endocytosis, liposomal degradation, and macro-autophagy.
c. Keratinocyte/melanocyte early melanoma interactions with demonstration of mutant p16 as critical to progression promoted by TERT.
d. Demonstration that the Pmel 117 (a differentiation antigen) has a non-structural role by binding FHL2.
e. Senescence of melanoma cells produces a secretory profile that has a protumoral and pro-metastatic profile.

Although early in our understanding of the mechanisms involved, each of these recently explored/discovered pathways was demonstrated to have different and diverse effects on proliferation, differentiation, senescence or autophagy. These studies overall open up entire new areas of investigation that will lead to better and more complete understanding of the remarkable cell that is the melanocyte and what happens at a fundamental level when transformation and progression occurs both intrinsically and in relationship to the surrounding cellular and extracellular matrix. Several early experiments presented in these presentations suggest that it is likely that we will soon see the development of new therapeutic approaches for the prevention and treatment of melanoma emanating from these basic observations.

CS19: Preclinical & Clinical advances in melanoma management (SMR-IPCC)

Chairs: P. Chapman – G. Ghanem – P. Saiag

Contributed by G. Ghanem

IL39: R.D. Carvajal reported an on going clinical selecting melanoma patients on the basis of the presence of kit mutations or amplification for treatment with the multikinase inhibitor Imatinib. The trial was conducted in GIST and melanoma. They observed better responses and improved survival specially where a positive selection of mutated kit (mut/wt ratio) in metastatic lesions.

IL40: The emerging role of Tyrp1 as a melanoma marker has been presented by G. Ghanem. Tyrp1 mRNA expression levels in metastatic tissue were correlated to patients' survival and to important prognosis markers at the primary lesion, namely Breslow thickness. Data and many arguments suggest the implication of Tyrp1 in proliferation and invasion.

C86: M. Hossain *et al.* studied telomeric crisis in cells overcoming senescence from primaries RGP-VGP and metastatic lesions. VGP and metastases shared many common features suggesting that cells may metastasize before full immortalization. However, it seems that very few cells in melanoma primaries are able to evade both senescence and telomeric crisis as very few can be expanded in culture.

C87: D.S. Widmer *et al.* Examined the role of hypoxia in phenotype switching in a model for melanoma progression. Microarray data showed an up-regulation of invasion-related genes and prolonged hypoxic conditions lead to increased invasion ability of cells belonging to the proliferative phenotype. Data in biopsies also suggest a loss of markers within hypoxic regions. In addition, based on gene expression profiles, they developed an algorithm able to define phenotypes and predict their switching.

C88: M. Tichet *et al.* studied metastatic process at the vascular level. They explored the role of SPARC that is overexpressed by melanoma cells and that have been previously shown to be associated with poor survival. The study provides evidence for SPARC promoting ability of transendothelial migration of tumor cells that is dependent on VCAM-1 expression by activated endothelial cells. The authors propose SPARC as a target for therapy to limit metastatic spread.

C89: A.P. Benaduce described a technique called nanoindentation to measure the mechanical properties of normal and malignant melanocytes. They found significant differences between these, suggesting that tumor cells were more elastic. When exposed to Endothelin 3, only normal melanocytes showed a decrease in their hardness and stiffness similar to those of melanoma cells. The authors believe that their technique may be useful to distinguish between normal and transformed cells.

CS21: DNA repair and melanoma molecular biology

Chairs: A. Sarasin – H. de Verneuil

Contributed by all Chairs

The symposium dealt with the role of DNA repair pathways in human diseases and their association with the risk of melanoma development.

A. Sarasin made a review on DNA repair-deficient diseases in terms of clinical and biological descriptions. The complicated relationship and interactions between at least 11 DNA repair genes involved in nucleotide excision repair (NER) and the four major diseases (xerodermapigmentosum, Cockayne syndrome, trichothiodystrophy and UV-sensitive syndrome) were described and explained in the context of clinical outcomes. The various technologies developed for a possible gene therapy of XP were detailed by A. Sarasin. The use of recombinant retroviruses makes it possible, now, to produce *in vitro* reconstructed XP-C skin fully complemented for the DNA repair deficiency. The use of homologous recombination, following the induction of a unique double-strand break induced by specific meganucleases and produced next to the *XPC* mutation to be corrected, allows to fully correct a given mutation in XP-C fibroblasts. These new technologies represent an open avenue toward a specific gene therapy treatment of XP-C patients.
H.R. Rezvani (Inserm U1035, Bordeaux, France) focused its talk on the implication of XP-C deficient cells (XPC^{KD}) in understanding of cancer initiation steps. H.R. Rezvani showed that knockdown of XPC in keratinocytes reduces mitochondrial oxidative phosphorylation and increases glycolysis, a hallmark observed in most cancer cells. The critical step in this process is the activation of bypass repair systems which helps cells with unrepaired DNA escape senescence and death. H.R. Rezvani showed that activation of DNA-dependent protein kinase results in upregulation of AKT and NADPH oxidase-1 (NOX1) with a concomitant increase in reactive oxygen species (ROS) production. The XPC^{KD} were able to form squamous cell carcinomas (SCCs) when implanted into immunodeficient mice. Impairment of AKT or NOX activation in XPC^{KD} cells blocks the formation of ROS and neoplastic transformation. The knowledge gained by studying XPC silencing-mediated tumoral transformation of normal human keratinocytes has given a greater insight into the contribution of metabolism alteration and ROS accumulation to skin cancer. Further elucidation of the molecular mechanisms involved in skin cancer formation may ultimately lead to implement new strategies for the prevention of skin cancers and also other cancers.

H.H. Hu (Inserm U976, Paris, France) focused its talk on the role of the various classes of melanocortin receptor-1 (MC1R) alleles, especially non-red hair colored (RHC) alleles and rare MC1R variants, as their role on melanoma risk is still debated. MC1R variants from 1131 cutaneous melanoma and 869 skin cancer patients were classified in two main categories: R (RHC) and r (non-RHC). All known frequent MC1R variants (including R and r alleles) were found in this study. R alleles were strongly associated with melanoma (P = 1.09E-33; OR = 3.295). Interestingly, r alleles were also associated, although less strongly, with melanoma (P = 1.1E-15; OR = 1.839). In addition, multivariate analysis showed that both R and r variants also associated with melanoma risks. R but not r variants associated with familial and multiple melanoma risks. These findings may have important consequences in defining high-risk melanoma factor sub-groups and/or help to the genetic council in melanoma families.
S. Corre (CNRS-UMR6061, Rennes, France) described the specific defense machinery of the cell to maintain the integrity of the genome. NER, one of the most versatile DNA repair systems, is divided into at least two sub-pathways depending on the localization of the distorded DNA: Global Genome Repair (GGR) and Transcription Coupled Repair (TCR). S. Corre showed that the expression of CSA and HR23A , two factors involved in TCR and GGR respectively, is up-regulated after UV induced DNA damage in the skin. This up-regulation is driven by a common p53

independent mechanism involving the stress responsive Upstream Stimulating Factor 1 (USF1). The current study therefore extends the repertoire of USF1 target genes mediating skin protection against UV. Importantly, this regulation of CSA and HR23A by USF1 is physiologically significant, as demonstrated by a genetic approach with knock-out mice. Results from this study provide compelling evidence that USF1 plays a key role in DNA-repair and in maintaining genome integrity.

C. Leikam (University of Wurzburg, Germany) described the process of evasion of melanocyte senescence as a major pathway toward transformation into melanoma. Senescence is a mean to avoid tumoral transformation partly linked to reactive oxygen stress. In melanocyte, a complex interaction between MYC and its suppressor partner MIZ1 regulates the senescence evasion *via* ROS production. The cystathionase gene (*CTH*) codes for a protein able to modulate the MYC effect during the production of endogenous ROS. Inhibition of CTH reconstitutes cellular senescence *in vitro*, enhances melanocyte senescence and reduces proliferation of melanoma cells. This result may represent a new target for avoiding melanoma appearance.

V.P. Swope (University of Cincinnati, USA) reported the regulation of γH2AX in human melanocytes treated by UV-light following or not the activation of the MC1R receptor by the αMSH hormone. γH2AX is induced in melanocyte by UV with a kinetics similar to the one describing repair of the UV-induced pyrimidine 6-4 pyrimidone photoproducts. αMSH pretreatment increases γH2AX production independently of free radicals, as well as CHK1 and CHK2 phosphorylation, probably through ATR/ATM stimulation. This result suggests that MC1R activation by αMSH associated with enhanced γH2AX production may explain the enhancement of DNA repair of some UV-induced DNA lesions observed in human melanocytes.

Plenary session VIII Translational research/Miscellaneous
Chairs: H. Arnheiter – D. Gawkrodger – F. Tison

Contributed by all Chairs

The Plenary Session VIII on “Translational Research” included a component “Miscellaneous” and took place in the morning of the last day of the IPCC. It was opened by the guest lecture delivered by **T. Luger**, Münster/Germany, who reported on the immunomodulatory role of alpha MSH. Previous studies have found that a carboxyl terminal fragment of alpha MSH has anti-inflammatory activities, and T. Luger presented results with KdPT, a tri-peptide with structural similarities to the carboxyl-terminal tripeptide KPV, that has potential for treatment of inflammatory disorders without affecting pigmentation. **L. Zecca**, Milan, Italy, gave the second guest lecture presenting an overview on chemistry, structure, distribution and functions of neuromelanins, which can be both protective as they remove reactive quinones and metals but also toxic when they are released from dying neurons, such as in Parkinson’s disease. The presentation by **C. Grill** from the University of Iceland centered on the interferon regulatory factor-4 (IRF4), a member of a family of proteins regulating interferon transcription. Interestingly, polymorphisms in the IRF4 gene are associated with variations in skin pigmentation and eye color in humans, and C. Grill presented results showing that IRF4 is regulated by MITF, and that MITF and IRF4 together regulate the expression of Tyrosinase though not other pigmentary genes. Moving from the molecular biology of melanocytes to their migratory behavior, **R. Mort**, Edinburgh, United Kingdom, presented live imaging of GFP labeled

melanoblasts in explant cultures of embryonic mouse skin and showed evidence for the previous in vitro observation that KITL regulates their migration. L. Mort then used mathematical models to explain the dispersal of melanoblasts during development. **T. Cheng**, New York, United States of America, examined the adaptation of melanocytes to endoplasmic reticulum (ER) stress, which triggers the unfolded protein response (UPR), using OCA2 null melanocytes, in which tyrosinase is partially retained in the ER. Such adaptive phenomena may play a role in resistance of melanoma cells to hypoxia or in vitiligo. T. Cheng showed that some of the downstream UPR signals are not activated in melanocytes as they normally are in other cells, suggesting that their analysis will become important in delineating potential targets for melanoma and vitiligo therapies. The final presentation in this interesting Plenary Session was given by **J. Pawelek**, New Haven, United States of America, who reported on a correlation between an autophagosome marker, L3CB, and the proliferative state/poor outcome of melanomas as well as breast cancers. The study underscores the importance of autophagy for cancer progression and suggests therapeutic avenues against these devastating diseases.

CS23: Albinism: Basic science and patient-oriented session, clinically-oriented.

Chairs: T. Suzuki – R. Aquaron – B. Arveiler

Contributed by all Chairs

First, Dr. Arveiler (France) talked on the genetics of oculocutaneous albinism. He summarized four types of non-syndromic OCA and X-linked ocular albinism (OA1), and also syndromic forms of albinism including Piebaldism, Waardenburg syndrome (WS1-4), Hermansky-Pudlak syndrome (HPS1-7), Chediak-Higashi syndrome (CHS), and the very rare Griscelli syndrome (GS1-3). The relative frequency in Caucasian and Asian patients of the different forms of OCA can be roughly estimated as follows: OCA1 50%, OCA2 30%, OCA3 3%, OCA4 17%, and about 20% of patients remain without diagnosis, suggesting that other albinism genes remain to be discovered.
Dr. Suzuki (Japan) summarized the characteristics of OCA found in Japanese population, and talked about two topics, a patient recently diagnosed as OCA3 which was very rare in non-African populations, and OCA2 gene contributing to the skin color of normally pigmented Japanese.
Dr. Li (China) spoke on the optimized strategy for genetic testing of the Chinese patients with oculocutaneous albinism. Among the 179 Chinese OCA patients, 115 (64.3%) were found mutations on TYR gene, 21 (11.7%) on OCA2, 28 (15.6%) on SLC45A2, 4 (2.2%) on HPS1, and 11 (6.2%) patients uncharacterized. Common alleles have been revealed in the TYR and SLC45A2 gene in the Chinese OCA patients.
Dr. Moltó (Spain) talked on the albinochip. More than 500 mutations have been reported in those 14 loci, causing albinism, as obtained from the literature and human gene mutation databases. They have developed a new technological strategy aiming to a universal genetic diagnosis of all types of albinism. This approach uses iPlex methodology, by Sequenom, which combines automated array processing of human subject's DNA samples with mass spectrometry (MALDI-TOF) and can be applied to discriminate, simultaneously, up to 1000 different alleles with known mutations, from a given DNA sample. The "albinochip" proposal aims to define a universal genetic

test for all known forms of albinism using massive genomic approaches with the aim of detecting any known mutation in any of the affected genes.
Dr. Aquaron (France) made a speech about a story of the p.Gly47Asp mutation on the tyrosinase gene and personal data about 5 cases with OCAIA and OCAIB. The history of the Sephardic (Spanish) and Moroccan Jewish populations may provide clues to the origin and spread of the p.Gly47Asp is (to suppress) mutation.

Finally, Dr. Cullinane (USA) presented about new HPS subtype, HPS-9, They identified homozygous nonsense mutations in PLDN, encoding the BLOC-1 subunit, pallidin, in a single individual with characteristic features of HPS. They also identified only the second mutation to date in BLOC3, causing HPS-8. These results help explain the patients' severe albinism and establish a common cellular defect within patients having BLOC-1 gene mutations.

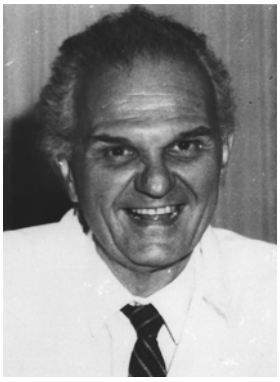
Then, the nice session was closed with cheers and claps.

CS24: Skin depigmenting / repigmenting agents, from basic mechanisms to application

Chairs: K. Al Ghamdi – N. Al Mutairi – MH. Lee

Contributed by Mu-Hyoung Lee

IL50: The most widely used combination for treating melasma is hydroquinone, retinoic acid and corticosteroid, namely the so-called Kligman formula.
Recently, the therapeutic effects of oral or intradermal injection of tranexamic acid, plasmin inhibitor, in the treatment of melasma have been reported in Asian countries.
C115: This paper proposed that fibroblasts in the dermis also play a role in regulating constitutive skin color of individuals ranging from very light to very dark via secretion of neuroregulin 1(NRG-1).
C116: This paper suggested that recent onset vitiligo are much more responsive to the repigmentation therapy, possibility due to higher numbers of residual melanocytes. Therefore, early initiation of therapy is an important therapeutic strategy for regaining lost pigmentation.
C117: This paper suggested that monobenzyl ether of hydroquinone is safe and effective depigmenting agent. Topical imatinib, imiquimod and diphecyprone may be considered as potential depigmenting agents.
C118: This paper said 0.2% rapamycin is effective for hypomelanotic macules arsing in tuberous sclerosis complex. The skin lesions were disappeared in patients after 12 weeks topical treatment without obvious side effects. Treatment with rapamycin influenced the expression of MITF in melanoma and melanocytes. These results indicate that activation of mTOR participates in hypomelanotic macules arising in TSC.



In Memoriam of Professor Jean Thivolet (4.2.1926 - 4.2.2011)

Professor Jean Thivolet, teacher, clinician and a world-renowned scientist, one of the twenty most cited dermatologists. He spent his entire life in his native Lyon. In 1962 he became the Head of the Department of Dermatology and five years later he became the Chairman of the Department of Hygiene and Immunology at the „Hôpital de l´Antiquaille". From 1972 to 1992 he was the Head of the Chair of Dermatology, Venerology and Allergology at the „Hôpital Edouard Herriot". In 1977 he founded and chaired the Laboratory of Cutaneous Immunopathology which became the first INSERM unit of dermatology with the highest reputation attracting scientists from all over the world.

The scientific interest of prof. Thivolet was centered in immunodermatology, particularly in autoimmune bullous diseases, islets of Langerhans and in epidermal cultures for the treatment of large burns. He published 810 papers, from which 11 dealt with melanoma and 4 with melanin pigmentation.

Prof. Thivolet believed that in order to be inventive and creative, to achieve medical and scientific excellency, researchers need to communicate and discuss their findings. And nothing is more stimulating in this respect than the personal interactions during scientific meetings. To convert his credo into practice, he founded French as well as European Societies for Dermatological Research. In 1989 he founded the European Journal of Dermatology and acted as its editor for twenty years.

Less known is his eternal impact on the European pigment cell community. The ESPCR members will forever remember him as the initiator of the 1st European Workshop on Melanin Pigmentation organized by him (in cooperation with Dr J.P. Ortonne and Dr C.Voulot) in Lyon in September 1979 (for more details see ESPCR Bull.No.62, pp.1825-26) which triggered a series of regular European Workshops and finally in 1985 it led to the official formation of the ESPCR (see ESPCR Bull. No.70 pp.2167-68).

Jan Borovanský

The help of prof. Robert Aquaron, dr Odile Berthier- Vergnes and dr Marek Haftek in the archive search is thankfully acknowledged.

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*, *ChemComm)* it was shown that a synthetic pheomelanin, CD melanin, is able to act as a prooxidant toward DOPA inducing the formation of a black eumelanic pigment which encapsulates the CD melanin core. This finding has interesting implications in relation to the casing model of in vivo mixed melanogenesis proposed for neuromelanin and iridial melanosomes. The absorption spectra of melanosomes of various origin were determined showing not unexpectedly significantly different features with respect to those of the 5,6-dihydroxyindole melanin precursors, but also noticeable differences in the absorption coefficients. (Peles and Simon *J.Phys. Chem*). Electrospray deposition is proposed as a new technique to obtain homogeneous films allowing investigation of the optical and electronic properties of melanins (Abbas *et al. J.Phys Chem B*). A further report by the group of Ball in Strasbourg shows how it is possible to obtain dopamine melanin films exhibiting different permeability and permselectivity to electrochemical probes by use of different procedure for pigment synthesis (Bernsman *et al Electrochim. Acta*)**.**
Among the numerous melanogenesis inhibitors are some kojic acid aminoacid amides (Kwak *et al*. *J. Peptide Sci*), arylthiosemicarbazones with varying aryl moiety (Yi *et al Eur. J. Med. Chem.*), PEGylated lipoic acid derivatives (Lu *et al. Eur J. Med Chem*) and as natural products mulberroside A, a glycosylated derivative of oxyresveratrol obtained from the roots of Morus alba (Park *et al. Food Chem Toxicol.*), and a 3-O'-glucosylated quercetin isolated from the skin of allium Cepa (Arung *et al J. Biosci*) Of interest also the melanogenesis stimulation by 2,4,6-octatrienoic acid as the result of upregulation of tyrosinase and Mitf expression (Flori *et al PCMR*) .
The ability of microbial melanin to ensure protection against gamma radiation was interpreted based on the results of chronoamperometry, chronopotentiometry and cyclic voltammetry as due to an alteration of oxidn.-redn. potential so that microbial melanin is continuously oxidized in the presence of gamma radiation (Turick *et al Bioelectrochem*) (2011).

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2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

The world of the stem cells regardless of embryonic or adult origin, represents a new and promising tool to understand the fine control of cell behavior and to define cell-based therapies. The people working on pigmentary system and the related disorders participate to this complex discussion. The team of **Lutolf** described a high-throughput hydrogel microwell system suitable to probe cell density, substrate mechanics, and protein incorporation. The system allows the simultaneous analysis of more than 2000 experiments on a single slide. The use of materials such as hydrogel permits the independent analysis of wide number of variable.
A previous article, from **Fuchs** group, already focused on epidermal stem differentiation using an animal model. The application of iRNA appeared to be effective in vivo mammalian systems to gain insights into how changes in spindle orientation during skin development. By this way, the knowledge in this filed opens interesting perspective in melanocyte cancer and dgeneration.
Regarding vitiligo, new epidemiological studies and therapy models have been recently proposed (**Katasambas A, Brochez L**). The immune-driven tribute to vitiligo pathogenesis was focused by the papers from **Le Poole** and **Erf** teams, respectively. The first one, according to long-time experience in the specific field, demonstrated that inducible hsp70 is necessary and sufficient to promote depigmentation in mouse vitiligo model through the activiation of the dendritic cells.
The research of **Erf** points on the specific polarization of T profile during the activation of the disease in the SL line chicken model of vitiligo. IFNg expression strongly correlates to parallel increase of IL-10 and IL-21, mainly during the early and active phase of the disease. All together these results suggested Erf team that Th1 polarization occurs.

Recently several interesting studies presented relevant results concerning melasma, a common disorder of hyperpigmentation affecting millions of people worldwide, including two extensive overview focusing on melasma pathogenesis, diagnosis, and treatment (**Sheth** and **Pandya**).
Kang and co-workers, presented a comparative histological and transcriptomic study on lesional and perilesional normal skin from melasma patients. By evaluating both histochemical and immmunohistochemical they demonstrated that melanocyte numbers were comparable between lesional and perilisional skin. By contrast, significantly higher amounts of melanin and 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. Interestingly, transcriptomic analysis showed upregulation of a subset of Wnt pathway modulators (Wnt5a, SFRP2 and WIF1). In particular, WIF1, which is an antagonist of Wnt signaling, was one of the most upregulated genes in lesional skin. The moderate increase of Dickkopf 1 and 2 also suggests the existence a fibroblast-driven negative feedback regulatory mechanism that play an important role melanogenesis regulation. This study also evidenced significant modifications of lipid metabolism-related genes in melasma. The biological role of lipid metabolism in the pathogenesis of melasma is also the topic of a second study of the same group published by **Lee** et al., that demonstrated impaired stratum corneum integrity associated with skin hyperpigmentation and photodamage.
Two interesting manuscripts reported a new genomic variant of MITF gene (E318K) that predisposes to melanoma. The study of **Yokoyama** et al., investigated both familial and sporadic melanoma whereas **Bertolotto** and co-workers described E318K variant, and its consequence in Mitf sumoylation, in melanoma and renal carcinoma. Both studies linked the modification of Mitf sumoylation consensus sequence to Mitf-dependent transcriptional activity and cancer.

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(Pr M. Böhm)

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- Yun JY, Roh E, Son JK, Lee SH, Seo CS, Hwang BY, Han SB, Kim Y. **Effect of saucerneol D on melanin production in cAMP-elevated melanocytes**. Arch Pharm Res. 2011; 34: 1339-45.

4. Photobiology

(Pr M-D Galibert)

- Afaq F, Katiyar SK.
  Polyphenols: Skin Photoprotection and Inhibition of Photocarcinogenesis. Mini Rev Med Chem. 2011 Oct 28.
  Abstract: Polyphenols are a large family of naturally occurring plant products and are widely distributed in plant foods, such as, fruits, vegetables, nuts, flowers, bark and seeds, etc. These polyphenols contribute to the beneficial health effects of dietary products. Clinical and epidemiological studies suggest that exposure of the skin to environmental factors/pollutants, such as solar ultraviolet (UV) radiation induce harmful effects and leads to various skin diseases including the risk of melanoma and non-melanoma skin cancers. The incidence of non-melanoma skin cancer, comprising of squamous cell carcinoma and basal cell carcinoma, is a significant public health concern world-wide. Exposure of the skin to solar UV radiation results in inflammation, oxidative stress, DNA damage, dysregulation of cellular signaling pathways and immunosuppression thereby resulting in skin cancer. The regular intake of natural plant products, especially polyphenols, which are widely present in fruits, vegetables, dry legumes and beverages have gained considerable attention as protective agents against the adverse effects of UV radiation. In this article, we first discussed the impact of polyphenols on human health based on their structure-activity relationship and bioavailability. We then discussed in detail the photoprotective effects of some selected polyphenols on UV-induced skin inflammation, proliferation, immunosuppression, DNA damage and dysregulation of important cellular signaling pathways and their implications in skin cancer management. The selected polyphenols include: green tea polyphenols, pomegranate fruit extract, grape seed proanthocyanidins, resveratrol, silymarin, genistein and delphinidin. The new information on the mechanisms of action of these polyphenols supports their potential use in skin photoprotection and prevention of photocarcinogenesis in humans.

- Bakthavatchalu V, Dey S, Xu Y, Noel T, Jungsuwadee P, Holley AK, Dhar SK, Batinic-Haberle I, St Clair DK.
  Manganese superoxide dismutase is a mitochondrial fidelity protein that protects Polγ against UV-induced inactivation. Oncogene. 2011 Sep 12. doi: 10.1038/onc.2011.407.
  Abstract: Manganese superoxide dismutase is a nuclear encoded primary antioxidant enzyme localized exclusively in the mitochondrial matrix. Genotoxic agents, such as ultraviolet (UV) radiation, generates oxidative stress and cause mitochondrial DNA (mtDNA) damage. The mtDNA polymerase (Polγ), a major constituent of nucleoids, is responsible for the replication and repair of the mitochondrial genome. Recent studies suggest that the mitochondria contain fidelity proteins and MnSOD constitutes an integral part of the nucleoid complex. However, it is not known whether or how MnSOD participates in the mitochondrial repair processes. Using skin tissue from C57BL/6 mice exposed to UVB radiation, we demonstrate that MnSOD has a critical role in preventing mtDNA damage by protecting the function of Polγ. Quantitative-PCR analysis shows an increase in mtDNA damage after UVB exposure. Immunofluorescence and immunoblotting studies demonstrate p53 translocation to the mitochondria and interaction with Polγ after UVB exposure. The mtDNA immunoprecipitation assay with Polγ and p53 antibodies in p53(+/+) and p53(-/-) mice demonstrates an interaction between MnSOD, p53 and Polγ. The results suggest that these proteins form a complex for the repair of UVB-associated mtDNA damage. The data also demonstrate that UVB exposure injures the mtDNA D-loop in a p53-dependent manner. Using MnSOD-deficient mice we demonstrate that UVB-induced mtDNA damage is MnSOD dependent. Exposure to UVB results in nitration and inactivation of Polγ, which is prevented by addition of the MnSOD mimetic Mn(III)TE-2-PyP(5+). These results demonstrate for the first time that MnSOD is a fidelity protein that maintains the activity of Polγ by preventing UVB-induced nitration and inactivation of Polγ. The data also demonstrate that MnSOD has a role along with p53 to prevent mtDNA damage.Oncogene advance online publication, 12 September 2011; doi:10.1038/onc.2011.407.

- Gaddameedhi S, Selby CP, Kaufmann WK, Smart RC, Sancar A.
  Control of skin cancer by the circadian rhythm. Proc Natl Acad Sci U S A. 2011 Nov 15;108(46):18790-5. Epub 2011 Oct 24.
  Abstract: Skin cancer is the most common form of cancer in the United States. The main cause of this cancer is DNA damage induced by the UV component of sunlight. In humans and mice, UV damage is removed by the nucleotide excision repair system. Here, we report that a rate-limiting subunit of excision repair, the xeroderma pigmentosum group A (XPA) protein, and the excision repair rate exhibit daily rhythmicity in mouse skin, with a minimum in the morning and a maximum in the afternoon/evening. In

parallel with the rhythmicity of repair rate, we find that mice exposed to UV radiation (UVR) at 4:00 AM display a decreased latency and about a fivefold increased multiplicity of skin cancer (invasive squamous cell carcinoma) than mice exposed to UVR at 4:00 PM. We conclude that time of day of exposure to UVR is a contributing factor to its carcinogenicity in mice, and possibly in humans.

- Hart PH, Gorman S, Finlay-Jones JJ.
  Modulation of the immune system by UV radiation: more than just the effects of vitamin D ? Nat Rev Immunol. 2011 Aug 19;11(9):584-96.
  Abstract: Humans obtain most of their vitamin D through the exposure of skin to sunlight. The immunoregulatory properties of vitamin D have been demonstrated in studies showing that vitamin D deficiency is associated with poor immune function and increased disease susceptibility. The benefits of moderate ultraviolet (UV) radiation exposure and the positive latitude gradients observed for some immune-mediated diseases may therefore reflect the activities of UV-induced vitamin D. Alternatively, other mediators that are induced by UV radiation may be more important for UV-mediated immunomodulation. Here, we compare and contrast the effects of UV radiation and vitamin D on immune function in immunopathological diseases, such as psoriasis, multiple sclerosis and asthma, and during infection.

- Jandova J, Eshaghian A, Shi M, Li M, King LE, Janda J, Sligh JE.
  Identification of an mtDNA Mutation Hot Spot in UV-Induced Mouse Skin Tumors Producing Altered Cellular Biochemistry. J Invest Dermatol. 2011 Oct 20.
  Abstract: There is increasing awareness of the role of mtDNA alterations in the development of cancer, as mtDNA point mutations are found at high frequency in a variety of human tumors. To determine the biological effects of mtDNA mutations in UV-induced skin tumors, hairless mice were irradiated to produce tumors, and the tumor mtDNAs were screened for single-nucleotide changes using temperature gradient capillary electrophoresis (TGCE), followed by direct sequencing. A mutation hot spot (9821insA) in the mitochondrially encoded tRNA arginine (mt-Tr) locus (tRNA(Arg)) was discovered in approximately one-third of premalignant and malignant skin tumors. To determine the functional relevance of this particular mutation in vitro, cybrid cell lines containing different mt-Tr (tRNA(Arg)) alleles were generated. The resulting cybrid cell lines contained the same nuclear genotype and differed only in their mtDNAs. The biochemical analysis of the cybrids revealed that the mutant haplotype is associated with diminished levels of complex I protein (CI), resulting in lower levels of baseline oxygen consumption and lower cellular adenosine triphosphate (ATP) production. We hypothesize that this specific mtDNA mutation alters cellular biochemistry, supporting the development of keratinocyte neoplasia

- Jung YS, Qian Y, Chen X.
  DNA polymerase eta is targeted by Mdm2 for polyubiquitination and proteasomal degradation in response to ultraviolet irradiation. DNA Repair (Amst). 2011 Nov 4.
  Abstract: DNA polymerase eta (PolH), the product of the xeroderma pigmentosum variant (XPV) gene and a Y-family DNA polymerase, plays a pivotal role in translesion DNA synthesis. Loss of PolH leads to early onset of malignant skin cancer in XPV patients and increases UV-induced carcinogenesis. Thus, the pathways by which PolH expression and activity are controlled may be explored as a strategy to prevent UV-induced cancer. In this study, we found that Mdm2, a RING finger E3 ligase, promotes PolH degradation. Specifically, we showed that knockdown of Mdm2 increases PolH expression in both p53-proficient and -deficient cells. In addition, we showed that UV-induced PolH degradation is attenuated by Mdm2 knockdown. In contrast, ectopically expression of Mdm2 decreases PolH expression, which can be abrogated by the proteasome inhibitor MG132. Moreover, we showed that Mdm2 physically associates with PolH and promotes PolH polyubiquitination in vivo and in vitro. Finally, we showed that knockdown of Mdm2 increases the formation of PolH replication foci and decreases the sensitivity of cells to UV-induced lesions in a PolH-dependent manner. Taken together, we uncovered that Mdm2 serves as an E3 ligase for PolH polyubiquitination and proteasomal degradation in cells under the basal condition and in response to UV irradiation.

- Kurdykowski S, Mine S, Bardey V, Danoux L, Jeanmaire C, Pauly G, Brabencova E, Wegrowski Y, Maquart FX.
  Ultraviolet-B irradiation induces epidermal up-regulation of heparanase expression and activity. J Photochem Photobiol B. 2011 Oct 31.
  Abstract: Heparan sulfate (HS) glycosaminoglycans are abundant components of basement membranes and cell surfaces where they are present associated with specific core-proteins to form proteoglycans, mainly perlecan, glypicans and syndecans. They play many roles such as modulation of cell proliferation and differentiation, cell-matrix adhesion and assembly. It was previously shown that HS content decreases

during skin aging. This decrease could be explained either by a decrease of HS synthesis or by an increased activity of its degrading enzyme, heparanase (Hpse-1). Since UV-B irradiation is one of the most important factor for skin photo-damage, we decided to study the effects of UV-B irradiation on heparanase expression and activity in human epidermal keratinocytes. Normal human keratinocytes and reconstructed epidermis were submitted to increasing doses of UV-B. HPSE1 mRNA levels were measured using real time PCR and heparanase enzymatic activity was quantified in human keratinocyte cultures using a microtiter-based assay. Expression and distribution of Hpse-1 were also studied in reconstructed epidermis by immunofluorescence. Both HPSE1 mRNA level and heparanase enzymatic activity were increased after UV-B irradiation of keratinocyte cultures in a time and dose-dependent manner. Protein expression of Hpse-1 was also up-regulated with increasing doses of UV-B in reconstructed epidermis. Increase of Hpse-1 expression and activity in the epidermis after UV-B irradiation could contribute to skin photo-aging.

- Li J, Malakhova M, Mottamal M, Reddy K, Kurinov I, Carper A, Langfald A, Oi N, Kim MO, Zhu F, Sosa CP, Zhou K, Bode AM, Dong Z.
  Norathyriol suppresses skin cancers induced by solar ultraviolet radiation by targeting ERK kinases. Cancer Res. 2011 Nov 14.
  Abstract: Ultraviolet (UV) irradiation is the leading factor in the development of skin cancer, prompting great interest in chemopreventive agents for this disease. In this study, we report the discovery of norathyriol, a plant-derived chemopreventive compound identified through an in silico virtual screening of the Chinese Medicine Library. Norathyriol is a metabolite of mangiferin found in mango, Hypericum elegans, and Tripterospermum lanceolatum and is known to have anticancer activity. Mechanistic investigations determined that norathyriol acted as an inhibitor of ERK1/2 kinase activity to attenuate UVB-induced phosphorylation in MAPK signaling cascades. We confirmed the direct and specific binding of norathyriol with ERK2 through a co-crystal structural analysis. The xanthone moiety in norathyriol acted as an adenine mimetic to anchor the compound by hydrogen bonds to the hinge region of the protein ATP-binding site on ERK2. Norathyriol inhibited in vitro cell growth in mouse skin epidermal JB6 P+ cells at the level of G2-M phase arrest. In mouse skin tumorigenesis assays, norathyriol significantly suppressed solar UV-induced skin carcinogenesis. Further analysis indicated that norathyriol mediates its chemopreventive activity by inhibiting the ERK-dependent activity of transcriptional factors AP-1 and NF-kB during UV-induced skin carcinogenesis. Taken together, our results identify norathyriol as a safe new chemopreventive agent that is highly effective against development of UV-induced skin cancer.

- Mouret S, Forestier A, Douki T.
  The specificity of UVA-induced DNA damage in human melanocytes. Photochem Photobiol Sci. 2011 Oct 10.
  Abstract: Exposure to solar UV radiation is the origin of most skin cancers, including deadly melanomas. Melanomas are quite different from keratinocyte-derived tumours and exhibit a different mutation spectrum in the activated oncogenes, possibly arising from a different class of DNA damage. In addition, some data suggest a role for UVA radiation in melanomagenesis. To get further insight into the molecular mechanisms underlying induction of melanoma, we quantified a series of UV-induced DNA damage in primary cultures of normal human melanocytes. The results were compared with those obtained in keratinocytes from the same donors. In the UVB range, the frequency and the distribution of pyrimidine dimers was the same in melanocytes and keratinocytes. UVA was also found to produce thymine cyclobutane dimer as the major DNA lesion with an equal efficiency in both cell types. In contrast, following UVA-irradiation a large difference was found for the yield of 8-oxo-7,8-dihydroguanine; the level of this product was 2.2-fold higher in melanocytes than in keratinocytes. The comet assay showed that the induction of strand breaks was equally efficient in both cell types but that the yield of Fpg-sensitive sites was larger in melanocytes. Our data show that, upon UVA irradiation, oxidative lesions contribute to a larger extent to DNA damage in melanocytes than in keratinocytes. We also observed that the basal level of oxidative lesions was higher in the melanocytes, in agreement with a higher oxidative stress that may be due to the production of melanin. The bulk of these results, combined with qPCR and cell survival data, may explain some of the differences in mutation spectrum and target genes between melanomas and carcinomas arising from keratinocytes.

- Mujtaba SF, Dwivedi A, Mudiam MK, Ali D, Yadav N, Ray RS.
  Production of ROS by photosensitized anthracene under sunlight and UV-R at ambient environmental intensities. Photochem Photobiol. 2011 Sep-Oct;87(5):1067-76.
  Abstract: The aim of this study was to analyze the photostability and phototoxicity mechanism of anthracene (ANT) in a human skin epidermal cell line (HaCaT) at ambient environmental intensities of sunlight/UV-R (UV-A and UV-B). Photomodification of ANT under sunlight/UV-R exposure produced two photoproducts, anthrone and 9,10 anthracenedione. Generation of (1)O(2), O(2)(•-) and (•)OH was measured under UV-

R/sunlight exposure. Involvement of reactive oxygen species (ROS) was further substantiated by their quenching with free radical quenchers. Photodegradation of 2-deoxyguanosine and linoleic acid peroxidation showed that ROS were mainly responsible for ANT phototoxicity. ANT generates significant amount of intracellular ROS in cell line. Maximum cell viability (85%) was reduced under sunlight exposure (30 min). Results of MTT assay accord NRU assay. ANT (0.01 μg mL(-1)) induced cell-cycle arrest at G1 phase. RT-PCR demonstrated constitutive inducible mRNA expression of CYP 1A1 and 1B1 genes. Photosensitive ANT upregulates CYP 1A1 (2.2-folds) and 1B1 (4.1-folds) genes. Thus, the study suggests that ROS and DNA damage were mainly responsible for ANT phototoxicity. ANT exposure may be deleterious to human health at ambient environmental intensities reaching the earth's surface through sunlight.

- Muschik D, Braspenning-Wesch I, Stockfleth E, Rösl F, Hofmann TG, Nindl I.
  Cutaneous HPV23 E6 Prevents p53 Phosphorylation through Interaction with HIPK2. PLoS One. 2011;6(11):e27655. Epub 2011 Nov 16.
  Abstract: Ultraviolet irradiation (UV) is the major risk factor for the development of skin cancer. Moreover, increasing evidence suggests cutaneotropic human papillomaviruses (HPV) from the beta genus to play a causal role as a co-factor in the development of cutaneous squamous cell carcinoma. Homeodomain-interacting protein kinase 2 (HIPK2) operates as a potential suppressor in skin tumorigenesis and is stabilized by UV-damage. HIPK2 is an important regulator of apoptosis, which forms a complex with the tumor suppressor p53, mediating p53 phosphorylation at Ser 46 and thus promoting pro-apoptotic gene expression. In our study, we demonstrate that cutaneous HPV23 E6 protein directly targets HIPK2 function. Accordingly, HPV23 E6 interacts with HIPK2 both in vitro and in vivo. Furthermore, upon massive UVB-damage HPV23 E6 co-localizes with endogenous HIPK2 at nuclear bodies. Functionally, we demonstrate that HPV23 E6 inhibits HIPK2-mediated p53 Ser 46 phosphorylation through enforcing dissociation of the HIPK2/p53 complex. In addition, HPV23 E6 co-accumulates with endogenous HIPK2 upon UV damage suggesting a mechanism by which HPV23 E6 keeps HIPK2 in check after UV damage. Thus, cutaneous HPV23 E6 prevents HIPK2-mediated p53 Ser 46 phosphorylation, which may favour survival of UV-damaged keratinocytes and skin carcinogenesis by apoptosis evasion.

- Paik SH, Kim HJ, Lee S, Im SW, Ju YS, Yeon JH, Jo SJ, Eun HC, Seo JS, Kim JI, Kwon OS.
  Linkage and association scan for tanning ability in an isolated Mongolian population. BMB Rep. 2011 Nov;44(11):741-6.
  Abstract: Tanning ability is important, because it represents the ability of the skin to protect itself against ultraviolet (UV) radiation. Here, we sought to determine genetic regions associated with tanning ability. Skin pigmentation was measured at the outer forearm and buttock areas to represent facultative and constitutive skin color, respectively. In our study population consisting of isolated Mongolian subjects, with common histories of environmental UV exposure during their nomadic life, facultative skin color adjusted by constitutive skin color was used to indicate tanning ability. Through linkage analysis and family-based association tests of 345 Mongolian subjects, we identified 2 potential linkage regions regulating tanning ability on 5q35.3 and 12q13.2, having 6 and 7 significant single nucleotide polymorphisms (SNPs), respectively. Those significant SNPs were located in or adjacent to potential candidate genes related to tanning ability: GRM6, ATF1, WNT1, and SILV/Pmel17.

- Pan X, Kane LA, Van Eyk JE, Coulombe PA.
  Type I Keratin 17 Protein Is Phosphorylated on Serine 44 by p90 Ribosomal Protein S6 Kinase 1 (RSK1) in a Growth- and Stress-dependent Fashion. J Biol Chem. 2011 Dec 9;286(49):42403-13.
  Abstract: Keratin 17 (K17) is a type I intermediate filament protein that is constitutively expressed in ectoderm-derived epithelial appendages and robustly induced in epidermis following injury, during inflammation, and in chronic diseases such as psoriasis and cancer. Mutations within K17 are responsible for two rare diseases related to ectodermal dysplasias. Studies in K17-null mice uncovered several roles for K17, including structural support, resistance to TNFα-induced apoptosis, regulation of protein synthesis, and modulation of cytokine expression. Yet, little is known about the regulation of K17 protein via post-translational modification. Here, we report that serine 44 in the N-terminal head domain of K17 (K17-Ser(44)) is phosphorylated in response to extracellular stimuli (serum, EGF, and the phorbol ester 12-O-tetradecanoylphorbol-13-acetate) that alter skin keratinocyte growth, and to cellular stresses (sorbitol-induced hyperosmotic shock, UV irradiation, and hydrogen peroxide-induced oxidative stress). It also occurs in basaloid skin tumors in situ. Upon its stimulation in skin keratinocytes, K17-Ser(44) phosphorylation is induced rapidly but stays on transiently. The majority of the phosphorylated K17-Ser(44) pool is polymer-bound and is not obviously related to a change in filament organization. The amino acid sequence surrounding K17-Ser(44) matches the consensus for the AGC family of basophilic kinases. We

show that p90 RSK1, an AGC kinase involved in the regulation of cell survival and proliferation, phosphorylates K17-Ser(44) in skin keratinocytes. These findings confirm and expand the tight link that has emerged between K17 up-regulation and growth and stress responses in the skin epithelium.

- Sullivan NJ, Tober KL, Burns EM, Schick JS, Riggenbach JA, Mace TA, Bill MA, Young GS, Oberyszyn TM, Lesinski GB.
  UV Light B-Mediated Inhibition of Skin Catalase Activity Promotes Gr-1(+)CD11b(+) Myeloid Cell Expansion. J Invest Dermatol. 2011 Oct 27. doi: 10.1038/jid.2011.329.
  Abstract: Skin cancer incidence and mortality are higher in men compared with women, but the causes of this sex discrepancy remain largely unknown. UV light exposure induces cutaneous inflammation and neutralizes cutaneous antioxidants. Gr-1(+)CD11b(+) myeloid cells are heterogeneous bone marrow-derived cells that promote inflammation-associated carcinogenesis. Reduced activity of catalase, an antioxidant present in the skin, has been associated with skin carcinogenesis. We used the outbred, immune-competent Skh-1 hairless mouse model of UVB-induced inflammation and non-melanoma skin cancer to further define sex discrepancies in UVB-induced inflammation. Our results demonstrated that male skin had relatively lower baseline catalase activity, which was inhibited following acute UVB exposure in both sexes. Further analysis revealed that skin catalase activity inversely correlated with splenic Gr-1(+)CD11b(+) myeloid cell percentage. Acute UVB exposure induced Gr-1(+)CD11b(+) myeloid cell skin infiltration, which was inhibited to a greater extent in male mice by topical catalase treatment. In chronic UVB studies, we demonstrated that the percentage of splenic Gr-1(+)CD11b(+) myeloid cells was 55% higher in male tumor-bearing mice compared with their female counterparts. Together, our findings indicate that lower skin catalase activity in male mice may at least in part contribute to increased UVB-induced generation of Gr-1(+)CD11b(+) myeloid cells and subsequent skin carcinogenesis.

- Warrick E, Garcia M, Chagnoleau C, Chevallier O, Bergoglio V, Sartori D, Mavilio F, Angulo JF, Avril MF, Sarasin A, Larcher F, Del Rio M, Bernerd F, Magnaldo T.
  Preclinical Corrective Gene Transfer in Xeroderma Pigmentosum Human Skin Stem Cells. Mol Ther. 2011 Nov 8. doi: 10.1038/mt.2011.233.
  Abstract: Xeroderma pigmentosum (XP) is a devastating disease associated with dramatic skin cancer proneness. XP cells are deficient in nucleotide excision repair (NER) of bulky DNA adducts including ultraviolet (UV)-induced mutagenic lesions. Approaches of corrective gene transfer in NER-deficient keratinocyte stem cells hold great hope for the long-term treatment of XP patients. To face this challenge, we developed a retrovirus-based strategy to safely transduce the wild-type XPC gene into clonogenic human primary XP-C keratinocytes. De novo expression of XPC was maintained in both mass population and derived independent candidate stem cells (holoclones) after more than 130 population doublings (PD) in culture upon serial propagation (>10(40) cells). Analyses of retrovirus integration sequences in isolated keratinocyte stem cells suggested the absence of adverse effects such as oncogenic activation or clonal expansion. Furthermore, corrected XP-C keratinocytes exhibited full NER capacity as well as normal features of epidermal differentiation in both organotypic skin cultures and in a preclinical murine model of human skin regeneration in vivo. The achievement of a long-term genetic correction of XP-C epidermal stem cells constitutes the first preclinical model of ex vivo gene therapy for XP-C patients.

5. Neuromelanins

(Pr M. d'Ischia)

Neuromelanin biosynthesis and biological roles are strictly related to the oxidative metabolism of dopamine via dopamine quinone and oxidized species downstream. The hypothesis that dopamine quinone reacts with DNA to give adducts that cause mutations and initiate Parkinson's disease was put forward by Zahid et al. (2011) in a review of quinone toxicity mechanisms.

In a study of synuclein expression in the brains of normal individuals Xuan et al. (2011) reported an increase of the protein with age in those neurones showing neumelanin accumulation, and suggested that age related accumulation of neuromelanin might induce synuclein over-expression and increased vulnerability of dopamine neurons to oxidative stress and related injuries.

Sian-Huelsmann et al. (2011) offered an updated review of the role of iron metabolism in the pathogenesis of Parkinson's disease and emphasized the possibility that certain discrepancies in the literature concerning the disease progression stage at which nigral iron change occurs may reflect in fact the occurrence of different sub-groups of the disease. This would explain why there a number of possible factors which may concur to the onset of the disease and it is difficult to draw a unified etiological and pathogenetic perspective.

Finally, He et al. (2011) provided evidence that PC12 cells exposed to a model of oxidative stress based on administration of levodopa and glucose oxidase exhibit higher tyrosine hydroxylase activity and neuromelanin production. On this basis they suggested that neuromelanin synthesis is part of the neuronal response to elevated levels of oxidative stress during L-DOPA treatment.

- He AY, Qiu LJ, Gao Y, Zhu Y, Xu ZW, Xu JM, Zhang ZH.
  The role of oxidative stress in neuromelanin synthesis in PC12 cells. Neuroscience (Amsterdam, Netherlands) (2011), 189 43-50.
  Abstract: Previous research has indicated that neuromelanin (NM) is involved in the pathogenesis of Parkinson's disease (PD). Increased reactive oxygen species (ROS) generation in PD sufferers is thought to be related to enhanced tyrosine hydroxylase (TH) activity and NM prodn. However, few reports have confirmed this hypothesis. In this study, PC12 cells of all expts. were exposed to 50 □mol/L levodopa (L-DOPA) to generate a model for NM synthesis. Meanwhile, PC12 cells were treated with glucose oxidase (GO) at different concns. to generate oxidative stress. Finally, cell viability, TH activity, and NM generation in PC12 cells were measured. The results showed that GO dose-dependently stimulated oxidative stress generation in PC12 cells. Moderate increases in oxidative stress enhanced the viability of PC12 cells. However, an excessive level of oxidative stress can lead to the degeneration of PC12 cells. Notably, in the surviving PC12 cells, ROS significantly increased the TH activity, and the NM prodn. was also upregulated. Thus, oxidative stress may upregulate the synthesis of NM, which may be a result of the increased TH activity obsd. in response to the elevated ROS in L-DOPA-treated PC12 cells.

- Sian-Hülsmann J, Mandel S, Youdim MB, Riederer P.
  The relevance of iron in the pathogenesis of Parkinson's disease. Journal of Neurochemistry (2011), 118(5 & 6), 939-957.
  Abstract: A review. Alterations of iron levels in the brain has been obsd. and documented in a no. of neurodegenerative disorders including Parkinson's disease (PD). The elevated nigral iron levels obsd. in PD may reflect a dysfunction of brain iron homeostasis. Under normal physiol. conditions excess iron can be sequestrated in ferritin and neuromelanin. Alternatively, the excess iron may represent a component of brain iron deposition assocd. with ageing. The etiol. of idiopathic PD largely remains an enigma. However, intensive investigations have provided a host of putative mechanisms that might contribute to the pathogenesis underlying the characteristic degeneration of the dopaminergic neurons in the substantia nigra (SN). The mechanisms proposed include oxidative (and nitrative) stress, inflammation, excitotoxicity, mitochondrial dysfunction, altered proteolysis and finally apoptotic induced cell death. Iron-mediated cellular destruction is mediated primarily via reactive oxygen or/and nitrogen species induced oxidative stress. Furthermore, these pathogenic mechanisms appear to be closely interlinked to the cascade of events leading to cellular death. There are conflicting reports about the stage during disease progression at which nigral iron change occurs in PD. Some have found that there are no changes in iron content SN in asymptomatic incidental Lewy body disease, suggesting it may represent a secondary event in the cascade of neuronal degeneration. In contrast, others have found an elevation of iron in SN in pre-clin. stages. These discrepancies may be attributed to the occurrence of different sub-groups of the disease. This concurs with the notion that PD represents a group of related diseases with a no. of potential pathogenic pathways.

- Xuan Q, Xu SL, Lu DH, Yu S, Zhou M, Uéda K, Cui YQ, Zhang BY, Chan P.
  Increase expression of □ -synuclein in aged human brain associated with neuromelanin accumulation. Journal of Neural Transmission (2011), 118(11), 1575-1583.
  Abstract: Although the increased prevalence of Parkinson's disease (PD) with aging suggests that aging processes predispose dopamine neurons to degeneration, the mechanism involved remains unknown. Dopamine neurons contain significant amts. of neuromelanin, and the amt. of neuromelanin increases with aging. In the present study, age-related changes in the no. of nigral neurons expressing neuromelanin (NM), □-synuclein, and tyrosine hydroxylase (TH) were stereol. analyzed in the postmortem brains of 28 healthy humans with an age range of 17-84 years. Stereol. counting of NM content, □-synuclein content, and TH immunoreactivity revealed significant accumulation of NM and □-synuclein in neurons during the aging process. In cells contg. a large amt. of NM, □-synuclein-immunoreactive cells in aged individuals outnumbered those of younger individuals. In non-NM cells, the □-synuclein expression profile was similar across age groups. Furthermore, TH-immunoreactive neurons decreased significantly with aging, which was assocd. with accumulation of NM and □-synuclein. Our results suggest that age related accumulation of NM might induce □-synuclein over-expression and thereby make dopamine neurons more vulnerable to injuries.

- Zahid M., Saeed M., Yang L, Beseler C., Rogan E., Cavalieri EL.
  Formation of dopamine quinone-DNA adducts and their potential role in the etiology of Parkinson's disease. IUBMB Life No pp. yet given. 2011 Nov 2. doi: 10.1002/iub.538.
  Abstract : The neurotransmitter dopamine is oxidized to its quinone (DA-Q), which at neutral pH undergoes intramol. cyclization by 1,4-Michael addn., followed by oxidn. to form leukochrome, then aminochrome, and finally neuromelanin. At lower pH, the amino group of DA is partially protonated, allowing the competitive intermol. 1,4-Michael addn. with nucleophiles in DNA to form the depurinating adducts, DA-6-N3Ade and DA-6-N7Gua. Catechol estrogen-3,4-quinones react by 1,4-Michael addn. to form the depurinating 4-hydroxyestrone(estradiol)-1-N3Ade [4-OHE1(E2)-1-N3Ade] and 4-OHE1(E2)-1-N7Gua adducts, which are implicated in the initiation of breast and other human cancers. The effect of pH was studied by reacting tyrosinase-activated DA with DNA and measuring the formation of depurinating adducts. The most adducts were formed at pH 4, 5, and 6, and their level was nominal at pH 7 and 8. The N3Ade adduct depurinated instantaneously, but N7Gua had a half-life of 3 H. The slow loss of the N7Gua adduct is analogous to that obsd. in previous studies of natural and synthetic estrogens. The antioxidants N-acetylcysteine and resveratrol efficiently blocked formation of the DA-DNA adducts. Thus, slightly acidic conditions render competitive the reaction of DA-Q with DNA to form depurinating adducts. We hypothesize that formation of these adducts could lead to mutations that initiate Parkinson's disease. If so, use of N-acetylcysteine and resveratrol as dietary supplements may prevent initiation of this disease. □ 2011 IUBMB IUBMB Life, 2011.

6. Genetics, molecular and developmental biology

(Dr. L. Montoliu)

- Berlin I, Luciani F, Gallagher SJ, Rambow F, Conde-Perez A, Colombo S, Champeval D, Delmas V, Larue L.
  General strategy to analyse coat colour phenotypes in mice. Pigment Cell Melanoma Res. 2011 Nov 16.

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  BRCA1 is a new MITF target gene. Pigment Cell Melanoma Res. 2011 Aug;24(4):725-7.

- Bonazzi VF, Nancarrow DJ, Stark MS, Moser RJ, Boyle GM, Aoude LG, Schmidt C, Hayward NK.
  Cross-platform array screening identifies COL1A2, THBS1, TNFRSF10D and UCHL1 as genes frequently silenced by methylation in melanoma. PLoS One. 2011;6(10):e26121.

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  Carcinogen treatment in mouse selectively expressing activated N-Ras(Q61K) in melanocytes recapitulates metastatic cutaneous melanoma development. Pigment Cell Melanoma Res. 2011 Nov 29.
  The incidence of melanoma has significantly increased and a better understanding its pathogenesis and development of new therapeutic strategies are urgently needed. Here we describe a murine model of metastatic cutaneous melanoma using C57BL/6 mice expressing a mutated human N-Ras gene under the control of a tyrosinase promoter (TyrRas). These mice were topically exposed to 7,12-dimethylbenzanthracene (DMBA) for brief exposure periods. Cutaneous melanoma developed at the site of exposure on average by 19 weeks of age and in 80% of mice. Importantly, as in humans, melanoma development was associated with subsequent metastasis to tumor-draining lymph nodes. Critically, such metastatic behavior is transplantable, as intradermal inoculation of melanoma cells from TyrRas-DMBA mice into non-transgenic mice led to the growth of melanoma and, again, metastasis to skin-draining lymph nodes. This metastatic melanoma model closely mimics human pathology and should be a useful tool for studying melanoma pathogenesis and developing new therapies.

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  A BLOC-1 mutation screen reveals that PLDN is mutated in Hermansky-Pudlak Syndrome type 9. Am J Hum Genet. 2011 Jun 10;88(6):778-87.
  Hermansky-Pudlak Syndrome (HPS) is an autosomal-recessive condition characterized by oculocutaneous albinism and a bleeding diathesis due to absent platelet delta granules. HPS is a genetically heterogeneous disorder of intracellular vesicle biogenesis. We first screened all our patients with HPS-like symptoms for mutations in the genes responsible for HPS-1 through HPS-6 and found no functional mutations in 38 individuals. We then examined all eight genes encoding the biogenesis of lysosome-related organelles complex-1, or BLOC-1, proteins in these individuals. This identified a homozygous nonsense mutation in PLDN in a boy with characteristic features of HPS. PLDN is mutated in the HPS mouse model pallid and encodes the protein pallidin, which interacts with the early endosomal t-SNARE syntaxin-13. We could not detect any full-length pallidin in our patient's cells despite normal mRNA expression of the mutant transcript. We could detect an alternative transcript that would skip the exon that harbored the mutation, but we demonstrate that if this transcript is translated into protein, although it correctly localizes to early endosomes, it does not interact with syntaxin-13. In our patient's melanocytes, the melanogenic protein TYRP1 showed aberrant localization, an increase in plasma-membrane trafficking, and a failure to reach melanosomes, explaining the boy's severe albinism and establishing his diagnosis as HPS-9.

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  An iterative genetic and dynamical modelling approach identifies novel features of the gene regulatory network underlying melanocyte development. PLoS Genet. 2011 Sep;7(9):e1002265.
  The mechanisms generating stably differentiated cell-types from multipotent precursors are key to understanding normal development and have implications for treatment of cancer and the therapeutic use of stem cells. Pigment cells are a major derivative of neural crest stem cells and a key model cell-type for our understanding of the genetics of cell differentiation. Several factors driving melanocyte fate specification have been identified, including the transcription factor and master regulator of melanocyte development, Mitf, and Wnt signalling and the multipotency and fate specification factor, Sox10, which drive mitf expression. While these factors together drive multipotent neural crest cells to become specified melanoblasts, the mechanisms stabilising melanocyte differentiation remain unclear. Furthermore, there is controversy over whether Sox10 has an ongoing role in melanocyte differentiation. Here we use zebrafish to explore in vivo the gene regulatory network (GRN) underlying melanocyte specification and differentiation. We use an iterative process of mathematical modelling and experimental observation to explore methodically the core melanocyte GRN we have defined. We show that Sox10 is not required for ongoing differentiation and expression is downregulated in differentiating cells, in response to Mitfa and Hdac1. Unexpectedly, we find that Sox10 represses Mitf-dependent expression of melanocyte differentiation genes. Our systems biology approach allowed us to predict two novel features of the melanocyte GRN, which we then validate experimentally. Specifically, we show that maintenance of mitfa expression is Mitfa-dependent, and identify Sox9b as providing an Mitfa-independent input to melanocyte differentiation. Our data supports our previous suggestion that Sox10 only functions transiently in regulation of mitfa and cannot be responsible for long-term maintenance of mitfa expression; indeed, Sox10 is likely to slow melanocyte differentiation in the zebrafish embryo. More generally, this novel approach to understanding melanocyte differentiation provides a basis for systematic modelling of differentiation in this and other cell-types.

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  Metastases are the major cause of death from melanoma, a skin cancer that has the fastest rising incidence of any malignancy in the Western world. Molecular pathways that drive melanoblast migration in development are believed to underpin the movement and ultimately the metastasis of melanoma. Here we show that mice lacking P-Rex1, a Rac-specific Rho GTPase guanine nucleotide exchange factor, have a melanoblast migration defect during development evidenced by a white belly. Moreover, these P-Rex1(-/-) mice are resistant to metastasis when crossed to a murine model of melanoma. Mechanistically, this is associated with P-Rex1 driving invasion in a Rac-dependent manner. P-Rex1 is elevated in the majority of human melanoma cell lines and tumour tissue. We conclude that P-Rex1 has an important role in melanoblast migration and cancer progression to metastasis in mice and humans.

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- Lu H, Li L, Watson ER, Williams RW, Geisert EE, Jablonski MM, Lu L.
  Complex interactions of Tyrp1 in the eye. Mol Vis. 2011;17:2455-68. Epub 2011 Sep 22.

- Luciani F, Champeval D, Herbette A, Denat L, Aylaj B, Martinozzi S, Ballotti R, Kemler R, Goding CR, De Vuyst F, Larue L, Delmas V.
  Biological and mathematical modeling of melanocyte development. Development. 2011 Sep;138(18):3943-54.

We aim to evaluate environmental and genetic effects on the expansion/proliferation of committed single cells during embryonic development, using melanoblasts as a paradigm to model this phenomenon. Melanoblasts are a specific type of cell that display extensive cellular proliferation during development. However, the events controlling melanoblast expansion are still poorly understood due to insufficient knowledge concerning their number and distribution in the various skin compartments. We show that melanoblast expansion is tightly controlled both spatially and temporally, with little variation between embryos. We established a mathematical model reflecting the main cellular mechanisms involved in melanoblast expansion, including proliferation and migration from the dermis to epidermis. In association with biological information, the model allows the calculation of doubling times for melanoblasts, revealing that dermal and epidermal melanoblasts have short but different doubling times. Moreover, the number of trunk founder melanoblasts at E8.5 was estimated to be 16, a population impossible to count by classical biological approaches. We also assessed the importance of the genetic background by studying gain- and loss-of-function β-catenin mutants in the melanocyte lineage. We found that any alteration of β-catenin activity, whether positive or negative, reduced both dermal and epidermal melanoblast proliferation. Finally, we determined that the pool of dermal melanoblasts remains constant in wild-type and mutant embryos during development, implying that specific control mechanisms associated with cell division ensure half of the cells at each cell division to migrate from the dermis to the epidermis. Modeling melanoblast expansion revealed novel links between cell division, cell localization within the embryo and appropriate feedback control through β-catenin.

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  Nitisinone improves eye and skin pigmentation defects in a mouse model of oculocutaneous albinism. J Clin Invest. 2011 Oct 3;121(10):3914-23.
  Mutation of the tyrosinase gene (TYR) causes oculocutaneous albinism, type 1 (OCA1), a condition characterized by reduced skin and eye melanin pigmentation and by vision loss. The retinal pigment epithelium influences postnatal visual development. Therefore, increasing ocular pigmentation in patients with OCA1 might enhance visual function. There are 2 forms of OCA1, OCA-1A and OCA-1B. Individuals with the former lack functional tyrosinase and therefore lack melanin, while individuals with the latter produce some melanin. We hypothesized that increasing plasma tyrosine concentrations using nitisinone, an FDA-approved inhibitor of tyrosine degradation, could stabilize tyrosinase and improve pigmentation in individuals with OCA1. Here, we tested this hypothesis in mice homozygous for either the Tyrc-2J null allele or the Tyrc-h allele, which model OCA-1A and OCA-1B, respectively. Only nitisinone-treated Tyrc-h/c-h mice manifested increased pigmentation in their fur and irides and had more pigmented melanosomes. High levels of tyrosine improved the stability and enzymatic function of the Tyrc-h protein and also increased overall melanin levels in melanocytes from a human with OCA-1B. These results suggest that the use of nitisinone in OCA-1B patients could improve their pigmentation and potentially ameliorate vision loss.

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  Identification of a melanocyte-specific, microphthalmia-associated transcription factor-dependent regulatory element in the intronic duplication causing hair greying and melanoma in horses. Pigment Cell Melanoma Res. 2011 Aug 30.

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  Modelling melanoma in mice. Pigment Cell Melanoma Res. 2011 Dec;24(6):1158-76.
  Phenotypic and molecular heterogeneity in human melanoma has impaired efforts to explain many of the clinically important features of melanoma. For example, many of the underlying mechanisms that might predict age-of-onset, time to metastasis and other key elements in melanoma progression remain unknown. Furthermore, melanoma staging used to predict outcome and treatment has not yet moved beyond a basic phenotypic classification. While molecularly targeted therapies show great promise for melanoma patients,

establishing accurate animal models that recapitulate human cutaneous melanoma progression remains a priority. We examine the relevance of mice as models for human melanoma progression and for key molecular and histopathologic variants of melanoma. These mice may be used as preclinical models to probe the relationships between causative mutations, disease progression and outcome for molecularly targeted therapeutics. We ask how new mouse models, or more detailed histopathologic and molecular analyses of existing mouse models, may be used to advance our understanding of genotype-phenotype correlations in this tumour type. This necessarily involves a consideration of the utility of mice as models for ultraviolet radiation-induced melanoma, and how this might be improved.

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  Oculocutaneous albinism type 3: A Japanese girl with novel mutations in TYRP1 gene. J Dermatol Sci. 2011 Dec;64(3):217-22.

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  Specific interaction of G*α*i3 with the Oa1 G-protein coupled receptor controls the size and density of melanosomes in retinal pigment epithelium. PLoS One. 2011;6(9):e24376.

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7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borron)

- Beberok A, Buszman E, Wrześniok D, Otręba M, Trzcionka J.
Interaction between ciprofloxacin and melanin: the effect on proliferation and melanization in melanocytes. Eur J Pharmacol. 2011 Nov 1;669(1-3):32-7. Epub 2011 Aug 19.
There have been described serious adverse events caused by ciprofloxacin in pigmented tissues. It is known that some fluoroquinolones bind well to melanin rich tissues, but the relation between their affinity to melanin and the skin or eye toxicity is not well documented. The aim of this study was to examine whether ciprofloxacin binds to melanin, and how this interaction affects the proliferation and melanization in melanocytes. We have demonstrated that complexes which ciprofloxacin forms with melanin possess at least two classes of independent binding sites. Their association constants are K(1)~10(5) M(-1) and K(2)~10(2) M(-1), respectively. Ciprofloxacin has induced evident concentration-dependent loss in melanocytes viability. The value of ED(50) was found to be ~0.5 mM. It has also been shown that ciprofloxacin reduces melanin content, and decreases tyrosinase activity in human skin melanocytes. The ability of ciprofloxacin to interact with melanin and its inhibitory effect on melanization in melanocytes in vitro may explain a potential role of melanin in the mechanisms of ciprofloxacin toxic effects in vivo.

- Dong Y, Wang H, Cao J, Ren J, Fan R, He X, Smith GW, Dong C.
Nitric oxide enhances melanogenesis of alpaca skin melanocytes in vitro by activating the MITF phosphorylation. Mol Cell Biochem. 2011 Jun;352(1-2):255-60.
Ultraviolet (UV) B radiation can cause skin-tanning via the synthesis of melanin which is synthesized by specific tyrosinase and tyrosinase-related enzymes expressed in melanocytes. It is reported that several melanogenic factors are released from keratinocytes and other cells surrounding melanocytes in the skin following UV radiation. Some of them are reported to up-regulate tyrosinase gene expression through a different pathway, but most regulate tyrosinase via microphthalmia-associated transcription factor (MITF). It is unknown whether an NO-induced pathway regulates melanogenesis via MITF in vitro. In this study, we investigated this problem because it is important for our understanding of how to enhance the coat color of alpaca. We set up three groups for experiments using alpaca melanocytes: the control cultures were allowed a total of 5 days growth; the UV group cultures were also allowed 5 days of growth like the control group, but were then irradiated once everyday with 312 mJ/cm(2) of UVB; the UV + L-NAME group was the same as the UV group, but with the addition of 300 μM L-NAME every 6 h. To determine the NO inhibition effect, NO product was measured. To determine the effect of NO on MITF, the expression levels of the MITF gene and protein were measured by immunofluorescence, quantitative real-time PCR and western immunoblotting. To determine the influence of NO on MITF phosphorylation, phosphorylated MITF protein (p-MITF) was measured by western immunoblotting. To determine the effect of NO on melanogenesis, the melanin content was measured. The results provide exciting new evidence that NO can enhance melanogenesis in alpaca skin melanocytes by stimulating MITF phosphorylation.

- Flori E, Mastrofrancesco A, Kovacs D, Ramot Y, Briganti S, Bellei B, Paus R, Picardo M.
2,4,6-Octatrienoic acid is a novel promoter of melanogenesis and antioxidant defence in normal human melanocytes via PPAR-γ activation. Pigment Cell Melanoma Res. 2011 Aug;24(4):618-30. doi: 10.1111/j.1755-148X.2011.00887.x.
Given the importance of the tanning response in protecting human skin from the harmful effects of UV radiation, one important research priority is to identify novel molecules that are capable of promoting pigmentation and/or antioxidant defence. Parrodienes share some structural features with carotenoids and retinoids, stimulate cell antioxidant defence and counteract senescence-like phenotype in fibroblasts. We selected the parrodiene-derivative 2,4,6-octatrienoic acid (Octa) to study its impact on key parameters of melanogenesis and antioxidant defence in organ-cultured human skin and in normal human melanocytes. Octa promoted melanogenesis by up-regulating tyrosinase and microphthalmia-associated transcription factor expression. This correlated with an increase of melanin content in both human epidermis in situ and cultured human epidermal melanocytes. Moreover, Octa increased the biological antioxidant potential content and the expression and activity of catalase. Activation of peroxisome proliferator-activated receptor (PPAR)-γ was necessary to evoke these effects. These data strongly encourage the systematic study of Octa as a novel candidate promoter of human skin photoprotection.

- Gáspár E, Nguyen-Thi KT, Hardenbicker C, Tiede S, Plate C, Bodó E, Knuever J, Funk W, Bíró T, Paus R.

Thyrotropin-Releasing Hormone Selectively Stimulates Human Hair Follicle Pigmentation. J Invest Dermatol. 2011 Dec;131(12):2368-2377. doi: 10.1038/jid.2011.221. Epub 2011 Sep 29.
In amphibians, thyrotropin-releasing hormone (TRH) stimulates skin melanophores by inducing secretion of α-melanocyte-stimulating hormone in the pituitary gland. However, it is unknown whether this tripeptide neurohormone exerts any direct effects on pigment cells, namely, on human melanocytes, under physiological conditions. Therefore, we have investigated whether TRH stimulates pigment production in organ-cultured human hair follicles (HFs), the epithelium of which expresses both TRH and its receptor, and/or in full-thickness human skin in situ. TRH stimulated melanin synthesis, tyrosinase transcription and activity, melanosome formation, melanocyte dendricity, gp100 immunoreactivity, and microphthalmia-associated transcription factor expression in human HFs in a pituitary gland-independent manner. TRH also stimulated proliferation, gp100 expression, tyrosinase activity, and dendricity of isolated human HF melanocytes. However, intraepidermal melanogenesis was unaffected. As TRH upregulated the intrafollicular production of "pituitary" neurohormones (proopiomelanocortin transcription and ACTH immunoreactivity) and as agouti-signaling protein counteracted TRH-induced HF pigmentation, these pigmentary TRH effects may be mediated in part by locally generated melanocortins and/or by MC-1 signaling. Our study introduces TRH as a novel, potent, selective, and evolutionarily highly conserved neuroendocrine factor controlling human pigmentation in situ. This physiologically relevant and melanocyte sub-population-specific neuroendocrine control of human pigmentation deserves clinical exploration, e.g., for preventing or reversing hair graying.

- Hirobe T, Yoshihara C, Takeuchi S, Wakamatsu K, Ito S, Abe H, Kawa Y, Soma Y.
A Novel Deletion Mutation of Mouse ruby-eye 2 Named ru2(d)/Hps5(ru2-d) Inhibits Melanocyte Differentiation and Its Impaired Differentiation is Rescued by L-tyrosine. Zoolog Sci. 2011 Nov;28(11):790-801.
In our laboratory, a single autosomal recessive mutation in a phenotype similar to ruby-eye (ru/Hps6(ru)) or ruby-eye 2 (ru2/Hps5(ru2)) spontaneously occurred in siblings of C57BL/10JHir (+/+, black) mice in 2006. RT-PCR analysis revealed that this novel mutation, named ru2(d)/Hps5(ru2-d), exhibited frameshift by 997G deletion in the Hps5 gene. To clarify the mechanism of the hypopigmentation, the characteristics of proliferation and differentiation of ru2(d)/ru2(d) epidermal melanoblasts and melanocytes cultured in a serum-free medium were investigated. The proliferation of ru2(d)/ru2(d) melanoblasts and melanocytes did not differ from that of +/+ melanoblasts and melanocytes. However, the differentiation of ru2(d)/ru2(d) melanocytes was greatly inhibited. Tyrosinase (TYR) activity, expression of TYR, TYR-related protein 1 (TRP1) and TRP2 (dopachrome tautomerase, DCT), eumelanin synthesis, and the number of stage IV melanosomes markedly decreased in ru2(d)/ru2(d) melanocytes. However, excess L-tyrosine (Tyr) added to culture media from initiation of the primary culture rescued the reduced differentiation through increase in TYR activity, expression of TYR, TRP1, TRP2 and Kit, eumelanin synthesis, and stage IV melanosomes. L-Tyr injected into ru2(d)/ru2(d) mice also stimulated melanocyte differentiation. These results suggest that the ru2(d) allele inhibits melanocyte differentiation, and that its impaired differentiation is rescued by excess Tyr.

- Jian D, Jiang D, Su J, Chen W, Hu X, Kuang Y, Xie H, Li J, Chen X.
Diethylstilbestrol enhances melanogenesis via cAMP-PKA-mediating up-regulation of tyrosinase and MITF in mouse B16 melanoma cells. Steroids. 2011 Nov;76(12):1297-304. Epub 2011 Jun 30.
BACKGROUND: It is well known that melanin synthesis in melanoma cells is controlled by melanogenic enzymes, which regulate the cAMP-PKA signaling pathway. Estrogen was previously reported to upregulate melanogenesis that is associated with human skin pigmentation. OBJECTIVE: To investigate the influence and mechanism of diethylstilbestrol (DES) on melanogenesis in mouse B16 melanoma cells. METHODS: The effects of diethylstilbestrol on cell viability, melanin content, tyrosinase activity, cAMP level, expression of the tyrosinase family and microphthalmia related transcription factor (MITF) were measured in B16 melanoma. Estrogen receptor (ER) expression were detected in B16 melanoma and A375 melanoma. Diethylstilbestrol-induced melanin synthesis were evaluated in the presence and absence of H89 (a PKA-specific inhibitor) and ICI182, 780 (a pure ER antagonist). Tyrosinase activity, the mRNA levels of tyrosinase and MITF were evaluated in the presence and absence of H89. RESULTS: In B16 cells, diethylstilbestrol increased cell proliferation, melanin synthesis, tyrosinase activity and expression of the tyrosinase family and MITF. ER expression have not difference in human and mouse melanoma. When ER were inhibited by ICI182, 780, DES-induced melanogenesis was significantly reduced. Diethylstilbestrol enhanced the level of cAMP. The upregulation of melanin content and tyrosinase activity stimulated by diethylstilbestrol was significantly attenuated in the presence of H89. Further, diethylstilbestrol-induced upregulation of tyrosinase and MITF were significantly attenuated when the PKA pathway was blocked. CONCLUSIONS: Diethylstilbestrol can enhance melanin synthesis in melanoma cells. This effect is

associated with activation of the cAMP-PKA pathway and upregulation of expression and activity of the melanogenesis-related enzyme tyrosinase and MITF.

- Jin ML, Park SY, Kim YH, Park G, Son HJ, Lee SJ.
  Suppression of α-MSH and IBMX-induced melanogenesis by cordycepin via inhibition of CREB and MITF, and activation of PI3K/Akt and ERK-dependent mechanisms. Int J Mol Med. 2012 Jan;29(1):119-24. doi: 10.3892/ijmm.2011.807. Epub 2011 Oct 3.
  Cordycepin has been a traditional medicine in China and Korea for centuries. This study explored the inhibitory effect of cordycepin on melanogenesis and the relative molecular mechanisms. Cordycepin inhibited melanin synthesis-related enzymes, such as tyrosinase, tyrosinase-related protein-1 (TRP1) and tyrosinase-related protein-2 (TRP2). α-MSH and IBMX were reported as melanin synthesis enhancers. Both of them could increase intracellular melanin synthesis by activation of the microphthalmia-associated transcription factor (MITF) signaling pathway. In the MITF pathway, the phosphorylation of cAMP related binding protein (CREB) activated the transcription of MITF, resulting in increasing melanin synthesis. Cordycepin also decreased the phosphorylation of CREB induced by α-MSH and IBMX in B16F10 melanoma cells. Accordingly, cordycepin inhibited melanogenesis signaling pathways by activating ERK and AKT signaling pathways to regulate the suppression of MITF and its downstream pathways including tyrosinase, TRP1 and TRP2. These results indicate the role of cordycepin as a potent depigmenting agent for cosmetics.

- Jung E, Hwang W, Kim S, Kim YS, Kim YS, Lee J, Park D.
  Depigmenting action of platycodin D depends on the cAMP/Rho-dependent signalling pathway. Exp Dermatol. 2011 20(12):986-91. doi: 10.1111/j.1600-0625.2011.01379.x. Epub 2011 Oct 13.
  The overproduction and accumulation of melanin in the skin could lead to a pigmentary disorders, such as melasma, freckle, postinflammatory melanoderma and solar lentigo. Therefore, this study was conducted to investigate the effects of platycodin D (PD) on melanogenesis and its action mechanisms. In this study, we found that PD significantly inhibited melanin synthesis at low concentrations. These effects were further demonstrated by the PD-induced inhibition of cAMP production, phosphorylation of the cAMP-response element-binding protein and expression of microphthalmia-associated transcription factor and its downstream genes, tyrosinase, tyrosinase-related proteins-1 and Dct/tyrosinase-related proteins-2, suggesting that PD inhibits melanogenesis through the downregulation of cAMP signalling. Furthermore, PD induced significant morphological changes in melanocytes, namely, the retraction of dendrites. A small GTPase assays revealed that PD stimulated an increase in GTP-bound Rho content, one of downstream molecules of cAMP, but not in Rac or CDC42 content. Moreover, a Rho inhibitor (C3 exoenzyme) and a Rho kinase inhibitor (Y27632) attenuated the dendrite retraction induced by PD. Taken together, these findings indicate that PD inhibits melanogenesis by inhibiting the cAMP-protein kinase A pathway and also suppresses melanocyte dendricity through activation of the Rho signal that is mediated by PD-induced reduction in cAMP production. Therefore, these results suggest that PD exerts its inhibitory effects on melanogenesis and melanocyte dendricity via suppression of cAMP signalling and may be introduced as an inhibitor of hyperpigmentation caused by UV irradiation or pigmented skin disorders.

- Kim B, Kim JE, Lee SM, Lee SH, Lee JW, Kim MK, Lee KJ, Kim H, Lee JD, Choi KY.
  N-Nicotinoyl dopamine, a novel niacinamide derivative, retains high antioxidant activity and inhibits skin pigmentation. Exp Dermatol. 2011 20(11):950-2. doi: 10.1111/j.1600-0625.2011.01345.x. Epub 2011 Aug 15.
  We synthesized a novel derivative of a well-known skin-lightening compound niacinamide, N-nicotinoyl dopamine (NND). NND did not show inhibitory effects of tyrosinase and melanin synthesis in B16F10 mouse melanoma cells. However, NND retains high antioxidant activity without affecting viability of cells. In a reconstructed skin model, topical applications of 0.05% and 0.1% NND induced skin lightening and decreased melanin production without affecting the viability and morphology of melanocytes and overall tissue histology. Moreover, no evidence for skin irritation or sensitization was observed when 0.1% NND emulsion was applied onto the skin of 52 volunteers. The effect of NND on skin lightening was further revealed by pigmented spot analyses of human clinical trial. Overall, NND treatment may be a useful trial for skin lightening and treating pigmentary disorders.

- 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. 2011 Aug;131(8):1701-9. doi: 10.1038/jid.2011.98.
  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 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 Nov; 303(9):669-78.
  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.

- Lu H, Li L, Watson ER, Williams RW, Geisert EE, Jablonski MM, Lu L.
  Complex interactions of Tyrp1 in the eye. Mol Vis. 2011;17:2455-68. Epub 2011 Sep 22.
  PURPOSE: To use a systems genetics approach to construct and analyze co-expression networks that are causally linked to mutations in a key pigmentation gene, tyrosinase-related protein 1 (Tyrp1), that is associated both with oculocutaneous albinism type 3 (OCA3) in humans and with glaucoma in mice. METHODS: Gene expression patterns were measured in whole eyes of a large family of BXD recombinant inbred (RI) mice derived from parental lines that encode for wildtype (C57BL/6J) and mutant (DBA/2J) Tyrp1. Protein levels of Tyrp1 were measured in whole eyes and isolated irides. Bioinformatics analyses were performed on the expression data along with our archived sequence data. Separate data sets were generated which were comprised of strains that harbor either wildtype or mutant Tyrp1 and each was mined individually to identify gene networks that covary significantly with each isoform of Tyrp1. Ontology trees and network graphs were generated to probe essential function and statistical significance of covariation. Genes with strong covariance in wildtype mice were assembled into genome-wide heatmaps for cohorts carrying either wildtype or mutant Tyrp1. RESULTS: Single nucleotide polymorphism (SNP) analysis verified the presence of the Tyrp1b mutation in the Tyrp1 gene. Message levels were greater in BXD strains with the mutant Tyrp1. Interval mapping of these BXD mice revealed a strong expression quantitative trait locus (eQTL) on Chr 4 at the location of the gene itself. Composite mapping revealed a suggestive eQTL on Chr 9 at the location of myosin-Va (Myo5a), mutations in which are known as dilute. Enriched biologic processes associated with wildtype Tyrp1 included pigmentation, melanin biosynthetic process, and mesenchymal cell development, while associations with the mutant gene included categories of neural crest cell development, protein metabolic processes and glycoprotein metabolic processes. Genome-wide heatmaps revealed strong candidate cis-eQTLs on Chr 4 at Tyrp1 and on Chr 9 at Myo5a in all mice. In the wildtype data set, Tyrp1 was an upstream regulator of six pigmentation and two mesenchyme genes. In addition, five genes, including Tyrp1, were at least partially regulated by Myo5a. Analyses of the strains harboring the mutant gene revealed significant loss of correlation to traditional genes and gain of correlation to genes with little or no functional relationship. CONCLUSIONS: These findings indicate that the Tyrp1(b) mutation modifies the pathways and gene networks in which Tyrp1 functions. Our results also indicate

direct and indirect regulatory control of Tyrp1 and other pigmentation and mesenchymal genes by Myo5a. Lastly, we find that the mutations reduce the ability of Tyrp1 to regulate expression of other genes that participate in pigmentation metabolism.

- Muñoz-Muñoz JL, Garcia-Molina F, Garcia-Ruiz PA, Varon R, Tudela J, Rodriguez-Lopez JN, Garcia-Canovas F.
  Catalytic oxidation of o-aminophenols and aromatic amines by mushroom tyrosinase. Biochim Biophys Acta. 2011 Dec;1814(12):1974-83. Epub 2011 Jul 22.
  The kinetics of tyrosinase acting on o-aminophenols and aromatic amines as substrates was studied. The catalytic constants of aromatic monoamines and o-diamines were both low, these results are consistent with our previous mechanism in which the slow step is the transfer of a proton by a hydroxyl to the peroxide in oxy-tyrosinase (Fenoll et al., Biochem. J. 380 (2004) 643-650). In the case of o-aminophenols, the hydroxyl group indirectly cooperates in the transfer of the proton and consequently the catalytic constants in the action of tyrosinase on these compounds are higher. In the case of aromatic monoamines, the Michaelis constants are of the same order of magnitude than for monophenols, which suggests that the monophenols bind better (higher binding constant) to the enzyme to facilitate the π-π interactions between the aromatic ring and a possible histidine of the active site. In the case of aromatic o-diamines, both the catalytic and Michaelis constants are low, the values of the catalytic constants being lower than those of the corresponding o-diphenols. The values of the Michaelis constants of the aromatic o-diamines are slightly lower than those of their corresponding o-diphenols, confirming that the aromatic o-diamines bind less well (lower binding constant) to the enzyme.

- Nakajima H, Fukazawa K, Wakabayashi Y, Wakamatsu K, Imokawa G.
  Withania somnifera extract attenuates stem cell factor-stimulated pigmentation in human epidermal equivalents through interruption of ERK phosphorylation within melanocytes. J Nat Med. 2011 Nov 16.
  We previously demonstrated that mitogen-activated protein kinase (MAPK) signaling, including microphthalmia-associated transcription factor (MITF) and cAMP response element-binding protein (CREB) phosphorylation, is a major pathway involved in up-regulating melanogenesis within human melanocytes in several hyperpigmentary disorders such as UVB melanosis and lentigo senilis. Recently, a redox imbalance was shown to be closely linked to a variety of altered cellular responses in which the precise balance between levels of oxidizing and reducing equivalents that reflect the intracellular redox condition profoundly affects intracellular signaling pathways, especially the MAPK pathway. To elucidate the effects of redox balance regulation on epidermal pigmentation, we used an antioxidant-rich extract of the herb Withania somnifera to assess its effect on stem cell factor (SCF)-stimulated pigmentation in human epidermal equivalents and analyzed its biological mechanism of action. Addition of the W. somnifera extract (WSE) caused a marked reduction in SCF-stimulated pigmentation in a dose-dependent manner after 14 days of treatment, which was accompanied by a significant decrease in eumelanin content. In WSE-treated human epidermal equivalents, melanocyte-specific proteins (including tyrosinase) were significantly suppressed at the gene and protein levels by WSE. Signaling analysis with immunoblots revealed that in human melanocytes or human melanoma cells treated with WSE, there was a marked deficiency in SCF-stimulated phosphorylation of ERK, MITF and CREB, but not of Raf-1 and MEK. Since WSE had no direct inhibitory effect on tyrosinase activity and no melano-cytotoxic effect on melanocytes present in the human epidermal equivalents or on cultured human melanocytes, the sum of these findings indicates that WSE attenuates SCF-stimulated pigmentation by preferentially interrupting ERK phosphorylation within melanocytes and can serve as a therapeutic tool for SCF-associated hyperpigmentary disorders.

- Niki Y, Yoshida M, Ando H, Wakamatsu K, Ito S, Harada N, Matsui MS, Yarosh D, Ichihashi M.**1-(2,4-Dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylpheny)propane inhibits melanin synthesis by dual mechanisms.** J Dermatol Sci. 2011 Aug;63(2):115-21.
  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.

- Onojafe IF, Adams DR, Simeonov DR, Zhang J, Chan CC, Bernardini IM, Sergeev YV, Dolinska MB, Alur RP, Brilliant MH, Gahl WA, Brooks BP.
  Nitisinone improves eye and skin pigmentation defects in a mouse model of oculocutaneous albinism. J Clin Invest. 2011 Oct 3;121(10):3914-23.
  Mutation of the tyrosinase gene (TYR) causes oculocutaneous albinism, type 1 (OCA1), a condition characterized by reduced skin and eye melanin pigmentation and by vision loss. The retinal pigment epithelium influences postnatal visual development. Therefore, increasing ocular pigmentation in patients with OCA1 might enhance visual function. There are 2 forms of OCA1, OCA-1A and OCA-1B. Individuals with the former lack functional tyrosinase and therefore lack melanin, while individuals with the latter produce some melanin. We hypothesized that increasing plasma tyrosine concentrations using nitisinone, an FDA-approved inhibitor of tyrosine degradation, could stabilize tyrosinase and improve pigmentation in individuals with OCA1. Here, we tested this hypothesis in mice homozygous for either the Tyrc-2J null allele or the Tyrc-h allele, which model OCA-1A and OCA-1B, respectively. Only nitisinone-treated Tyrc-h/c-h mice manifested increased pigmentation in their fur and irides and had more pigmented melanosomes. High levels of tyrosine improved the stability and enzymatic function of the Tyrc-h protein and also increased overall melanin levels in melanocytes from a human with OCA-1B. These results suggest that the use of nitisinone in OCA-1B patients could improve their pigmentation and potentially ameliorate vision loss.

- Orio M, Bochot C, Dubois C, Gellon G, Hardré R, Jamet H, Luneau D, Philouze C, Réglier M, Serratrice G, Belle C.
  The Versatile Binding Mode of Transition-State Analogue Inhibitors of Tyrosinase towards Dicopper(II) Model Complexes: Experimental and Theoretical Investigations. Chemistry. 2011 Nov 25;17(48):13482-94. doi: 10.1002/chem.201100665. Epub 2011 Oct 25.
  We describe 2-mercaptopyridine-N-oxide (HSPNO) as a new and efficient competitive inhibitor of mushroom tyrosinase (K(IC) =3.7 μM). Binding studies of HSPNO and 2-hydroxypyridine-N-oxide (HOPNO) on dinuclear copper(II) complexes [Cu(2) (BPMP)(μ-OH)](ClO(4))(2) (1; HBPMP=2,6-bis[bis(2-pyridylmethyl)aminomethyl]-4-methylphenol) and [Cu(2) (BPEP)(μ-OH)](ClO(4))(2)) (2; HBPEP=2,6-bis{bis[2-(2-pyridyl)ethyl]aminomethyl}-4-methylphenol), known to be functional models for the tyrosinase diphenolase activity, have been performed. A combination of structural data, spectroscopic studies, and DFT calculations evidenced the adaptable binding mode (bridging versus chelating) of HOPNO in relation to the geometry and chelate size of the dicopper center. For comparison, binding studies of HSPNO and kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone) on dinuclear complexes were performed. A theoretical approach has been developed and validated on HOPNO adducts to compare the binding mode on the model complexes. It has been applied for HSPNO and kojic acid. Although results for HSPNO were in line with those obtained with HOPNO, thus reflecting their chemical similarity, we showed that the bridging mode was the most preferential binding mode for kojic acid on both complexes.

- Pinon A, Limami Y, Micallef L, Cook-Moreau J, Liagre B, Delage C, Duval RE, Simon A.
  A novel form of melanoma apoptosis resistance: melanogenesis up-regulation in apoptotic B16-F0 cells delays ursolic acid-triggered cell death. Exp Cell Res. 2011 Jul 15;317(12):1669-76.
  Melanoma is one of the most aggressive forms of cancer with a continuously growing incidence worldwide and is usually resistant to chemotherapy agents, which is due in part to a strong resistance to apoptosis. The resistance mechanisms are complex and melanoma cells may have diverse possibilities for regulating apoptosis to generate apoptotic deficiencies. In this study, we investigated the relationship between melanogenesis and resistance to apoptosis induced by ursolic acid, a natural chemopreventive agent, in B16-F0 melanoma cells. We demonstrated that cells undergoing apoptosis are able to delay their own death. It appeared that tyrosinase and TRP-1 up-regulation in apoptotic cells and the subsequent production of melanin were clearly implicated in an apoptosis resistance mechanism; while TRP-2, a well known mediator of melanoma resistance to cell death, was repressed. Our results confirm the difficulty of treating melanomas, since, even undergoing apoptosis, cells are nevertheless able to trigger a resistance mechanism to delay death.

- Um JM, Kim HJ, Lee Y, Choi CH, Hoang Nguyen D, Lee HB, Shin JH, Tai No K, Kim EK.
  A small molecule inhibitor of Mitf-E-box DNA binding and its depigmenting effect in melan-a cells. J Eur Acad Dermatol Venereol. 2011 Sep 29. doi: 10.1111/j.1468-3083.2011.04286.x. [Epub ahead of print]
  Background: Microphthalmia associated transcription factor (Mitf) is a key regulatory transcriptional factor of pigmentation-related genes including tyrosinase. Inhibition of tyrosinase transcription by blocking the binding of Mitf with its promoter E-box DNA can control the pigmentation. However, no such chemicals were reported so far. Objective: To discover and evaluate the small molecule inhibitors of Mitf-E-box DNA. Methods Candidate chemicals were screened by virtual screening from pharmacophore data followed by Mitf E-box DNA protein chip. After selecting the chemical, its inhibitory activity on binding interaction between Mitf and E-box DNA, electrophoretic mobility shift assay (EMSA) was performed. To evaluate the depigmenting activity of Compound #17, cellular melanin assa, and Western blot were performed in melan-a cells. Results: Among 27 chemicals selected from a pharmacophore data by virtual screening, Compound #17 was screened, which showed the most potent inhibitory activity against Mitf-E-box DNA binding in protein chip. EMSA results confirmed the specific inhibition of Compound #17 on Mitf-E-box DNA binding. In melan-a cells, Compound #17 reduced tyrosinase expression and melanin synthesis (62.5% at 25 μM). Conclusions: The results show that Compound #17 is the first small molecule inhibitor of Mitf-E-box DNA binding with depigmenting activity.

- 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 Sep;20(9):755-7. doi: 10.1111/j.1600-0625.2011.01297.x.
  We investigated the involvement of MAPK pathways in the melanogenic effect of apigenin in B16 cells. Apigenin treatment for 48 dose (5-20 μm)-dependently 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 MAPK. Treatment with 10 μm apigenin time (6-48 h)-dependently elevated the 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.

- Zarivi O, Bonfigli A, Colafarina S, Aimola P, Ragnelli AM, Pacioni G, Miranda M.
  Tyrosinase expression during black truffle development: From free living mycelium to ripe fruit body. Phytochemistry. 2011 Dec;72(18):2317-24. Epub 2011 Sep 23.
  The present work studies the expression of tyrosinase (monophenol:diphenol oxygen oxidoreductase, EC 1.14.18.1) during the development of the black truffle Tuber melanosporum Vittad., an ectomycorrhizal fungus of great biological and economic interest. As widely reported in the literature, melanins and the enzymes that synthesize them, are of paramount importance in fungal development and sexual differentiation. Tyrosinase and laccase are the enzymes that produce melanins from monophenols and diphenols. We have detected tyrosinase expression from the stage of free living mycelium, through the mychorrizal stage and the six fruit body developmental stages by measuring the levels of tyrosinase mRNA by quantitative PCR (q-PCR), spectrophotometry, histochemistry, immunohistochemistry and electrophoresis. Tyrosinase is always expressed, from the free living mycelium to the ripe fruit body developmental stages, when it is very low. The switching off of the tyrosinase gene during T. melanosporum development when the fruit body is ripe and no more cell walls are to be built is discussed in relation of thioflavour production. Specific primers, prepared from the cloned T. melanosporum tyrosinase cDNA were used for the q-PCR and the deduced aminoacid sequences of the CuA and CuB binding sites were compared to those of various ascomycetes and basidiomycetes.

8. Melanosomes

(Pr J. Borovansky)

Reviews were devoted to theTyrp-1 (*Ghanem&Fabrice*) and to the melanosome transport (Hume&Seabra).

Melanosomal proteins. Knockout mouse models were used to obtain information on Pmel (*Hellstrőm et al.*) and MART-1 (*Aydin et al*) functions.
Wang et al studied Mart-1 expression in uveal melanoma cells. Functional consequences of PMEL mutations found in Dominant White chicken and Silver horse were characterized by *Watt et al*. *Wang et al*. found a different Mart-1 expression in uveal melanoma lines.
Tarafder et al investigated mechanisms of targetting of Rab27a to melanosomes and *Hume et al* studied a participation of Rab27a and Rab32/38 in melanosome transport.
Cho& Celis emphasized the possibility of employing the Tyrp-1, Tyrp-2 and and gp100 epitopes in the vaccination against melanoma.

Melanosome loss. *Ebanks et al* demonstrated that the loss of melanosomes from the light keratinocytes is quicker compared to the dark skin keratinocytes.Gouras et al
distinquished two types of lysosomes engaged in melanosome digestion in the RPE.

Melanosome interaction(s) with light. Photobleaching of RPE melanosomes was reported by *Saha et al*., and *Kerimo et al. Peles and Simon* measured UV absorption spectra of uveal and RPE melanosomes.

Melanosome in fossil series has continued by articles by *Barden et al* and *Knight et al*.

Melanosomes under pathological situations were subject of a study by *Deviller et al*. (hypomelanosis of Ito) and by *Peterson et al* (HMB45 positive renal tumour).

- Aydin IT, Hummler E, Smit NPM, Beermann F.
  Coat color dilution in mice because of inactivation of the melanoma antigen MART-1. Pigment Cell Melanoma Res Sep 21. doi: 10.1111/j.1755-148X.2011.00910.x. [Epub ahead of print], 2011.
  To understand the function of MART-1 in pigmentation, the authors developed a knockout mouse model. The loss of MART-1 affected the morphology of hair follicle Stage III and IV melanosomes: they displayed blebbing of the limiting membrane and vesicles were observed between the pigment and the limiting membrane. The loss of MART-1 did not affect the ultrastructure of the RPE melanosomes.
  Lack of MART-1 did not affect the localization of melanocyte specific proteins nor the maturation of Pmel17 (which is contrary to Pmel17 changes in human melanoma cells - see J Biol Chem 281:21198-21208, 2005)

- Barden HE, Wogelius RA, Li D, Manning PL, Edwards NP, van Dongen BE
  Morphological and geochemical evidence of eumelanin preservation in the feathers of the early Cretaceous bird, Gansus yumenensis. PLOS One 6(10), e25494, 2011.
  The study combining morphological observation with geochemical analyses demonstrated the preservation of the endogenous pigmentation within circa 100 million year old fossilized feathers. Scanning EM revealed structures concordant with eumelanosomes. Infrared analysis (FTIR) strenghtened the conclusion by demonstrating the presence of characteristic eumelanic functional groups in the fossilized feather in contrast to the surrounding matrix.

- Cho HI, Celis E.
  Design of immunogenic and effective multi-epitope DNA vaccines for melanoma. Cancer Immunol Immunother [Sep 14, e-pub ahead of print], 2011.
  Multiepitope DNA vaccines containing sequences of Tyrp1, Tyrp2 and gp100 were constructed. Vaccination induced both prophylactic and antitumour responses against the B16 melanoma.

- Devillers C, Quatresooz P, Hermanns-Le T., Szepetiuk G, Lemaire R, Piérard-Franchimont C, Piérard GE.
  Hypomelanosis of Ito: pigmentary mosaicism with immature melanosome in keratinocytes. Int J Dermatol 50(10): 1234-1239, 2011.
  EM study of a biopsy from a young child showed a striking difference in the hypomelanized skin. Melanocytes contained fewer premature-looking single or compound melanosomes stage II-III

melanosomes) with a weak tyrosinase immunoreactivity.. Some melanosomes contained striated or zig-zag pleated coiled inclusions, others were distorted. Those immature or aberrant melanosomes present in keratinocytes were engulfed in prominent phagosomes.

- Ebanks, LP, Koshoffer A, Wickett RR, Schwemberger S, Bbcock G, Hakozaki T, Boissy RE, **Epidermal keratinocytes from light vs dark skin exhibit differential degradation of melanosomes**. J Invest Dermatol 131(6): 1226-1233, 2011.
  In a model system the loss of fluorescently labelled isolated melanosomes taken up by cultured human keratinocytes was traced. Melanosomes were incorporated into the cytoplasm both of light and dark keratinocytes and trafficked to perinuclear region. Confocal microscopy images and the measurement both of CFDA and MEL5/phycoerythrin fluorescence showed that within 48 hours the light keratinocytes have lost melanosomes more efficiently than the dark ones. TEM micrographs revealed ultrastructural disruption of some melanosomes.

- Ghanem G, Fabrice J.
  Tyrosinase related protein 1 (Tyrp1/gp75) in human cutaneous melanoma. Molecular Oncology 5(2): 150-155, 2011.
  A comprehensive review of the melanosomal proteinTyrp1/gp75 covers its gene, subcellular localization, its role in melanocytes (role in pigmentation, marker of differentiation, relation to oxidative stress) and in melanoma cells (anti-Tyrp1 antibodies, Tyrp1/gp75 as target for therapy, TYRP1 expression and in melanoma progression).

- Gouras P, Brown K, Ivert L, Neuringer M.
  A novel melano-lysosome in the retinal epithelium of rhesus monkeys. Exp Eye Res [epub-ahead of print] 2011.
  Morphometric parameters of the RPE melanosomes were recorded in relation to the monkeys' age. With age, melanosomes are lost from the RPE. According to the authors much of the loss is attributed to two types of lysosomes. One, not defined as unique before, appears to be autophagic in digesting its own melanin (=Type 1 lysosome decreasing with age). The other, a more canonical lysosome, is both heterophagic in digesting phagosomes and autophagic in digesting local melanosomes (= Type 2 lysosome increasing with age). There is no attempt to discuss the chemical nature of melanosome degradation.

- Hellström AR, Watt B, Fard SS, Tenza D, Mannström P, Narfström K, Ekesten B, Ito S, Wakamatsu K, Larsson J, Ulfendahl M, Kullander K, Raposo G, Kerje S, Halbőők F, Marks MS, Andersson L.
  Inactivation of Pmel alters melanosome shape but has only a subtle effect on visible pigmentation. PLOS Genetics 7(9): e1002285, 2011.
  Pmel knockout mouse was developed to gain an insight into the function(s) of this protein. Mice lacking PMEL have almost normal visible pigmentation. A loss of PMEL had a dramatic impact on the melanosome morphometry parameters: Epidermal and hair rod-shaped melanosomes and oblong choroid and RPE melanosomes were spherical in Pmel -/ - mice. The knockout mice had a substantial reduction of the black pigment in hair. In the discussion the authors confirmed our view that the shape of the melanosome has no relation to the type of the pigment produced.(cf. Folia Morphol.(Prague), 20: 82-84, 1972).

- Hume AN, Seabra MC
  Melanosome on the move: a model to understand organelle dynamics. Biochem Soc Trans 39(5): 1191-1196, 2011.

- Hume AN, Wilson MS, Ushakov DS, Ferenczi MA, Seabra MC.
  Semiautomated analysis of organelle movement and membrane content: Understanding Rab-motor complex transport function. Traffic 12(12):1686-16701, 2011.
  A semiautomated method for the quantitative analysis of the melanosome movement and the Rab27a-melanophilin (Mlph)-myosinVA complex recruitment revealed: Rab27a is enriched on slow moving and static melanosomes, whereas Rab32/38 on fast moving melanosomes that have small melanin cores. The correlation between the Rab27a status and the melanosome speed is dependent upon effector Mlph. Rab27a recruitment switches melanosomes from microtubule to actin-dependent transport.

- Kasraee B, Pataky M, Nikolic DS, Carraux P, Piguet V, Salomon D, Sorg O, Saurat JH.
  A new spectrophotometric method for simple quantification of melanosomal transfer from melanocytes to keratinocytes. Exp Dermatol 20: 938-942, 2011.

A new method for a 3-dimensional coculture of melanocytes and keratinocytes , that permits a direct transfer of melanosomes from melanocytes to keratiocytes, followed by a simple and reliable cell separation and a simple spectrophotometric quantification of melanosomes (accomplished by an ELISA method using gp100 as antigen) was developed.

- Kerimo J, Rajadhyaksha M, DiMarzio CA
  Enhanced melanin fluorescence by stepwise three-photon excitation. Photochem. Photobiol. 87(5), 1042-1049, 2011.
  The fluorescence of eumelanin (in Sepia officinalis granules and in black hair melanosomes) was activated and enhanced by exposure to a near infrared radiation The near infrared radiation caused obvious changes to the pigment organelles that could be seen by the fluorescence and bright field imaging. The photobleaching was reduced in a nitrogen atmosphere. .The authors declare that this is the first reported evidence of three-photon fluorescence from melanin.

- Knight TK, Bingham PS, Lewis RD, Savrda CE.
  Feathers of the Ingersoll shale, eutaw formation (upper Cretaceous), Eastern Alabama: The largest collection of feathers from North American mesozoic rocks. Palaios 26: 364-376, 2011.
  Fourteen fossil feather specimens representing a number of different individuals and probably a number of different species found in clay lens was studied by means of scanning EM. Preservation of the feather ultrastructure was very good, with distinct ovate to rod shaped elements paralleling the feather branches. These elements are most probably melanin bodies (melanosomes). The analysis of their morphology and density indicates original colours ranging from gray and brownish gray to black.

- Peles DN, Simon JD.
  UV-absorption spectra of melanosomes containing varying 5,6-dihydroxyindole and 5,6-dihydroxyindole-2 carboxylic acid content. J Phys Chem B 115(43): 12624-12631, 2011.
  The absorption spectra of eumelanosomes were found to differ significantly from their constituent precursor molecules DHI and DHICA. The absorption coefficients of
  melanosomes isolated from Z CEHO ISOLOVANYCH ??? were quantified by means of the photoemission EM technique. The coefficients of the RPE melanosomes were □1.5 larger than those of uveal melanosomes. It means that melanosomes of different embryonic origin may have different pigment densities or differences in the structure of their constituent eumelanin. (see also J Phys Chem Letters 1: 2391-2395, 2010)

- Peterson F, Vaněček T, Michal M, Martignoni G, Brunelli M, Halbhuber Z, Spagnolo D, Kuroda N, Yang X, Cabrero IA, Hora M, Branžovský J, Trivunic S, Kacerovská D, Steiner P, Hes O.
  A distinctive translocation carcinoma of the kidney; rosette forming", t(6;11), HMB45-positive renal tumor: a histomorpologic, immunohistochemical, ultrastructural and molecular genetic study of 4 cases. Human Pathol – epub ahead of print, 2011.
  Tumour cells displayed a focal immunoreactivity for the melanocytic marker HMB45, cathepsin K and vimentin. Melan A, tyrosinase, cytokeratins, CD10, and microphthalmia transcription factor were each positive in 3 of 4 cases. On an ultrastructural examination numerous electron-dense secretory cytoplasmic granules with some resemblance to melanosomes were observed. However, no definitive melanosomes or premelanosomes of any developmental stage were identified.

- Saha A, Arora R, Yakovlev VV, Burke JM.
  Raman microspectroscopy of melanosomes: the effect of long term light irradiation. J Biophotonics 4(11-12): 805-813, 2011.
  Isolated RPE melanosomes were exposed to green light 532nm and the chemical changes leading to their photobleaching were recorded by Raman microspectroscopy.

- TarafderAK, Wasmeier C, Figueiredo AC, Booth AEG, Orihara A, Ramalho JS, Hme AN, Seabra MC.
  Rab27a targeting to melanosomes requires nucleotide exchange but not effector binding. Traffic 12(8): 1056-1066, 2011.
  Rab 27a regulates the motility of lysosome-related organelles and secretory granules. Using
  Rab27a mutants that show impaired binding to the representatives of four Rab27a effector subgroups, the authors demonstrated that effector binding is not essential for targetting
  Rab27a to melanosomes unlike Rab3-guanine nucleotide exchange factor.

- Wang J, Jia RB , Yao YT , Cun BY , Huang XL , Gu P, Ge SF, Fan XQ

Differential expression of Mart-1 in human uveal melanoma cells. Molecular Medicine Rep. 4(5):799-803, 2011.
Differential expression of Mart-1 was investigated in four human uveal melanoma cells (SP6.5, VUP, OCM-1 and OM431) on three levels of analysis: mRNA, protein and, morphology. The expression of Mart-1 varied among the lines studied.

– Watt B, Tenza D, Lemmon MA, Kerje S, Raposo G, Andersson L, Marks MS.
Mutations in or near the transmembrane domain alter PMEL amyloid formation from functional to pathogenic. PLOS Genetics 7(9), e1002286, 2011.
Whereas animal models that entirely lack the PMEL expression have modest pigment loss, PMEL alleles found in Dominant White (DW) chicken and Silver horse (HoSi), which bear mutations that alter the non-amyloidogenic region of PMEL, are associated with a striking loss of pigmentation.The authors showed that the DW and HoSi mutations altered the PMEL transmembrane domain oligomerization and/or association with membranes , with
consequent formation of aberrantly packed fibrils. The aberrant fibrils were associated with loss of pigmentation in cultured melanocytes.

9. Melanoma experimental, cell culture

(Dr R. Morandini)

Most of in vitro studies in experimental skin biology have been done in 2-dimensional monocultures, while accumulating evidence suggests that cells have a different phenotype when they are grown within a 3D extracellular matrix and also interact with other cells in the matrix. *Li et al*. has published a complete protocol to generate a 3D skin reconstructs with normal human melanocytes, dermal stem cells and melanoma cells. They have used viral vector in order to activate or inactivate gene function for a better understanding of the dynamics and functional significance of genes expressed by each cell type. This seems to be a good model to study transformation mechanisms of melanocytes, but also progression of melanoma
An important remark has been done by the Authors: "*Mouse models have been broadly utilized to study tissue morphogenesis in vivo. However mouse and human skin have significant differences in cellular architecture and physiology, which makes it difficult to extrapolate mouse studies to humans.*"

The concomitant expression of MMP-2 and MMP-9, their inhibitors TIMP-1 and TIMP-2, the p53 protein, and Ki67 in melanoma has been studied by Väisänen *et al*. in a series of 157 cases of skin melanoma. They evaluate a novel immunohistochemical prognostic index that could help triage melanoma patients at increased risk of recurrence.

A. Signal transduction and cell culture

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ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events
2011 ESPCR Members

Calendar of Events

2012 PTEN Pathways & Targets Meeting
March 13-16, Cold Spring Harbor, NY 11724
Contact: Web: http://meetings.cshl.edu/meetings/pten12.shtml

2012 XVIIth Meeting of the ESPCR
September 11-14, Geneva, Switzerland
Contact: Dr Bernhard WEHRLE-HALLER

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

2012 PASPCR Meeting
September 19-22, Park City, UT
Contact: Web: www.huntsmancancer.org/paspcr2012

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: E-mail: congress@worldmelanoma2013.com
Web: www.worldmelanoma2013.com

2014 XXIInd IPCC Meeting

Mid 2014, Singapore
Contact: Web: www.ipcc2014.org

2015 45th Annual ESDR Meeting

September 9-12, Rotterdam, The Netherlands

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