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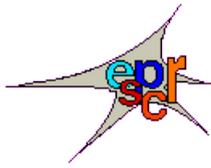
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**LETTER TO THE EDITOR
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Editor's Highlights

**A Landscape of Driver Mutations in Melanoma
Cell 150, 251–263, July 20, 2012**

Eran Hodis, Ian R. Watson, Gregory V. Kryukov, Stefan T. Arold, Marcin Imielinski, Jean-Philippe Theurillat, Elizabeth Nickerson, Daniel Auclair, Liren Li, Chelsea Place, Daniel DiCara, Alex H. Ramos, Michael S. Lawrence, Kristian Cibulskis, Andrey Sivachenko, Douglas Voet, Gordon Saksena, Nicolas Stransky, Robert C. Onofrio, Wendy Winckler, Kristin Ardlie, Nikhil Wagle, Jennifer Wargo, Kelly Chong, Donald L. Morton, Katherine Stemke-Hale, Guo Chen, Michael Noble, Matthew Meyerson, John E. Ladbury, Michael A. Davies, Jeffrey E. Gershenwald, Stephan N. Wagner, Dave S.B. Hoon, Dirk Schadendorf, Eric S. Lander, Stacey B. Gabriel, Gad Getz, Levi A. Garraway, and Lynda Chin.

SUMMARY

Despite recent insights into melanoma genetics, systematic surveys for driver mutations are challenged by an abundance of passenger mutations caused by carcinogenic UV light exposure. We developed a permutation-based framework to address this challenge, employing mutation data from intronic sequences to control for passenger mutational load on a per gene basis. Analysis of large-scale melanoma exome data by this approach discovered six novel melanoma genes (PPP6C, RAC1, SNX31, TACC1, STK19, and ARID2), three of which—RAC1, PPP6C, and STK19—harbored recurrent and potentially targetable mutations. Integration with chromosomal copy number data contextualized the landscape of driver mutations, providing oncogenic insights in BRAF- and NRAS-driven melanoma as well as those without known NRAS/BRAF mutations. The landscape also clarified a mutational basis for RB and p53 pathway deregulation in this malignancy.

Finally, **the spectrum of driver mutations provided unequivocal genomic evidence for a direct mutagenic role of UV light in melanoma pathogenesis.**



1. Chemistry of Melanins and other Pigments

(Pr A. Napolitano)

The ability of melanin to protect tissues from gamma rays is emerging as a topic of increasing interest. In a paper appeared in this term (Ghannan and Mady, J. Phys Sci.) the effect of irradiation with 5 to 50 Gy gamma rays on the pigment structure was investigated by different techniques showing a substantial modification of properties such as the extinction coefficient or conductivity. This result was interpreted as due to the formation of aggregates and an increase of the cross link favoured by the growing number of radicals generated by the radiation.

The binding of melanin to the antibiotic netilmicin and how this can be modified by the presence of metal ions of physiological relevance was evidenced by the study appeared on Acta Poloniae Pharmaceutica, whereas the strong binding ability of melanin toward heavy metals like lead suggested a possible exploitation for water remediation (Sono et al Bioorg Chem and Appl).

A paper appeared on PNAS (Glass et al) report evidence for the presence of melanin in the ink of a cephalopod dating back to the Jurassic period, pointing to an unbelievable stability of the pigment to degradation.

Another PNAS report by groups of T. Sarna and P. Meredith reinforces the discovery recently published that hydration of melanin has a dramatic effect on the comproportionation equilibrium (hydroxyindole-quinone/semiquinone) doping electrons and protons into the system. On this basis melanin should be regarded as electronic ionic hybrid conductor rather than an amorphous semiconductor as originally proposed.

Melanin-like pigment obtained from various fungi were investigated by X-ray analysis in comparison with synthetic melanins and neuromelanins and were found to show differences in the distances between the planar assemblies giving stacking interactions. (Casadevall et al PLoS One). It is proposed that assessment of the stacking that appears to be a general feature of melanin pigments and measurement of interplanar distance may represent an innovative identification tool.

Structure, Reactivity and Properties

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Plant and fungal pigments

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Characterization of melanin pigment produced by Aspergillus nidulans. *World Journal of Microbiology & Biotechnology* (2012), 28(4): 1467-1474.

2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

The American Joint Committee on Cancer has indicated the mitotic rate (number of mitoses per mm²) as the major prognostic criterion for the classification of thin melanoma (Leibberg *et al.* 2009). Leibberg *et al.* performed an immunohistochemical dual staining evaluation on 15 melanomas with a tumor thickness <1.0 mm using phosphohistone-H3 (PHH3) and melan-A to identify mitoses in melanocytic cells. In fact, PHH3 specifically and sensitively detects cells through the mitotic cycle from prophase to telophase and it is already used in the detection of mitotic rate in several tumours. Based on the author's findings, to combine PHH3 and melan-A double labelling in the staging of melanoma allows concordant results to those obtained from E&E stained sections with the advantage to be much more faster. Double immunohistochemical staining may be therefore helpful to standardize the evaluation of the mitotic rate and may represent a useful tool in the management of challenging cases. In the work published on the Journal of Cutaneous Pathology, Mills *et al.* report their institutional experience with sentinel lymph node biopsy (SNLB) in difficult-to-diagnose atypical melanocytic neoplasms of pediatric patients, evaluating 24 subjects with a median age of 15.5 years (range 4-21 years). Atypical melanocytic neoplasms were defined as melanocytic proliferations of uncertain biologic potential that did not reach a definitive diagnosis, even after an outside consultative opinion. Although the author's analysis showed a lower proportion of patients with positive SLNs (29%) than the published average (58%), it confirmed the long-term disease-free survival already reported for pediatric patients, suggesting that atypical melanocytic proliferations in childhood may be less aggressive than conventional melanoma. However, the meaning of nodal involvement still produces unsolved controversies and further works with long-term follow up on larger number of patients are required to ascertain the correct prognostic value of SNLB.

Melasma in men is less frequent than in women. UV exposure, sex hormones and genetic influences have been largely considered in the development of this hyperpigmentary disorder. In addition, several studies focused on the pro-melanogenic paracrine network of cytokines and growth factors existing among the different skin cell population as an important factor influencing the onset of the lesions. The aim of the study by Jang *et al.* was to better define the histopathological features of melasma in men in comparison to those of women to highlight the main predisposing factors for the pathogenesis of male melasma. Specimens obtained from lesional and non lesional skin of 8 men with melasma, 10 women with melasma and 5 men and women each with solar lentigo were evaluated for the amount of melanin, number of melanocytes and the expression of estrogen receptor, progesterone receptor, factor VIIIa-related antigen, stem cell factor (SCF) and c-kit. The results showed a prominent solar elastosis in male melasma skin associated with an increased vascularity and a significant increase of SCF and c-kit expression compared with female melasma and solar lentigo, suggesting that accumulation of chronic sun exposure together with activation of paracrine cytokine signalling pathways may represent the most important factors leading to the hyperpigmentation of male melasma. Nakajima *et al.* evaluated melanocyte localization and dendricity in solar lentigines (SL) utilizing in vivo reflectance confocal microscopy (RCM). This technique represents a non invasive imaging tool which allows skin visualization with no alteration of the tissue. Seventeen female with a solar lentigo on the face with a diameter larger than 1 cm were included in the study. The authors observed that melanocyte dendrites were rarely present in the center of the lesion but they were very frequently seen in the outer rim. These results suggest an activated status of melanocytes at the borders of SL with a higher melanin production and an horizontally spreading of their dendrites. As for melasma, several cytokines and growth factors are involved in the development of these pigmented spots. However, the mechanisms underlying the onset of SL are not completely defined and the Authors suggest that their findings using RCM may be the result of different biological activities exerted by melanocytes in the center and outer border of solar lentigines. Among the numerous paracrine factors released by the different skin cell types contributing to regulate human skin pigmentation, neuregulin-1 (NRG1) is expressed and secreted at high level by fibroblasts from type VI dark skin and it is involved in the control of constitutive pigmentation. It has been previously demonstrated using melanocyte cultures and 3D skin reconstructs that recombinant human NRG1 increases melanocytes proliferation and pigmentation. Choi *et al.* characterized in their work the smallest bioactive motif of NRG1 specifically involved in increasing melanin production in melanocytes without affecting cell proliferation. The discovery of this small bioactive peptide may represent an advantage in clinical application both in terms of a more efficient skin barrier delivery and of a specific modulation of pigmentation without affecting melanocyte proliferation. Exposure of skin to UV results in the upregulation of COX-2 expression and increased production of its primary product, prostaglandin (PG) E₂. Melanocytes have the machinery for PGE₂ production and to express COX-2 mRNA and PGE₂ released from melanocytes in response to UV radiation is known to stimulate cAMP release and tyrosinase activity in an autocrine manner. To establish the role of COX-2 in melanogenesis, Kim *et al.* investigated the effect of COX-2 knock-down in melanocytes on melanin production and expression of melanogenic molecules by transfecting melanocytes with COX-2 short interfering RNA (siRNA). The authors showed that COX-2 knock-down in melanocytes resulted in decreased expression of tyrosinase, TRP-1 and MITF, as well as reduced tyrosinase enzyme activity. Moreover, COX-2 siRNA-transfected melanocytes showed markedly reduced melanocyte-stimulating hormone (α-MSH)-enhanced melanin production. These results indicate that COX-2 inhibitors might be effective at treating hyperpigmentation disorders such as melasma, postinflammatory hyperpigmentation and solar lentigo. Cryptoxanthin (CPX) is a carotenoid that is widely contained in the fruits of citrus plants. Shimoda and co-workers evaluated the effect of CPX on UVB-induced pigmentation and mRNA expression related to

melanogenesis in mouse skin. In addition, changes in melanogenic molecules were evaluated in cultured melanocytes stimulated with PGE₂, α -MSH and endothelin (ET)-1. Oral administration of α -MSH was found to suppress UVB-induced melanogenesis. Suppression of melanogenic enzymes, receptors of melanogenic stimulators, expression and phosphorylation of CREB are involved in the depigmenting mechanism. Vitiligo is an acquired depigmenting disorder featured by loss of melanocytes from the epidermis. Medical and surgical approaches are used for the treatment of the disease with the aim to repigment the lesional skin areas. Disease stability is considered the main parameter to proceed for any melanocyte transplantation procedures. In the work of **Rao *et al.***, the authors analyze the clinical, biochemical and immunological factors controlling disease stability in patients with generalized vitiligo with the aim to select the patients for melanocyte transplantation and to better highlight the mechanisms at the base of disease activity. Thirtythree patients with generalized vitiligo were enrolled in the study and divided in three groups based on disease clinical stability (group 1: stability > 3 months but < 1 year; group 2: □ 1 year b years). Melanocyte transplantation was performed using suction blister epidermal grafting (SBEG) on a single patch taking a punch biopsy on the day of transplantation from the margin of the macule to perform immunohistochemical analysis of CD4, CD8, CD45RO, CD45RA and FoxP3. The results showed that the percentage of repigmentation and the success rate increased parallel to the increased duration of disease stability. Moreover, the non responders displayed a significantly higher number of CD8 and CD45RO cells in comparison to the nonresponders, suggesting that the percentage of these cells in the lesional skin may be used as an helpful tool to establish the stability of the disease and predict a successful outcome of melanocyte transplantation. **Toosi *et al*** investigated the mechanism of vitiligo initiated by exposure to a chemical trigger such as 4-tertiary butyl phenol (4-TBP) and monobenzyl ether of hydroquinone (MBEH). The authors hypothesized that oxidative stress, induced inside melanocytes by contact with 4-TBP and MBEH, leads to disruption of homeostasis in the endoplasmic reticulum (ER), subsequent accumulation of misfolded proteins and activation of the unfolded protein response (UPR). Signaling via the UPR enhances the nuclear factor erythroid 2-related factor (NRF2)/heme oxygenase-1 (HMOX1) antioxidant response and allows restoration of homeostasis. However, due to their genetic background individuals susceptible to vitiligo are unable to sufficiently combat the oxidative stress and the sustained UPR activity leads to increased expression of pro-inflammatory cytokines, such as IL-6 and IL-8 that may contribute to autoimmune-mediated progression of vitiligo. In this study the authors suggested UPR as a key link between oxidative stress and activation of inflammatory response, improving the understanding of the mechanisms that link environmental stressors and autoimmunity. Due to the fact that vitiligo is not a disease confined to melanocytes, keratinocytes in depigmented epidermis may constitute a different microenvironment compared to those in normally pigmented epidermis. **Lee** revised the role of keratinocytes in the development of vitiligo, focusing on the structural changes in vitiliginous keratinocytes which may result in loss of melanocytes. In particular altered pathways able to explain the increased vulnerability to apoptosis of keratinocytes in the depigmented epidermis were addressed. The authors illustrated main mechanisms for apoptosis of vitiliginous keratinocytes, namely impaired Phosphatidylinositol 3-kinase (PI3K)/serine/threonine protein kinase (Akt) activation followed by reduced nuclear factor- κ B (NF- κ B) activation under increased tumor necrosis factor- α levels, down-regulation of aquaporin 3 expression connected to reduced phosphorylation of PI3K/AKT1 and expression of E-cadherin-catenin complex, and decreased production of keratinocyte-derived melanocytes growth factors, including stem-cell-factor, resulting in passive melanocytes death with the development of vitiligo. The development of vitiligo therapies has been impeded by a paucity of animal models, since mice lack interfollicular melanocytes, the primary targets in vitiligo. **Harris and coworkers** described a mouse model in which interfollicular melanocytes are retained by Kit ligand overexpression and an immune response is initiated by transplanting melanocyte-targeting CD8 β T cells. The new animal model does not shed light on the factors that lead to the onset of vitiligo and activation of the immune response, but it does recapitulate the autoimmune destruction of epidermal melanocytes making it especially useful in the development of immunomodulatory therapies for this common and disfiguring condition. Polymorphisms in IL-4 gene are known to increase its expression thereby implicating its role in vitiligo susceptibility. **Imran *et al*** explored intron 3 VNTR (IVS3) and -590 C/T (rs2243250) promoter polymorphism in IL-4 gene in Gujarat population. The authors suggested that these polymorphisms of IL-4 gene may be genetic risk factors for susceptibility towards vitiligo and the up-regulation of IL-4 transcript, protein and IgE levels in individuals with susceptible haplotypes reveal the crucial role of interleukin-4 in the pathogenesis of vitiligo. In the work by **Duval *et al.*** the authors developed a pigmented reconstructed skin model encompassing normal human melanocytes, keratinocytes and dermal fibroblasts capable to develop a real constitutive pigmentation, normalizing keratinocyte differentiation by the supplementation of keratinocyte growth factor (KGF) instead of epidermal growth factor (EGF). This model was able to produce melanin, transfer it to the neighbouring keratinocytes and induce pigmentation in response to propigmenting agents such as forskolin and α -MSH. This model may therefore represent an important tool to better highlight the complex epithelial-mesenchymal cross-talk controlling and regulating skin pigmentation.

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3. MSH, MCH, other hormones, differentiation

(Pr M. Böhm)

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4. Photobiology

(Pr M-D Galibert)

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Abstract: Melanoma is notable for its metastatic propensity, lethality in the advanced setting and association with ultraviolet exposure early in life. To obtain a comprehensive genomic view of melanoma in humans, we sequenced the genomes of 25 metastatic melanomas and matched germline DNA. A wide range of point mutation rates was observed: lowest in melanomas whose primaries arose on non-ultraviolet-exposed hairless skin of the extremities (3 and 14 per megabase (Mb) of genome), intermediate in those originating from hair-bearing skin of the trunk (5-55 per Mb), and highest in a patient with a documented history of chronic sun exposure (111 per Mb). Analysis of whole-genome sequence data identified PREX2 (phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2)--a PTEN-interacting protein and negative regulator of PTEN in breast cancer--as a significantly mutated gene with a mutation frequency of approximately 14% in an independent extension cohort of 107 human melanomas. PREX2 mutations are biologically relevant, as ectopic expression of mutant PREX2 accelerated tumour formation of immortalized human melanocytes in vivo. Thus, whole-genome sequencing of human melanoma tumours revealed genomic evidence of ultraviolet pathogenesis and discovered a new recurrently mutated gene in melanoma.
- Centore RC, Yazinski SA, Tse A, Zou L. **Spartan/C1orf124, a Reader of PCNA Ubiquitylation and a Regulator of UV-Induced DNA Damage Response.** *Mol Cell*. 2012 Jun 8;46(5):625-35.
Abstract: PCNA is a key component of DNA replication and repair machineries. DNA damage-induced PCNA ubiquitylation serves as a molecular mark to orchestrate postreplication repair. Here, we have identified and characterized Spartan, a protein that specifically recognizes ubiquitylated PCNA and plays an important role in cellular resistance to UV radiation. In vitro, Spartan engages ubiquitylated PCNA via both a PIP box and a UBZ domain. In cells, Spartan is recruited to sites of UV damage in a manner dependent upon the PIP box, the UBZ domain, and PCNA ubiquitylation. Furthermore, Spartan colocalizes and interacts with Rad18, the E3 ubiquitin ligase that modifies PCNA. Surprisingly, while Spartan is recruited by ubiquitylated PCNA, knockdown of Spartan compromised chromatin association of Rad18, monoubiquitylation of PCNA, and localization of Pol η to UV damage. Thus, as a "reader" of ubiquitylated PCNA, Spartan promotes an unexpected feed-forward loop to enhance PCNA ubiquitylation and translesion DNA synthesis.
- Furney SJ, Turajlic S, Fenwick K, Lambros MB, Mackay A, Ricken G, Mitsopoulos C, Kozarewa I, Hakas J, Zvelebil M, Lord CJ, Ashworth A, Reis-Filho JS, Herlyn M, Murata H, Marais R. **Genomic characterisation of acral melanoma cell lines.** *Pigment Cell Melanoma Res*. 2012 Jul;25(4):488-92.
Abstract : Acral melanoma is a rare melanoma subtype with distinct epidemiological, clinical and genetic features. To determine if acral melanoma cell lines are representative of this melanoma subtype, six lines were analysed by whole-exome sequencing and array comparative genomic hybridisation. We demonstrate that the cell lines display a mutation rate that is comparable to that of published primary and metastatic acral melanomas and observe a mutational signature suggestive of UV-induced mutagenesis in two of the cell lines. Mutations were identified in oncogenes and tumour suppressors previously linked to melanoma including BRAF, NRAS, KIT, PTEN and TP53, in cancer genes not previously linked to melanoma and in genes linked to DNA repair such as BRCA1 and BRCA2. Our findings provide strong circumstantial evidence to suggest that acral melanoma cell lines and acral tumours share genetic features in common and that these cells are therefore valuable tools to investigate the biology of this aggressive melanoma subtype.
- Haruta N, Kubota Y, Hishida T. **Chronic low-dose ultraviolet-induced mutagenesis in nucleotide excision repair-deficient cells.** *Nucleic Acids Res*. 2012 Jun 28.
Abstract: UV radiation induces two major types of DNA lesions, cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidine photoproducts, which are both primarily repaired by nucleotide excision repair (NER). Here, we investigated how chronic low-dose UV (CLUV)-induced mutagenesis occurs in rad14 \square NER-deficient yeast cells, which lack the yeast orthologue of human xeroderma pigmentosum A (XPA). The results show that rad14 \square induced mutations, most of which are C \square T transitions, occur in the non-template strand for transcription. Unexpectedly, many of the CLUV-induced C \square T mutations are dependent on translesion synthesis (TLS) DNA polymerase η , encoded by RAD30, despite its previously

T transition
 T mutation

established role in error-free TLS. Furthermore, we demonstrate that deamination of cytosine-containing CPDs contributes to CLUV-induced mutagenesis. Taken together, these results uncover a novel role for Pol η in the induction of C [Containing CPDs in CLUV-exposed NER-deficient cells. More generally, our data suggest that Pol η can act as both an error-free and a mutagenic DNA polymerase, depending on whether the NER pathway is available to efficiently repair damaged templates.

- Hodis E, Watson IR., Kryukov GV., Arold ST., Imielinski M, Theurillat J-P, Nickerson E, Auclair D, Li L, Place C, DiCara D, Ramos AH., Lawrence MS., Cibulskis K, Sivachenko A, Voet D, Saksena G, Stransky N, Onofrio RC., Winckler W, Ardlie K, Wagle N, Wargo J, Chong K, Morton DL., Stemke-Hale K, Chen G, Noble M, Meyerson M, Ladbury JE., Davies MA., Gershenwald JE., Wagner SN., Hoon D.S.B., Schadendorf D, Lander ES., Gabriel S.B., Getz G, Garraway L.A., Chin L.

A Landscape of Driver Mutations in Melanoma. CELL Volume 150, Issue 2, 2012, Pages 251–263.

Summary: Despite recent insights into melanoma genetics, systematic surveys for driver mutations are challenged by an abundance of passenger mutations caused by carcinogenic UV light exposure. We developed a permutation-based framework to address this challenge, employing mutation data from intronic sequences to control for passenger mutational load on a per gene basis. Analysis of large-scale melanoma exome data by this approach discovered six novel melanoma genes (PPP6C, RAC1, SNX31, TACC1, STK19, and ARID2), three of which—RAC1, PPP6C, and STK19—harbored recurrent and potentially targetable mutations. Integration with chromosomal copy number data contextualized the landscape of driver mutations, providing oncogenic insights in BRAF- and NRAS-driven melanoma as well as those without known NRAS/BRAF mutations. The landscape also clarified a mutational basis for RB and p53 pathway deregulation in this malignancy. Finally, the spectrum of driver mutations provided unequivocal genomic evidence for a direct mutagenic role of UV light in melanoma pathogenesis.

- Jarrett SG, Novak M, Harris N, Merlino G, Slominski A, Kaetzel DM.
NM23 deficiency promotes metastasis in a UV radiation-induced mouse model of human melanoma. Clin Exp Metastasis. 2012 Jun 15

Abstract: Cutaneous malignant melanoma is the most lethal form of skin cancer, with 5-year survival rates of <5 % for patients presenting with metastatic disease. Mechanisms underlying metastatic spread of UVR-induced melanoma are not well understood, in part due to a paucity of animal models that accurately recapitulate the disease in its advanced forms. We have employed a transgenic mouse strain harboring a tandem deletion of the nm23-m1 and nm23-m2 genes to assess the combined contribution of these genes to suppression of melanoma metastasis. Crossing of the nm23-h1/nm23-h2 knockout in hemizygous-null form ([m1m2](+/-)) to a transgenic mouse strain (hepatocyte growth factor/scatter factor-overexpressing, or HGF(+) strain) vulnerable to poorly-metastatic, UVR-induced melanomas resulted in UVR-induced melanomas with high metastatic potential. Metastasis to draining lymph nodes was seen in almost all cases of back skin melanomas, while aggressive metastasis to lung, thoracic cavity, liver and bone also occurred. Interestingly, no differences were observed in the invasive characteristics of primary melanomas of HGF(+) and HGF(+) X [m1m2](+/-) strains, with both exhibiting invasion into the dermis and subcutis, indicating factors other than simple invasive activity were responsible for metastasis of HGF(+) X [m1m2](+/-) melanomas. Stable cell lines were established from the primary and metastatic melanoma lesions from these mice, with HGF(+) X [m1m2](+/-) lines exhibiting increased single cell migration and genomic instability. These studies demonstrate for the first time in vivo a potent metastasis suppressor activity of NM23 in UVR-induced melanoma, and have provided new tools for identifying molecular mechanisms that underlie melanoma metastasis.

- Kadekaro AL, Chen J, Yang J, Chen S, Jameson J, Swope VB, Cheng T, Kadakia M, Abdel-Malek Z.
Alpha-Melanocyte-Stimulating Hormone Suppresses Oxidative Stress through a p53-Mediated Signaling Pathway in Human Melanocytes. Mol Cancer Res. 2012 Jun;10(6):778-86.

Abstract: Epidermal melanocytes are skin cells specialized in melanin production. Activation of the melanocortin 1 receptor (MC1R) on melanocytes by α -melanocyte-stimulating hormone (α -MSH) induces synthesis of the brown/black pigment eumelanin that confers photoprotection from solar UV radiation (UVR). Contrary to keratinocytes, melanocytes are slow proliferating cells that persist in the skin for decades, in an environment with high levels of UVR-induced reactive oxygen species (ROS). We previously reported that in addition to its role in pigmentation, α -MSH also reduces oxidative stress and enhances the repair of DNA photoproducts in melanocytes, independent of melanin synthesis. Given the significance of ROS in carcinogenesis, here we investigated the mechanisms by which α -MSH exerts antioxidant effects in melanocytes. We show that activation of the MC1R by α -MSH contributes to phosphorylation of p53 on serine 15, a known requirement for stabilization and activation of p53, a major sensor of DNA damage. This effect is mediated by the cAMP/PKA pathway and by the activation of phosphoinositide 3-kinase (PI3K) ATR and DNA protein kinase (DNA-PK). α -MSH increases the levels of 8-oxoguanine DNA glycosylase (OGG1) and apurinic apyrimidinic endonuclease 1 (APE-1/Ref-1), enzymes essential for base excision repair. Nutlin-3, an HDM2 inhibitor, mimicked the effects of α -MSH resulting in reduced phosphorylation of H2AX (g-H2AX), a marker of DNA damage. Conversely, the p53 inhibitor pifithrin-a or silencing of p53 abolished the effects of α -MSH and augmented oxidative stress. These

results show that p53 is an important target of the downstream MC1R signaling that reduces oxidative stress and possibly malignant transformation of melanocytes. *Mol Cancer Res*; 10(6); 778-86. ©2012 AACR.

- Laura Dántola M, Gojanovich AD, Thomas AH.
Inactivation of tyrosinase photoinduced by pterin. *Biochem Biophys Res Commun.* 2012 Jul 7.
Abstract: Tyrosinase catalyzes in mammals the first and rate-limiting step in the biosynthesis of the melanin, the main pigment of the skin. Pterins, heterocyclic compounds able to photoinduce oxidation of DNA and its components, accumulate in the skin of patients suffering from vitiligo, a chronic depigmentation disorder in which the protection against UV radiation fails due to the lack of melanin. Aqueous solutions of tyrosinase were exposed to UV-A irradiation (350nm) in the presence of pterin, the parent compound of oxidized pterins, under different experimental conditions. The enzyme activity in the irradiated solutions was determined by spectrophotometry and HPLC. In this work, we present data that demonstrate unequivocally that the enzyme is photoinactivated by pterin. The mechanism of the photosensitized process involves an electron transfer from tyrosinase to the triplet excited state of pterin, formed after UV-A excitation of pterin. The biological implications of the results are discussed.

- Nasti TH, Timares L.
Inflammasome Activation of IL-1 Family Mediators in Response to Cutaneous Photodamage(†). *Photochem Photobiol.* 2012 May 26. doi: 10.1111/j.1751-1097.2012.01182.x. [Epub ahead of print]
Abstract : Although keratinocytes are relatively resistant to ultraviolet radiation (UVR) induced damage, repeated UVR exposure result in accumulated DNA mutations that can lead to epidermal malignancies. Keratinocytes play a central role in elaborating innate responses that lead to inflammation and influence the generation of adaptive immune responses in skin. Apart from the minor cellular constituents of the epidermis, specifically Langerhans cells and melanocytes, keratinocytes are the major source of cytokines. UVR exposure stimulates keratinocytes to secrete abundant pro-inflammatory IL-1-family proteins, IL-1 α , IL-1 β , IL-18, and IL-33. Normal skin contains only low levels of inactive precursor forms of IL-1 β and IL-18, which require caspase 1-mediated proteolysis for their maturation and secretion. However, caspase-1 activation is not constitutive, but depends on the UV-induced formation of an active inflammasome complex. IL-1 family cytokines can induce a secondary cascade of mediators and cytokines from keratinocytes and other cells resulting in wide range of innate processes including infiltration of inflammatory leukocytes, induction of immunosuppression, DNA repair or apoptosis. Thus, the ability of keratinocytes to produce a wide repertoire of proinflammatory cytokines can influence the immune response locally as well as systematically, and alter the host response to photodamaged cells. We will highlight differential roles played by each IL-1 family molecule generated by UV-damaged keratinocytes, and reveal their complementary influences in modulating acute inflammatory and immunological events that follow cutaneous UV exposure.

- Noonan FP, Zaidi MR, Wolnicka-Glubisz A, Anver MR, Bahn J, Wielgus A, Cadet J, Douki T, Mouret S, Tucker MA, Popratiloff A, Merlino G, De Fabo EC.
Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. *Nat Commun.* 2012 Jun 6;3:884.
Abstract: Malignant melanoma of the skin (CMM) is associated with ultraviolet radiation exposure, but the mechanisms and even the wavelengths responsible are unclear. Here we use a mammalian model to investigate melanoma formed in response to precise spectrally defined ultraviolet wavelengths and biologically relevant doses. We show that melanoma induction by ultraviolet A (320-400 nm) requires the presence of melanin pigment and is associated with oxidative DNA damage within melanocytes. In contrast, ultraviolet B radiation (280-320 nm) initiates melanoma in a pigment-independent manner associated with direct ultraviolet B DNA damage. Thus, we identified two ultraviolet wavelength-dependent pathways for the induction of CMM and describe an unexpected and significant role for melanin within the melanocyte in melanomagenesis.

- Schuch AP, Lago JC, Yagura T, Menck CF.
DNA dosimetry assessment for sunscreen genotoxic photoprotection. *PLoS One.* 2012;7(6):e40344. Source Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.
Abstract:
BACKGROUND:
Due to the increase of solar ultraviolet radiation (UV) incidence over the last few decades, the use of sunscreen has been widely adopted for skin protection. However, considering the high efficiency of sunlight-induced DNA lesions, it is critical to improve upon the current approaches that are used to evaluate protection factors. An alternative approach to evaluate the photoprotection provided by sunscreens against daily UV radiation-induced DNA damage is provided by the systematic use of a DNA dosimeter.
METHODOLOGY/PRINCIPAL FINDINGS:
The Sun Protection Factor for DNA (DNA-SPF) is calculated by using specific DNA repair enzymes, and it is defined as the capacity for inhibiting the generation of cyclobutane pyrimidine dimers (CPD) and oxidised DNA bases compared with unprotected control samples. Five different commercial brands of sunscreen were initially

evaluated, and further studies extended the analysis to include 17 other products representing various formulations and Sun Protection Factors (SPF). Overall, all of the commercial brands of SPF 30 sunscreens provided sufficient protection against simulated sunlight genotoxicity. In addition, this DNA biosensor was useful for rapidly screening the biological protection properties of the various sunscreen formulations.

CONCLUSIONS/SIGNIFICANCE:

The application of the DNA dosimeter is demonstrated as an alternative, complementary, and reliable method for the quantification of sunscreen photoprotection at the level of DNA damage.

- Yanagida J, Hammiller B, Al-Matouq J, Behrens M, Trempus CS, Repertinger SK, Hansen LA.
Accelerated elimination of ultraviolet-induced DNA damage through apoptosis in CDC25A deficient skin. Carcinogenesis. 2012 Jul 4
Abstract: Cell division cycle 25A (CDC25A) is a dual-specificity phosphatase that removes inhibitory phosphates from cyclin-dependent kinases, allowing cell cycle progression. Activation of cell cycle checkpoints following DNA damage results in degradation of CDC25A, leading to cell cycle arrest. Ultraviolet (UV) irradiation, which causes most skin cancer, results in both DNA damage and CDC25A degradation. We hypothesized that ablation of CDC25A in the skin would increase cell cycle arrest following UV irradiation, allowing for improved repair of DNA damage and decreased tumorigenesis. Cdc25a^{fl/fl}/Krt14-Cre recombinase mice, with decreased CDC25A in the epithelium of the skin, were generated and exposed to UV. UV-induced DNA damage, in the form of cyclopyrimidine dimers and 8-oxo-deoxyguanosine adducts, was eliminated earlier from CDC25A deficient epidermis. Surprisingly, loss of CDC25A did not alter epidermal proliferation or cell cycle after UV exposure. However, the UV-induced apoptotic response was prolonged in CDC25A deficient skin. Double-labeling of cleaved-caspase 3 and the DNA damage marker γH2A.X revealed many of the apoptotic cells in UV-exposed Cdc25a mutant skin had high levels of DNA damage. Induction of skin tumors by UV-irradiation of Cdc25a mutant and control mice on a skin tumor susceptible v-rasHa Tg.AC mouse background revealed UV-induced papillomas in Cdc25a mutants were significantly smaller than in controls in the first 6 weeks following UV exposure although there was no difference in tumor multiplicity or incidence. Thus, deletion of Cdc25a increased apoptosis and accelerated the elimination of DNA damage following UV, but did not substantially alter cell cycle regulation or tumorigenesis.

- Zaidi MR, De Fabo EC, Noonan FP, Merlino G.
Shedding light on melanocyte pathobiology in vivo. Cancer Res. 2012 Apr 1;72(7):1591-5.
Abstract : Cutaneous malignant melanoma is rapidly increasing in the developed world and continues to be a challenge in the clinic. Although extensive epidemiologic evidence points to solar UV as the major risk factor for melanoma, there is a significant gap in our knowledge about how this most ubiquitous environmental carcinogen interacts with the largest organ of the mammalian body (skin) at the microenvironmental and molecular level. We review some recent advances that have started to close this gap.

5. Neuromelanins

(Pr M. d'Ischia)

The role of neuromelanin in Parkinson's disease is still obscure despite continuing interest. Li et al. (2012) examined the effects of neuromelanin on a human neuroblastoma cell line (SK-N-SH) over-expressing α -synuclein in the presence and in the absence of the Fenton reagent as a source of free radicals. The results indicated that neuromelanin enhances the effects of the oxidant in suppressing cell viability and inducing apoptosis, and that cells overexpressing synuclein suffer from more profound damaging effects. It can be argued that neuromelanin enhances vulnerability of dopaminergic cells by acting as an amplifier of toxic factors.

Two related papers by Munoz et al., 2012. identify nicotinic receptors and two proteins, vesicular monoamine transporter 2 (VMAT-2) and DT diaphorase, as effective contributors to protection against the toxic effects of aminochrome, the oxidative cyclization product of dopamine, in RCSN-3 cells.

The detailed mechanisms of the protective action remain however to be elucidated.

- Li J, Yang J, Zhao P, Li S, Zhang R, Zhang X, Liu D, Zhang B.

Neuromelanin enhances the toxicity of α -synuclein in SK-N-SH cells. Journal of Neural Transmission. 119(6), 685-691, 2012.

The key pathol. feature of Parkinson's disease (PD) is selective degeneration of the neuromelanin (NM)-pigmented dopaminergic neurons in the substantia nigra (SN). NM, like other risk factors, such as oxidative stress (OS) and α -synuclein (α -syn), is involved in the pathogenesis of PD. But whether or not NM synergizes with α -syn or OS in the pathogenesis of PD remains unexplored. In the present study, we examd. the effects of NM on cellular viability, apoptosis and free radical prodn. in α -syn over-expressing human neuroblastoma cell line (SK-N-SH) in the presence or absence of the oxidizer Fenton's Reagent (FR). We showed that NM synergized with FR in suppressing cell viability, and in inducing apoptosis and hydroxyl radical prodn. in all SK-N-SH cell lines. α -Syn over-expressing cells exhibited more pronounced effect, esp. the A53T mutation. Our findings suggest that NM synergizes with both OS and α -syn in conferring dopaminergic vulnerability, adding to our understanding of the pathogenesis of PD

- Munoz P, Huenchuguala S, Paris I, Cuevas C, Villa M, Caviedes P, Segura-Aguilar J, Tizabi Y.

Protective Effects of Nicotine Against Aminochrome-Induced Toxicity in Substantia Nigra Derived Cells: Implications for Parkinson's Disease. Neurotoxicity Research 22(2), 177-180, 2012.

Parkinson's disease is a debilitating progressive neurodegenerative disorder that results from the loss of or damage to dopaminergic cells contg. neuromelanin in the substantia nigra (SN). The underlying neurodegenerative mechanism(s), however, remain elusive. Aminochrome, the precursor of neuromelanin is an endogenous substance capable of inducing selective neurotoxicity to dopaminergic neurons in SN. Nicotine, on the other hand, may offer protective effects against dopaminergic cell damage induced by various neurotoxins including MPTP and salsolinol. In this study, we sought to det. whether nicotine may also protect against aminochrome-induced toxicity in SN derived RCSN-3 cells. Exposure of RCSN-3 cells to a combination of aminochrome (50 μ M) and dicoumarol (50 μ M) for 48 h induced approx. 70 % cell death. Pretreatment with nicotine, dose-dependently blocked this toxicity. The effects of nicotine in turn were dose-dependently blocked by mecamylamine, a non-selective nicotinic receptor antagonist. These results suggest involvement of nicotinic receptors in protective effects of nicotine against aminochrome-induced toxicity and provide further evidence for possible therapeutic effects of nicotine or nicotinic agonists in Parkinson's disease.

- Munoz P, Paris I, Sanders LH, Greenamyre JT, Segura-Aguilar J.

Overexpression of VMAT-2 and DT-diaphorase protects substantia nigra-derived cells against aminochrome neurotoxicity. From Biochimica et Biophysica Acta, Molecular Basis of Disease 1822(7), 1125-1136, 2012.

We tested the hypothesis that both VMAT-2 and DT-diaphorase are an important cellular defense against aminochrome-dependent neurotoxicity during dopamine oxidn. A cell line with VMAT-2 and DT-diaphorase over-expressed was created. The transfection of RCSN-3 cells with a bicistronic plasmid coding for VMAT-2 fused with GFP-IRES-DT-diaphorase cDNA induced a significant increase in protein expression of VMAT-2 (7-fold; $P < 0.001$) and DT-diaphorase (9-fold; $P < 0.001$), accompanied by a 4- and 5.5-fold significant increase in transport and enzyme activity, resp. Studies with synaptic vesicles from rat substantia nigra revealed that VMAT-2 uptake of 3H-aminochrome 6.3 ± 0.4 nmol/min/mg was similar to dopamine uptake 6.2 ± 0.3 nmol/min/mg that which were dependent on ATP. Interestingly, aminochrome uptake was inhibited by 2 μ M lobeline but not reserpine (1 and 10 μ M). Incubation of cells overexpressing VMAT-2 and DT-diaphorase with 20 μ M aminochrome resulted in (i) a significant decrease in cell death (6-fold, $P < 0.001$); (ii) normal ultra structure detd. by transmission electron microscopy contrasting with a significant increase of autophagosome and a dramatic remodeling of the mitochondrial inner membrane in wild type cells; (iii) normal level of ATP (256 ± 11 μ M) contrasting with a significant decrease in wild type cells (121 ± 11 μ M, $P < 0.001$); and (iv) a significant

decrease in DNA laddering (21 ± 8 pixels, $P < 0.001$) cells in comparison with wild type cells treated with $20 \mu\text{M}$ aminochrome (269 ± 9). These results support our hypothesis that VMAT-2 and DT-diaphorase are an important defense system against aminochrome formed during dopamine oxidn.

6. Genetics, molecular and developmental biology

(Dr. L. Montoliu)

- Anistoroaei R, Krogh AK, Christensen K.
A frameshift mutation in the LYST gene is responsible for the Aleutian color and the associated Chédiak-Higashi syndrome in American mink. *Anim Genet.* 2012 Jul 5. doi: 10.1111/j.1365-2052.2012.02391.x.
- Chen L, Ren X, Liang F, Li S, Zhong H, Lin S.
Characterization of two novel small molecules targeting melanocyte development in zebrafish embryogenesis. *Pigment Cell Melanoma Res.* 2012 Jul;25(4):446-53.
- Choi W, Kolbe L, Hearing VJ.
Characterization of the bioactive motif of neuregulin-1, a fibroblast-derived paracrine factor that regulates the constitutive color and the function of melanocytes in human skin. *Pigment Cell Melanoma Res.* 2012 Jul;25(4):477-81.
- Guo H, Yang K, Deng F, Xing Y, Li Y, Lian X, Yang T.
Wnt3a inhibits proliferation but promotes melanogenesis of melan-a cells. *Int J Mol Med.* 2012 Sep;30(3):636-42
- Hugall AF, Stuart-Fox D.
Accelerated speciation in colour-polymorphic birds. *Nature.* 2012 May 9;485(7400):631-4.
- Inoue Y, Hasegawa S, Yamada T, Date Y, Mizutani H, Nakata S, Matsunaga K, Akamatsu H.
Bimodal effect of retinoic acid on melanocyte differentiation identified by time-dependent analysis. *Pigment Cell Melanoma Res.* 2012 May;25(3):299-311.
- Kenny EE, Timpson NJ, Sikora M, Yee MC, Moreno-Estrada A, Eng C, Huntsman S, Burchard EG, Stoneking M, Bustamante CD, Myles S.
Melanesian blond hair is caused by an amino acid change in TYRP1. *Science.* 2012 May 4;336(6081):554.
Abstract: Naturally blond hair is rare in humans and found almost exclusively in Europe and Oceania. Here, we identify an arginine-to-cysteine change at a highly conserved residue in tyrosinase-related protein 1 (TYRP1) as a major determinant of blond hair in Solomon Islanders. This missense mutation is predicted to affect catalytic activity of TYRP1 and causes blond hair through a recessive mode of inheritance. The mutation is at a frequency of 26% in the Solomon Islands, is absent outside of Oceania, represents a strong common genetic effect on a complex human phenotype, and highlights the importance of examining genetic associations worldwide.
- Kronforst MR, Barsh GS, Kopp A, Mallet J, Monteiro A, Mullen SP, Protas M, Rosenblum EB, Schneider CJ, Hoekstra HE.
Unraveling the thread of nature's tapestry: the genetics of diversity and convergence in animal pigmentation. *Pigment Cell Melanoma Res.* 2012 Jul;25(4):411-433.
Abstract: Animals display incredibly diverse color patterns yet little is known about the underlying genetic basis of these phenotypes. However, emerging results are reshaping our view of how the process of phenotypic evolution occurs. Here, we outline recent research from three particularly active areas of investigation: melanin pigmentation in *Drosophila*, wing patterning in butterflies, and pigment variation in lizards. For each system, we highlight (i) the function and evolution of color variation, (ii) various approaches that have been used to explore the genetic basis of pigment variation, and (iii) conclusions regarding the genetic basis of convergent evolution which have emerged from comparative analyses. Results from these studies indicate that natural variation in pigmentation is a particularly powerful tool to examine the molecular basis of evolution, especially with regard to convergent or parallel evolution. Comparison of these systems also reveals that the molecular basis of convergent evolution is heterogeneous, sometimes involving conserved mechanisms and sometimes not. In the near future, additional work in other emerging systems will substantially expand the scope of available comparisons.
- Mäthger LM, Hanlon RT.
Pigmentation or transparency? Camouflage tactics in deep-sea cephalopods. *Pigment Cell Melanoma Res.* 2012 May;25(3):295-6.
- Pshenichnaya I, Schouwey K, Armario M, Larue L, Knoepfler PS, Eisenman RN, Trumpp A, Delmas V, Beermann F.

Constitutive gray hair in mice induced by melanocyte-specific deletion of c-Myc. *Pigment Cell Melanoma Res.* 2012 May;25(3):312-25.

Abstract: c-Myc is involved in the control of diverse cellular processes and implicated in the maintenance of different tissues including the neural crest. Here, we report that c-Myc is particularly important for pigment cell development and homeostasis. Targeting c-Myc specifically in the melanocyte lineage using the floxed allele of c-Myc and Tyr::Cre transgenic mice results in a congenital gray hair phenotype. The gray coat color is associated with a reduced number of functional melanocytes in the hair bulb and melanocyte stem cells in the hair bulge. Importantly, the gray phenotype does not progress with time, suggesting that maintenance of the melanocyte through the hair cycle does not involve c-Myc function. In embryos, at E13.5, c-Myc-deficient melanocyte precursors are affected in proliferation in concordance with a reduction in numbers, showing that c-Myc is required for the proper melanocyte development. Interestingly, melanocytes from c-Myc-deficient mice display elevated levels of the c-Myc paralog N-Myc. Double deletion of c-Myc and N-Myc results in nearly complete loss of the residual pigmentation, indicating that N-Myc is capable of compensating for c-Myc loss of function in melanocytes.

- Ruiz Y, Phillips C, Gomez-Tato A, Alvarez-Dios J, Casares de Cal M, Cruz R, Maroñas O, Söchtig J, Fondevila M, Rodriguez-Cid MJ, Carracedo A, Lareu MV.

Further development of forensic eye color predictive tests. *Forensic Sci Int Genet.* 2012 Jun 17

- Rutschmann S, Crozat K, Li X, Du X, Hanselman JC, Shigeoka AA, Brandl K, Popkin DL, McKay DB, Xia Y, Moresco EM, Beutler B.

Hypopigmentation and maternal-zygotic embryonic lethality caused by a hypomorphic mbtps1 mutation in mice. *G3 (Bethesda).* 2012 Apr;2(4):499-504.

7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borrón)

- Bultema JJ, Ambrosio AL, Burek CL, Di Pietro SM.
BLOC-2, AP-3, and AP-1 Proteins Function in Concert with Rab38 and Rab32 Proteins to Mediate Protein Trafficking to Lysosome-related Organelles. *J Biol Chem.* 2012 Jun 1;287(23):19550-63.
Lysosome-related organelles (LROs) are synthesized in specialized cell types where they largely coexist with conventional lysosomes. Most of the known cellular transport machinery involved in biogenesis are ubiquitously expressed and shared between lysosomes and LROs. Examples of common components are the adaptor protein complex-3 (AP-3) and biogenesis of lysosome-related organelle complex (BLOC)-2. These protein complexes control sorting and transport of newly synthesized integral membrane proteins from early endosomes to both lysosomes and LROs such as the melanosome. However, it is unknown what factors cooperate with the ubiquitous transport machinery to mediate transport to LROs in specialized cells. Focusing on the melanosome, we show that the ubiquitous machinery interacts with cell type-specific Rab proteins, Rab38 and Rab32, to facilitate transport to the maturing organelle. BLOC-2, AP-3, and AP-1 coimmunoprecipitated with Rab38 and Rab32 from MNT-1 melanocytic cell extracts. BLOC-2, AP-3, AP-1, and clathrin partially colocalized with Rab38 and Rab32 by confocal immunofluorescence microscopy in MNT-1 cells. Rab38- and Rab32-deficient MNT-1 cells displayed abnormal trafficking and steady state levels of known cargoes of the BLOC-2, AP-3, and AP-1 pathways, the melanin-synthesizing enzymes tyrosinase and tyrosinase-related protein-1. These observations support the idea that Rab38 and Rab32 are the specific factors that direct the ubiquitous machinery to mediate transport from early endosomes to maturing LROs. Additionally, analysis of tyrosinase-related protein-2 and total melanin production indicates that Rab32 has unique functions that cannot be carried out by Rab38 in melanosome biogenesis.
- Chiaverini C, Sillard L, Flori E, Ito S, Briganti S, Wakamatsu K, Fontas E, Berard E, Cailliez M, Cochat P, Foulard M, Guest G, Niaudet P, Picardo M, Bernard FX, Antignac C, Ortonne JP, Ballotti R.
Cystinosis is a melanosomal protein that regulates melanin synthesis. *FASEB J.* 2012 May 30. [Epub ahead of print]
Cystinosis is a rare autosomal recessive disease characterized by cystine crystal accumulation leading to multiorgan dysfunctions and caused by mutation in CTNS. CTNS encodes cystinosisin, a cystine/H(+) symporter that exports cystine out of the lysosomes. Patients with cystinosis frequently exhibit blond hair and fair complexion, suggesting an alteration in melanogenesis. However, the pigmentation singularities of these patients have not been studied, and the role of cystinosisin in melanogenesis has remained unknown. In our study, a clinical evaluation of 27 patients with cystinosis showed that 44% had a cutaneous pigmentation dilution compared to their relatives. Analysis of the hair melanin content in these patients by HPLC demonstrated a 50% decrease in eumelanin (4360 vs. 9360 ng/mg), and a 2-fold increase in pheomelanin (53 vs. 20 ng/mg), the yellow/red pigments. Cystinosisin-deficient mice also showed a 4-fold increase in hair pheomelanin content. In vitro studies showed that cystinosisin was located at melanosomes. CTNS silencing led to a 75% reduction of melanin synthesis that was caused by a degradation of tyrosinase by lysosomal proteases. Our results objectify the pigmentation defect in patients with cystinosis. We also identify the role of CTNS in melanogenesis and add a new gene to the list of the genes involved in the control of skin and hair pigmentation.
- Garcia-Molina Mdel M, Muñoz-Muñoz JL, Garcia-Molina F, García-Ruiz PA, Garcia-Canovas F.
Action of tyrosinase on ortho-substituted phenols: possible influence on browning and melanogenesis. *J Agric Food Chem.* 2012 Jun 27;60(25):6447-53.
The action of tyrosinase on ortho-substituted monophenols (thymol, carvacrol, guaiacol, butylated hydroxyanisole, eugenol, and isoeugenol) was studied. These monophenols inhibit melanogenesis because they act as alternative substrates to l-tyrosine and l-Dopa in the monophenolase and diphenolase activities, respectively, despite the steric hindrance on the part of the substituent in ortho position with respect to the hydroxyl group. We kinetically characterize the action of tyrosinase on these substrates and assess its possible effect on browning and melanogenesis. In general, these compounds are poor substrates of the enzyme, with high Michaelis constant values, $K(m)$, and low catalytic constant values, $k(cat)$, so that the catalytic efficiency $k(cat)/K(m)$ is low: thymol, $161 \pm 4 \text{ M}^{-1} \text{ s}^{-1}$; carvacrol, $95 \pm 7 \text{ M}^{-1} \text{ s}^{-1}$; guaiacol, $1160 \pm 101 \text{ M}^{-1} \text{ s}^{-1}$.
- Ginsbach JW, Kieber-Emmons MT, Nomoto R, Noguchi A, Ohnishi Y, Solomon EI.
Structure/function correlations among coupled binuclear copper proteins through spectroscopic and reactivity studies of NspF. *Proc Natl Acad Sci U S A.* 2012 Jul 3;109(27):10793-7.
The terminal step of 4-hydroxy-3-nitrosobenzamide biosynthesis in *Streptomyces murayamaensis* is performed by NspF, a mono-oxygenase that converts o-aminophenols to the corresponding nitroso product (hydroxyaniline activity). Previous biochemical characterization of the resting form of NspF suggested that this enzyme belonged to the coupled binuclear copper enzyme (CBC) family. Another member of this enzyme family,

tyrosinase, is able to mono-oxygenate monophenols (monophenolase activity) but not o-aminophenols. To gain insight into the unique reactivity of NspF, we have generated and characterized the oxy form of its active site. The observation of spectral features identical to those of oxy-tyrosinase indicates that oxy-NspF contains a Cu(2)O(2) core where peroxide is coordinated in a μ - η (2) η (2) mode, confirming that NspF is a CBC enzyme. This oxy form is found to react with monophenols, indicating that, like tyrosinase, NspF also possesses monophenolase activity. A comparison of the two electrophilic mechanisms for the monophenolase and hydroxyanilase activity indicates a large geometric change between their respective transition states. The potential for specific interactions between the protein pocket and the substrate in each transition state is discussed within the context of the differential reactivity of this family of enzymes with equivalent μ - η (2) η (2) peroxy bridged coupled binuclear copper active sites.

- Goldfeder M, Egozy M, Shuster Ben-Yosef V, Adir N, Fishman A.
Changes in tyrosinase specificity by ionic liquids and sodium dodecyl sulfate. Appl Microbiol Biotechnol. 2012 Apr 28. [Epub ahead of print]
Tyrosinase is a member of the type 3 copper enzyme family involved in the production of melanin in a wide range of organisms. The ability of tyrosinases to convert monophenols into diphenols has stimulated studies regarding the production of substituted catechols, important intermediates for the synthesis of pharmaceuticals, agrochemicals, polymerization inhibitors, and antioxidants. Despite its enormous potential, the use of tyrosinases for catechol synthesis has been limited due to the low monophenolase/diphenolase activity ratio. In the presence of two water miscible ionic liquids, [BMIM][BF₄] and ethylammonium nitrate, the selectivity of a tyrosinase from *Bacillus megaterium* (TyrBm) was altered, and the ratio of monophenolase/diphenolase activity increased by up to 5-fold. Furthermore, the addition of sodium dodecyl sulphate (SDS) at levels of 2-50 mM increased the activity of TyrBm by 2-fold towards the natural substrates L: -tyrosine and L: -Dopa and 15- to 20-fold towards the non-native phenol and catechol. The R209H tyrosinase variant we previously identified as having a preferential ratio of monophenolase/diphenolase activity was shown to have a 45-fold increase in activity towards phenol in the presence of SDS. We propose that the effect of SDS on the ability of tyrosinase to convert non-natural substrates is due to the interaction of surfactant molecules with residues located at the entrance to the active site, as visualized by the newly determined crystal structure of TyrBm in the presence of SDS. The effect of SDS on R209 may enable less polar substrates such as phenol and catechol, to penetrate more efficiently into the enzyme catalytic pocket.
- Guo H, Yang K, Deng F, Ye J, Xing Y, Li Y, Lian X, Yang T.
Wnt3a promotes melanin synthesis of mouse hair follicle melanocytes. Biochem Biophys Res Commun. 2012 Apr 20;420(4):799-804.
Although the importance of Wnt3a in melanocyte development has been well recognized, the effect of Wnt3a in normal HF melanocytes has not been clearly elucidated yet. Thus, we sought to examine the presence and location of Wnt3a in HF during hair cycle. By using melanocyte-targeted Dct-LacZ transgenic mice, we found that Wnt3a signaling is activated in mouse HF melanocytes during anagen of hair cycle. To further explore the potential functions of Wnt3a in mouse melanocytes, we infected melan-a cells with AdWnt3a to serve as the production source of Wnt3a protein. We demonstrated that Wnt3a promoted melanogenesis through upregulation of MITF and its downstream genes, tyrosinase and TRP1, in melanocytes. In vivo, AdWnt3a rescued the effects of AdsimMITF on HF melanocytes and promoted melanin synthesis. Our results suggest that Wnt3a plays an important role in mouse HF melanocytes homeostasis.
- Guo H, Yang K, Deng F, Xing Y, Li Y, Lian X, Yang T.
Wnt3a inhibits proliferation but promotes melanogenesis of melan-a cells. Int J Mol Med. 2012 Sep;30(3):636-42
Melanocytes are pigment-producing cells responsible for coloration of skin and hair. Although the importance of Wnt3a in melanocyte development has been well recognized, the role of Wnt3a in mature melanocytes has not been elucidated. This study was conducted to further explore the effects of Wnt3a on melanocyte proliferation and melanogenesis, and to elucidate the possible mechanisms involved. We infected melan-a cells with AdWnt3a to serve as the production source of the Wnt3a protein. MTT assay, 5-bromodeoxyuridine incorporation assay and flow cytometric analysis showed that Wnt3a inhibited the proliferation of melan-a cells and this was associated with decrease of cells in the S phase and increase of cells in the G1 phase. Melanin content and tyrosinase activity assay revealed that Wnt3a significantly promoted melanogenesis of melan-a cells. Furthermore, western blot analysis showed that Wnt3a upregulated the expression of microphthalmia-associated transcription factor and its downstream target genes, tyrosinase and tyrosinase-related protein 1 in melan-a cells. Collectively, our results suggest that Wnt3a plays an important role in melanocyte homeostasis.
- Kenny EE, Timpson NJ, Sikora M, Yee MC, Moreno-Estrada A, Eng C, Huntsman S, Burchard EG, Stoneking M, Bustamante CD, Myles S.
Melanesian blond hair is caused by an amino acid change in TYRP1. Science. 2012 May 4;336(6081):554.

Naturally blond hair is rare in humans and found almost exclusively in Europe and Oceania. Here, we identify an arginine-to-cysteine change at a highly conserved residue in tyrosinase-related protein 1 (TYRP1) as a major determinant of blond hair in Solomon Islanders. This missense mutation is predicted to affect catalytic activity of TYRP1 and causes blond hair through a recessive mode of inheritance. The mutation is at a frequency of 26% in the Solomon Islands, is absent outside of Oceania, represents a strong common genetic effect on a complex human phenotype, and highlights the importance of examining genetic associations worldwide.

- Kim JY, Shin JY, Kim MR, Hann SK, Oh SH.
siRNA-mediated knock-down of COX-2 in melanocytes suppresses melanogenesis. *Exp Dermatol.* 2012 Jun;21(6):420-5. doi: 10.1111/j.1600-0625.2012.01483.x.
Cyclooxygenase-2 (COX-2) is an enzyme induced in response to multiple mitogenic and inflammatory stimuli, including UV light. UV-induced COX-2 expression induces production of prostaglandin E2 (PGE2) in keratinocytes, which mediates inflammation and cell proliferation. Until recently, studies regarding COX-2 and PGE2 in the skin have focused on keratinocytes and skin cancer and the effect of PGs produced by keratinocytes on melanocytes. However, the effects of COX-2 itself or COX-2 inhibitors on melanogenesis are not well known. Therefore, to establish the role of COX-2 in melanogenesis, we investigated the effects of knock-down of COX-2 in melanocytes on melanin production and the expression of melanogenic molecules through silencing of COX-2 expression with COX-2 short interfering RNA (siRNA). COX-2 knock-down in melanocytes decreased the expressions of tyrosinase, TRP-1, TRP-2, gp100 and MITF and also reduced tyrosinase enzyme activity. Furthermore, COX-2 siRNA-transfected melanocytes showed markedly reduced alpha-melanocyte stimulating hormone (α -MSH)-induced melanin production. In addition, α -MSH-induced COX-2 expression in both scrambled siRNA-transfected and COX-2 siRNA-transfected melanocytes was greater than α -MSH-untreated cells. Our results suggest that COX-2 might be a candidate target for the development of anti-melanogenic agents and α -MSH-induced pigmentation could be closely associated with COX-2 expression. COX-2 inhibitors might therefore be of particular use in whitening cosmetics for hyperpigmentation disorders such as melasma, postinflammatory hyperpigmentation and solar lentigo.
- Kim NH, Cheong KA, Lee TR, Lee AY.
PDZK1 Upregulation in Estrogen-Related Hyperpigmentation in Melasma. *J Invest Dermatol.* 2012 Jun 14. doi: 10.1038/jid.2012.175.
The pathogenesis of melasma is unknown, although the potential role of estrogen has been considered. Microarray and real-time PCR analyses revealed that upregulation of PDZ domain protein kidney 1 (PDZK1) is clinically correlated with melasma. Although there has been no report that PDZK1 is involved in pigmentation and/or melanogenesis, PDZK1 expression can be induced by estrogen. In this study, the role of PDZK1 upregulation in melasma was examined, particularly in connection with estrogen, using biopsied skin specimens from 15 patients and monocultures and cocultures of melanocytes and keratinocytes with or without overexpression or knockdown of PDZK1. Estrogen upregulated PDZK1. Overexpression of PDZK1 increased tyrosinase expression and melanosome transfer to keratinocytes, whereas PDZK1 knockdown reduced estrogen-induced tyrosinase expression, through regulation of expression of estrogen receptors (ERs) ER- α and ER- β . The PDZK1-induced tyrosinase expression and melanosome transfer was regulated by ion transporters such as sodium-hydrogen exchanger (NHE), cystic fibrosis transmembrane conductance regulator (CFTR), and SLC26A3, which showed a specific association with each ER subtype. In the melanosome transfer, PDZK1 also increased phosphorylation of ezrin/radixin/moesin (ERM) and ras-related C3 botulinum toxin substrate 1, but not the expression of proteinase-activated receptor-2. Collectively, upregulation of PDZK1 could have an important role in the development of melasma in connection with estrogen through NHE, CFTR, and SLC26A3.
- Laura Dántola M, Gojanovich LA, Thomas AH.
Inactivation of tyrosinase photoinduced by pterin. *Biochem Biophys Res Commun.* 2012 Jul 7. [Epub ahead of print]
Tyrosinase catalyzes in mammals the first and rate-limiting step in the biosynthesis of the melanin, the main pigment of the skin. Pterins, heterocyclic compounds able to photoinduce oxidation of DNA and its components, accumulate in the skin of patients suffering from vitiligo, a chronic depigmentation disorder in which the protection against UV radiation fails due to the lack of melanin. Aqueous solutions of tyrosinase were exposed to UV-A irradiation (350 nm) in the presence of pterin, the parent compound of oxidized pterins, under different experimental conditions. The enzyme activity in the irradiated solutions was determined by spectrophotometry and HPLC. In this work, we present data that demonstrate unequivocally that the enzyme is photoinactivated by pterin. The mechanism of the photosensitized process involves an electron transfer from tyrosinase to the triplet excited state of pterin, formed after UV-A excitation of pterin. The biological implications of the results are discussed.
- Lee MS, Yoon HD, Kim JI, Choi JS, Byun DS, Kim HR.
Dioxinohydroeckol inhibits melanin synthesis through PI3K/Akt signalling pathway in α -melanocyte-stimulating hormone-treated B16F10 cells. *Exp Dermatol.* 2012 Jun;21(6):471-3.

Antimelanogenic activity has previously been reported in ethyl acetate fraction of *Ecklonia stolonifera*. In this study, using the isolated dioxinohydroeckol from the fraction, we sought to investigate an antimelanogenic signalling pathway in α -melanocyte-stimulating hormone (α -MSH)-stimulated B16F10 melanoma cells. Treatment with dioxinohydroeckol inhibited the cellular melanin contents and expression of melanogenesis-related proteins, including microphthalmia-associated transcription factor (MITF), tyrosinase and tyrosinase-related proteins TRP-1 and TRP-2. Moreover, dioxinohydroeckol stimulated phosphorylation of Akt in a dose-dependent manner without affecting phosphorylation of ERK. These data suggest that dioxinohydroeckol reduces melanin synthesis through the MITF regulation dependent upon PI3K/Akt signalling pathway.

- Li S, Wang C, Yu W, Zhao S, Gong Y.

Identification of genes related to white and black plumage formation by RNA-Seq from white and black feather bulbs in ducks. PLoS One. 2012;7(5):e36592. Epub 2012 May 15.

To elucidate the genes involved in the formation of white and black plumage in ducks, RNA from white and black feather bulbs of an F(2) population were analyzed using RNA-Seq. A total of 2,642 expressed sequence tags showed significant differential expression between white and black feather bulbs. Among these tags, 186 matched 133 annotated genes that grouped into 94 pathways. A number of genes controlling melanogenesis showed differential expression between the two types of feather bulbs. This differential expression was confirmed by qPCR analysis and demonstrated that Tyr (Tyrosinase) and Tyrp1 (Tyrosinase-related protein-1) were expressed not in W-W (white feather bulb from white dorsal plumage) and W-WB (white feather bulb from white-black dorsal plumage) but in B-B (black feather bulb from black dorsal plumage) and B-WB (black feather bulb from white-black dorsal plumage) feather bulbs. Tyrp2 (Tyrosinase-related protein-2) gene did not show expression in the four types of feather bulbs but expressed in retina. C-kit (The tyrosine kinase receptor) expressed in all of the samples but the relative mRNA expression in B-B or B-WB was approximately 10 fold higher than that in W-W or W-WB. Additionally, only one of the two *Mitf* isoforms was associated with plumage color determination. Downregulation of c-Kit and *Mitf* in feather bulbs may be the cause of white plumage in the duck.

- Li Z, Ptak D, Zhang L, Walls EK, Zhong W, Leung YF.

Phenylthiourea specifically reduces zebrafish eye size. PLoS One. 2012;7(6):e40132.

Phenylthiourea (PTU) is commonly used for inhibiting melanization of zebrafish embryos. In this study, the standard treatment with 0.2 mM PTU was demonstrated to specifically reduce eye size in larval fish starting at three days post-fertilization. This effect is likely the result of a reduction in retinal and lens size of PTU-treated eyes and is not related to melanization inhibition. This is because the eye size of *tyr*, a genetic mutant of tyrosinase whose activity is inhibited in PTU treatment, was not reduced. As PTU contains a thiocarbamide group which is presented in many goitrogens, suppressing thyroid hormone production is a possible mechanism by which PTU treatment may reduce eye size. Despite the fact that thyroxine level was found to be reduced in PTU-treated larvae, thyroid hormone supplements did not rescue the eye size reduction. Instead, treating embryos with six goitrogens, including inhibitors of thyroid peroxidase (TPO) and sodium-iodide symporter (NIS), suggested an alternative possibility. Specifically, three TPO inhibitors, including those that do not possess thiocarbamide, specifically reduced eye size; whereas none of the NIS inhibitors could elicit this effect. These observations indicate that TPO inhibition rather than a general suppression of thyroid hormone synthesis is likely the underlying cause of PTU-induced eye size reduction. Furthermore, the tissue-specific effect of PTU treatment might be mediated by an eye-specific TPO expression. Compared with treatment with other tyrosinase inhibitors or bleaching to remove melanization, PTU treatment remains the most effective approach. Thus, one should use caution when interpreting results that are obtained from PTU-treated embryos.

- Liao S, Shang J, Tian X, Fan X, Shi X, Pei S, Wang Q, Yu B.

Up-regulation of melanin synthesis by the antidepressant fluoxetine. Exp Dermatol. 2012 Aug;21(8):635-7.

Fluoxetine, a member of the class of selective serotonin reuptake inhibitors, is a potent antidepressant commonly used in clinical practice. Here, we report that fluoxetine increases cellular tyrosinase (TYR) activity, enhances the protein levels of microphthalmia-associated transcription factor (MITF), TYR and tyrosinase-related protein-1 (TRP-1) and eventually leads to a dramatic increase in melanin production in both murine B16F10 melanoma cells and normal human melanocytes (NHMCs). In well-characterized C57BL/6 mouse models, systemic application of fluoxetine increased hair pigmentation by up-regulating hair follicular MITF, TYR, TRP-1 and tyrosinase-related protein-2 (TRP-2) protein levels. Using a serotonin 1A receptor (SR1A) antagonist and RNA interference (RNAi) technique, we revealed that SR1A appears to be one of the involved pathways in the fluoxetine-induced melanogenesis in B16F10 cells. These results suggest that fluoxetine may hold a significant therapeutic potential for treating skin hypopigmentation disorders, and SR1A may serve as a novel target in modulating melanogenesis.

- Lin YS, Chuang MT, Chen CH, Chien MY, Hou WC.

Nicotinic acid hydroxamate downregulated the melanin synthesis and tyrosinase activity through activating the MEK/ERK and AKT/GSK3 β signaling pathways. J Agric Food Chem. 2012 May 16;60(19):4859-64.

In this study, nicotinic acid hydroxamate (NAH), a nicotinic acid derivative, was found to show dose-dependent inhibition of melanin content and tyrosinase activity of murine melanoma B16F10 cells with or without being cotreated with cAMP stimulators. In the studies on signaling pathways for skin whitening, NAH-treated B16F10 cells resulted in a decrease in the expression of tyrosinase, tyrosinase-related protein-1, and microphthalmia-associated transcription factor (MITF). PD98059 and LY294002 additions were obviously to increase melanin contents in B16F10 cells; however, they were reversed by NAH cotreatments. NAH-mediated increases in the phosphorylation of mitogen-activated protein kinase kinase (MEK)/ERK and AKT/glycogen synthase kinase-3 β (GSK3 β) were also found, which in turn led to the inhibition of MITF expression and then downregulated tyrosinase and tyrosinase-related protein (TRP)-1 expressions. These results suggest that NAH may be an active component in the inhibition of melanogenesis, which will have potential uses as cosmetics for whitening and need further investigation.

- Liu GS, Peshavariya H, Higuchi M, Brewer AC, Chang CW, Chan EC, Dusting GJ.
Microphthalmia-associated transcription factor modulates expression of NADPH oxidase type 4: a negative regulator of melanogenesis. Free Radic Biol Med. 2012 May 1;52(9):1835-43.
How signaling via reactive oxygen species (ROS) influences skin pigmentation is unclear. We have investigated how NADPH oxidase-derived ROS modulates the expression of the key pigment "melanin" synthesizing enzymes in B16 mouse melanoma cells. A melanin inducer α -melanocyte-stimulating hormone (α -MSH) caused ROS generation that was inhibited by the NADPH oxidase inhibitor Diphenyleneiodonium (DPI) and was insensitive to antagonists of other ROS-producing enzyme systems including mitochondrial enzymes, cyclooxygenase, and xanthine oxidase. NADPH oxidase 4 (Nox4) was found to be the most abundant isoform expressed in B16 cells, and its gene levels, as well as ROS generation, were enhanced by α -MSH. Interestingly, silencing Nox4 gene expression with Nox4 siRNA augmented melanin formation under basal conditions and after α -MSH stimulation, demonstrating that constitutive or stimulated Nox4-dependent ROS inhibits melanin formation. This process may be mediated by targeting the promoter region of a melanin synthesizing enzyme tyrosinase, because Nox4 siRNA enhanced tyrosinase promoter activity. Moreover, inhibition of tyrosinase mRNA expression in Nox4 siRNA-treated cells by blocking de novo mRNA and protein synthesis with actinomycin D and cycloheximide respectively indicates that Nox4 repression induces melanogenesis by increasing tyrosinase gene expression. We also found that α -MSH activated its downstream signal transducer microphthalmia-associated transcription factor (MITF) to stimulate Nox4 gene expression. We thus identified a novel mechanism by MITF signaling that in turn stimulates Nox4 to drive ROS generation, thereby repressing melanin synthesis. Such sequence of actions appears to act as an internal feedback mechanism to fine-tune melanin synthesis in response to exogenous challenges such as UV radiation.
- Martínez A, Membrillo I, Ugalde-Saldívar VM, Gasque L.
Dinuclear Copper Complexes with Imidazole Derivative Ligands: A Theoretical Study Related to Catechol Oxidase Activity. J Phys Chem B. 2012 Jul 6. [Epub ahead of print]
Catechol oxidase is a very important and interesting metalloprotein. In spite of the efforts to understand the reaction mechanism of this protein, there are important questions that remain unanswered concerning the catalytic mechanism of this enzyme. In this article, dinuclear copper compounds are used as biomimetic models of catechol oxidase to study plausible reaction paths. These dinuclear copper(II) complexes have distant metal centers (of 7.5 Å approximately) and superior catalytic activity to that of many dicopper complexes with shorter Cu-Cu distances. One mononuclear copper(II) complex is also analyzed in this investigation in order to see the influence of the two metal centers in the catalytic activity. Density functional theory calculations were performed to obtain optimized structures, vertical ionization energies, vertical electron affinities, the electrodonating power ($\omega(-)$), the electroaccepting power ($\omega(+)$) and the energy difference of several reaction paths. The K(M) experimental results that were previously reported compare well with the electroaccepting power ($\omega(+)$) of the copper compounds that are included in this article, indicating that this index is useful for the interpretation of the electron transfer capacity and therefore the catalytic activity. The catechol moiety coordinates to only one Cu ion, but two metal atoms are needed in order to have a good electron acceptor capacity of the biomimetic models.
- Muñoz-Muñoz JL, Berna J, García-Molina MD, García-Molina F, García-Ruiz PA, Varon R, Rodríguez-Lopez JN, García-Canovas F.
Hydroxylation of p-substituted phenols by tyrosinase: Further insight into the mechanism of tyrosinase activity. Biochem Biophys Res Commun. 2012 Jun 22. [Epub ahead of print]
A study of the monophenolase activity of tyrosinase by measuring the steady state rate with a group of p-substituted monophenols provides the following kinetic information: $k(\text{cat})(m)$ and the Michaelis constant, $K(M)(m)$. Analysis of these data taking into account chemical shifts of the carbon atom supporting the hydroxyl group (δ) and $\sigma(p)(+)$, enables a mechanism to be proposed for the transformation of monophenols into o-diphenols, in which the first step is a nucleophilic attack on the copper atom on the form E(ox) (attack of the

oxygen of the hydroxyl group of C-1 on the copper atom) followed by an electrophilic attack (attack of the hydroperoxide group on the ortho position with respect to the hydroxyl group of the benzene ring, electrophilic aromatic substitution with a reaction constant ρ of -1.75). These steps show the same dependency on the electronic effect of the substituent groups in C-4. Furthermore, a study of a solvent deuterium isotope effect on the oxidation of monophenols by tyrosinase points to an appreciable isotopic effect. In a proton inventory study with a series of p-substituted phenols, the representation of $\log(k/k_0)$ / $\log(n)$ against n (atom fractions of deuterium), where k is the catalytic constant for a molar fraction of deuterium (n) and k_0 is the corresponding kinetic parameter in a water solution, was linear for all substrates. These results indicate that only one of the proton transfer processes from the hydroxyl groups involved the catalytic cycle is responsible for the isotope effects. We suggest that this step is the proton transfer from the hydroxyl group of C-1 to the peroxide of the oxytyrosinase form (E(ox)). After the nucleophilic attack, the incorporation of the oxygen in the benzene ring occurs by means of an electrophilic aromatic substitution mechanism in which there is no isotopic effect.

- Panich U, Onkoksoong T, Limsaengurai S, Akarasereenont P, Wongkajornsilp A.
UVA-induced melanogenesis and modulation of glutathione redox system in different melanoma cell lines: the protective effect of gallic acid. J Photochem Photobiol B. 2012 Mar 1;108:16-22. doi: 10.1016/j.jphotobiol.2011.12.004.
 Oxidative stress has been suggested to play a role in ultraviolet A (UVA)-mediated melanogenesis. Glutathione (GSH) and GSH-related enzymes including γ -glutamyl cysteine ligase (γ -GCL) and glutathione S-transferase (GST) are important antioxidant defenses responsible for maintaining cellular redox balance. Hence, improving GSH redox system to cope with oxidative insults may be essential for attenuation of abnormal melanin production. Gallic acid (GA), a dietary phenolic, has been shown to provide beneficial effects against hyperpigmentation possibly through its antioxidant properties. This study thus aimed to assess the antimelanogenic action of GA with regard to modulation of GSH-GCL system and GST in two melanoma cell lines, lightly pigmented G361 human melanoma and more pigmented B16F10 mouse melanoma cells, irradiated with UVA. G361 cells were shown to have lower basal GSH content and GST activity than B16F10 cells. Moreover, GA provided antimelanogenic effects in correlation with promotion of GSH levels, GST activity as well as γ -GCL and GST mRNA in both G361 and B16F10 cells at 2-h post-irradiation. In summary, GA exhibits protective effects on UVA-mediated melanogenesis possibly through improvement of GSH-related antioxidant defenses. Furthermore, different redox state in G361 and B16F10 cells may affect the responses of melanoma cells to GA.

- Pucci M, Pasquariello N, Battista N, Di Tommaso M, Rapino C, Fezza F, Zuccolo M, Jourdain R, Finazzi Agrò A, Breton L, Maccarrone M.
Endocannabinoids stimulate human melanogenesis via type-1 cannabinoid receptor. J Biol Chem. 2012 May 4;287(19):15466-78.
 We show that a fully functional endocannabinoid system is present in primary human melanocytes (normal human epidermal melanocyte cells), including anandamide (AEA), 2-arachidonoylglycerol, the respective target receptors (CB(1), CB(2), and TRPV1), and their metabolic enzymes. We also show that at higher concentrations AEA induces normal human epidermal melanocyte apoptosis (~ 3-fold over controls at 5 μ M) through a TRPV1-mediated pathway that increases DNA fragmentation and p53 expression. However, at lower concentrations, AEA and other CB(1)-binding endocannabinoids dose-dependently stimulate melanin synthesis and enhance tyrosinase gene expression and activity (~ 3- and ~ 2-fold over controls at 1 μ M). This CB(1)-dependent activity was fully abolished by the selective CB(1) antagonist SR141716 or by RNA interference of the receptor. CB(1) signaling engaged p38 and p42/44 mitogen-activated protein kinases, which in turn activated the cyclic AMP response element-binding protein and the microphthalmia-associated transcription factor. Silencing of tyrosinase or microphthalmia-associated transcription factor further demonstrated the involvement of these proteins in AEA-induced melanogenesis. In addition, CB(1) activation did not engage the key regulator of skin pigmentation, cyclic AMP, showing a major difference compared with the regulation of melanogenesis by α -melanocyte-stimulating hormone through melanocortin 1 receptor.

- Rompel A, Büldt-Karentzopoulos K, Molitor C, Krebs B.
Purification and spectroscopic studies on catechol oxidase from lemon balm (*Melissa officinalis*). Phytochemistry. 2012 Jun 22. [Epub ahead of print]
 A catechol oxidase from lemon balm (*Melissa officinalis*) moCO which only catalyzes the oxidation of catechols to quinones without hydroxylating tyrosine was purified. The molecular mass of the *M. officinalis* enzyme of 39,370Da was obtained by MALDI mass spectrometry and the isoelectric point was determined to be 3.4. Addition of 2 eq. H₂O(2) to the enzyme leads to oxy catechol oxidase. In the UV/Vis spectrum two new absorption bands occur at 343nm ($\epsilon=8510\text{M}^{-1}\text{cm}^{-1}$) and 580nm ($\epsilon=580\text{M}^{-1}\text{cm}^{-1}$) due to O(2)(2-)Cu (II) charge transfer transitions in accordance with the oxy forms of other type 3 copper proteins. The N-terminal sequence has been determined by Edman degradation to NPVQAPELDKCGTAT, exhibiting a proline at the second and sixth position conserved in other polyphenol oxidases.

- Shen T, Wang MH.
Involvement of the p38 MAPK and ERK signaling pathway in the anti-melanogenic effect of methyl 3,5-dicaffeoyl quinate in B16F10 mouse melanoma cells. Chem Biol Interact. 2012 Jun 15. [Epub ahead of print]
 Methyl 3,5-dicaffeoyl quinate (MDQ), an active compound present in *Kalopanax pictus*, *Salicornia herbacea* L., *Aster oharai* and *Solidago virga-aurea* var. *gigantea*, is a dicaffeoylquinic acid derivative esterified by methanol. Recent studies have revealed that MDQ possesses multiple pharmacological activities, such as antitumor, antioxidative and cytoprotective activities. To date, there has been no attempt to test the action of MDQ in melanocytes. In this study, we investigated the effect of MDQ on melanogenesis in B16F10 mouse melanoma cells. MDQ inhibited melanin production and tyrosinase activity in B16F10 mouse melanoma cells without a direct inhibitory effect on mushroom tyrosinase activity. Furthermore, we also found that MDQ decreased protein expression levels of microphthalmia-associated transcription factor (MITF) and tyrosinase in B16F10 melanin cells. Meanwhile, phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) was significantly reduced after 6h MDQ treatment, and this expression recovered at 48h. The phosphorylation of extracellular signal-regulated kinase (ERK) was significantly enhanced at 12-48h, whereas no effect was observed in the phosphorylation of Akt. In addition, MDQ treatment did not significantly alter the expression levels of total p38 MAPK, ERK, and Akt. Thus, it seems that inhibition of phospho-p38 MAPK and activation of phospho-ERK may lead to the suppression of melanogenesis in MDQ-treated B16F10 mouse melanoma cells.

- Shimoda H, Shan SJ, Tanaka J, Maoka T.
 β -Cryptoxanthin suppresses UVB-induced melanogenesis in mouse: involvement of the inhibition of prostaglandin E(2) and melanocyte-stimulating hormone pathways. J Pharm Pharmacol. 2012 Aug;64(8):1165-76.
 Objective β -cryptoxanthin (β -CPX) is a carotenoid that is widely contained in the fruits of citrus plants. We evaluated the effect of β -CPX on UVB-induced pigmentation and mRNA expression related to melanogenesis in mouse skin. In addition, changes in melanogenic molecules were evaluated in cultured melanocytes stimulated with prostaglandin (PG) E(2), melanocyte-stimulating hormone (MSH) and endothelin (ET)-1. Methods Mice were irradiated with UVB and were given β -CPX (0.1, 1 and 10 mg/kg) orally for 14 days. Pigmentation was evaluated by skin colour change and microscopic observation. Total RNA was obtained from the skin and the expression of melanogenic mRNA was evaluated by RT-PCR. In cell culture studies, human melanocytes were cultured with β -CPX and melanogenic stimulants (PGE(2), MSH and ET-1) for 6-10 days. Melanin contents, dendricity, melanogenic mRNA and phosphorylation of cyclic AMP response element-binding protein (CREB) were evaluated. Key findings β -CPX (10 mg/kg) significantly suppressed skin pigmentation and mRNA expression of cyclooxygenase-2, ET-1 receptors, low-affinity neurotrophin receptor, PGE(2) receptor (EP1), melanocortin 1 receptor (MC1R), tyrosinase (Tyr), tyrosinase-related protein (Tyrrp) 1 and microphthalmia transcription factor. β -CPX (10 μ g/ml) suppressed melanogenesis induced by PGE(2), MSH and ET-1. In the PGE(2)-stimulated melanocytes, mRNA expressions of EP-1, Tyr and Tyrrp1 and phosphorylation of CREB protein were suppressed. In the ET-1-stimulated cells, only expression of CREB protein was suppressed. In the MSH-induced cells, mRNA expression of MC1R and Tyrrp1 and protein expression of CREB were suppressed. Conclusion Oral administration of β -CPX was found to suppress UVB-induced melanogenesis. Suppression of melanogenic enzymes, receptors of melanogenic stimulators, expression and phosphorylation of CREB are thought to be involved in the mechanism.

- Silavi R, Divsalar A, Saboury AA.
A short review on the structure-function relationship of artificial Catecholase/Tyrosinase and Nuclease activities of Cu-complexes. J Biomol Struct Dyn. 2012 Jun 26. [Epub ahead of print]
 The synthesis of metal complexes has vastly increased the scope of research for many scientists during the two last decades. Among these compounds, artificial tyrosinases, catecholases, proteases, and nucleases are some of the most studied due to their importance as modern tools in the fields of medicine, scientific research, and industry. Transition metals such as Zn(2+), Cu(2+), Fe(3+), Co(3+), Ni(2+), and lanthanide ions are the most commonly used. Among these ions, copper complexes have been the focus of the majority of studies thanks to their significant activity in comparison with other ions. Studies of copper-based tyrosinases, catecholases, and nucleases have revealed some of the overarching factors affecting reactions of all three types, which has led to improved activity and efficiency for all. Key factors include proper core-core distance, (Cu \cdots Cu distance 2.90-2.99 Å), suitable solvent, and ligands with proper hydrophobic structure and geometry. In the present investigation, we review and introduce the proposed mechanisms and the kinetically effective factors of natural catecholase, tyrosinase, and nuclease and their Cu-based synthetic mimics.

- Singh SK, Abbas WA, Tobin DJ.
Bone morphogenetic proteins differentially regulate pigmentation in human skin cells. J Cell Sci. 2012 May 28. [Epub ahead of print]
 Bone morphogenetic proteins (BMPs) represent a large family of multi-functional secreted signaling molecules. Previously BMP2/4 were shown to inhibit skin pigmentation by down-regulating tyrosinase expression and

activity in epidermal melanocytes (MC). However, a possible role for other BMP family members and their antagonists in melanogenesis has not yet been explored. In this study we show that BMP4 and BMP6, from two different BMP subclasses, and their antagonists noggin and sclerostin were variably expressed in MC and keratinocytes (KC) in human skin. We further examined their involvement in melanogenesis and melanin transfer using fully-matched primary cultures of adult human MC and keratinocyte (KC). BMP6 markedly stimulated melanogenesis by up-regulating tyrosinase expression and activity, and also stimulated the formation of filopodia and Myosin-X expression in MC, which was associated with increased melanosome transfer from MC to KC. BMP4, by contrast, inhibited melanin synthesis and transfer to below baseline levels. These findings were confirmed using siRNA knockdown of BMP receptors BMPRI1/2 or of Myosin-X, as well as by incubating cells with the antagonists noggin and sclerostin. While BMP6 was found to use the p38MAPK pathway to regulate melanogenesis in human MC independently of the Smad pathway, p38MAPK, PI-3K and Smad pathways were all involved in BMP6-mediated melanin transfer. This suggests that pigment formation may be regulated independently of pigment transfer. These data reveal a complex involvement of regulation of different members of the BMP family, their antagonists and inhibitory Smads, in MC behaviour.

- Sitaram A, Dennis MK, Chaudhuri R, De Jesus-Rojas W, Tenza D, Setty SR, Wood CS, Sviderskaya EV, Bennett DC, Raposo G, Bonifacino JS, Marks MS.

Differential recognition of a dileucine-based sorting signal by AP-1 and AP-3 reveals a requirement for both BLOC-1 and AP-3 in delivery of OCA2 to melanosomes. *Mol Biol Cell.* 2012 Jun 20. [Epub ahead of print]

Cell types that generate unique lysosome-related organelles (LROs), such as melanosomes in melanocytes, populate nascent LROs with cargoes that are diverted from endosomes. Cargo sorting toward melanosomes correlates with binding via cytoplasmically-exposed sorting signals to the heterotetrameric adaptors AP-1 or AP-3. Some cargoes bind both adaptors, but the relative contribution of each adaptor to cargo recognition and their functional interactions with other effectors during transport to melanosomes are not clear. Here, we exploit targeted mutagenesis of the acidic dileucine-based sorting signal in the pigment cell-specific protein OCA2 to dissect the relative roles of AP-1 and AP-3 in transport to melanosomes. We show that binding to AP-1 or AP-3 depends on the primary sequence of the signal and not its position within the cytoplasmic domain. Mutants that preferentially bound either AP-1 or AP-3 each trafficked toward melanosomes and functionally complemented OCA2 deficiency, but AP-3 binding was necessary for steady-state melanosome localization. Unlike tyrosinase, which also engages AP-3 for optimal melanosomal delivery, both AP-1- and AP-3-favoring OCA2 variants required BLOC-1 for melanosomal transport. These data provide evidence for distinct roles of AP-1 and AP-3 in OCA2 transport to melanosomes and indicate that BLOC-1 can cooperate with either adaptor during cargo sorting to LROs.

- Stanisz H, Stark A, Kilch T, Schwarz EC, Müller CS, Peinelt C, Hoth M, Niemeyer BA, Vogt T, Bogeski I. **ORAI1 Ca(2+) channels control endothelin-1-induced mitogenesis and melanogenesis in primary human melanocytes.** *J Invest Dermatol.* 2012 May;132(5):1443-51. doi: 10.1038/jid.2011.478.

UV radiation of the skin triggers keratinocytes to secrete endothelin-1 (ET-1) that binds to endothelin receptors on neighboring melanocytes. Melanocytes respond with a prolonged increase in intracellular Ca(2+) concentration ([Ca(2+)]_i), which is necessary for proliferation and melanogenesis. A major fraction of the Ca(2+) signal is caused by entry through Ca(2+)-permeable channels of unknown identity in the plasma membrane. ORAI Ca(2+) channels are molecular determinants of Ca(2+) release-activated Ca(2+) (CRAC) channels and are expressed in many tissues. Here, we show that ORAI1-3 and their activating partners stromal interaction molecules 1 and 2 (STIM1 and STIM2) are expressed in human melanocytes. Although ORAI1 is the predominant ORAI isoform, STIM2 mRNA expression exceeds STIM1. Inhibition of ORAI1 by 2-aminoethoxydiphenyl borate (2-APB) or downregulation of ORAI1 by small interfering RNA (siRNA) reduced Ca(2+) entry and CRAC current amplitudes in activated melanocytes. In addition, suppression of ORAI1 caused reduction in the ET-1-induced cellular viability, melanin synthesis, and tyrosinase activity. Our results imply a role for ORAI1 channels in skin pigmentation and their potential involvement in UV-induced stress responses of the human skin.

- Stratford MR, Ramsden CA, Riley PA.

The influence of hydroquinone on tyrosinase kinetics. *Bioorg Med Chem.* 2012 Jul 15;20(14):4364-70.

In vitro studies, using combined spectrophotometry and oximetry together with hplc/ms examination of the products of tyrosinase action demonstrate that hydroquinone is not a primary substrate for the enzyme but is vicariously oxidised by a redox exchange mechanism in the presence of either catechol, 1-3,4-dihydroxyphenylalanine or 4-ethylphenol. Secondary addition products formed in the presence of hydroquinone are shown to stimulate, rather than inhibit, the kinetics of substrate oxidation.

- Swope VB, Jameson JA, McFarland KL, Supp DM, Miller WE, McGraw DW, Patel MA, Nix MA, Millhauser GL, Babcock GF, Abdel-Malek ZA.

Defining MC1R Regulation in Human Melanocytes by Its Agonist α -Melanocortin and Antagonists Agouti Signaling Protein and β -Defensin 3. *J Invest Dermatol.* 2012 May 10. doi: 10.1038/jid.2012.135.

The melanocortin 1 receptor (MC1R), a G(s) protein-coupled receptor, has an important role in human pigmentation. We investigated the regulation of expression and activity of the MC1R in primary human melanocyte cultures. Human β -defensin 3 (HBD3) acted as an antagonist for MC1R, inhibiting the α -melanocortin (α -melanocyte-stimulating hormone (α -MSH))-induced increase in the activities of adenylate cyclase and tyrosinase, the rate-limiting enzyme for melanogenesis. α -Melanocortin and forskolin, which activate adenylate cyclase, and 12-O-tetradecanoylphorbol-13-acetate, which activates protein kinase C, increased, whereas exposure to UV radiation reduced, MC1R gene and membrane protein expression. Brief treatment with α -MSH resulted in MC1R desensitization, whereas continuous treatment up to 3 hours caused a steady rise in cAMP, suggesting receptor recycling. Pretreatment with agouti signaling protein or HBD3 prohibited responsiveness to α -MSH, but not forskolin, suggesting receptor desensitization by these antagonists. Melanocytes from different donors expressed different levels of the G protein-coupled receptor kinases (GRKs) 2, 3, 5, and 6, as well as β -arrestin 1. Therefore, in addition to the MC1R genotype, regulation of MC1R expression and activity is expected to affect human pigmentation and the responses to UV.

- Ubeid AA, Do S, Nye C, Hantash BM.

Potent low toxicity inhibition of human melanogenesis by novel indole-containing octapeptides. *Biochim Biophys Acta.* 2012 May 17;1820(10):1481-1489.

BACKGROUND: Abnormal production and accumulation of melanin are characteristics of a number of skin disorders, including postinflammatory hyperpigmentation and melasma. Our objective was to develop and validate novel oligopeptides with potent inhibitory activity against mushroom and human tyrosinase with minimal toxicity toward melanocytes, keratinocytes, and fibroblasts.

METHODS: A library of short sequence oligopeptides was docked against the crystal structure of mushroom tyrosinase to screen for favorable binding free energies and direct interaction with the catalytic pocket. The inhibitory activity of the octapeptides and hydroquinone (HQ) was assessed using mushroom and human tyrosinase and melanin content via human primary melanocytes. Effects on cell viability and proliferation were determined using the MTT assay and cytotoxicity via trypan blue exclusion.

RESULTS: Octapeptides P16-18 outperformed HQ, the benchmark of hypopigmenting agents, in all tested categories. Prolonged incubation of human keratinocytes, fibroblasts, or melanocytes with 30-3000 μ M HQ led to 8- to 65-fold greater cell death than with octapeptides. After 6d of incubation with 30 μ M HQ, we observed 70 \pm 3% and 60 \pm 2% cell death in melanocytes and fibroblasts, respectively, versus minimal toxicity up to an octapeptide concentration of 3mM.

CONCLUSION: Octapeptides P16-18 are potent competitive tyrosinase inhibitors with minimal toxicity toward the major cell types of human skin.

GENERAL SIGNIFICANCE: The findings in our study suggest that all three novel octapeptides may serve as safe and efficacious replacements of HQ for the treatment of pigmentary disorders.

- Yu M, Bell RH, Ho MM, Leung G, Haegert A, Carr N, Shapiro J, McElwee KJ.

Deficiency in nucleotide excision repair family gene activity, especially ERCC3, is associated with non-pigmented hair fiber growth. *PLoS One.* 2012;7(5):e34185.

We conducted a microarray study to discover gene expression patterns associated with a lack of melanogenesis in non-pigmented hair follicles (HF) by microarray. Pigmented and non-pigmented HFs were collected and micro-dissected into the hair bulb (HB) and the upper hair sheaths (HS) including the bulge region. In comparison to pigmented HS and HBs, nucleotide excision repair (NER) family genes ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, XPA, NTPBP, HCNP, DDB2 and POLH exhibited statistically significantly lower expression in non-pigmented HS and HBs. Quantitative PCR verified microarray data and identified ERCC3 as highly differentially expressed. Immunohistochemistry confirmed ERCC3 expression in HF melanocytes. A reduction in ERCC3 by siRNA interference in human melanocytes in vitro reduced their tyrosinase production ability. Our results suggest that loss of NER gene function is associated with a loss of melanin production capacity. This may be due to reduced gene transcription and/or reduced DNA repair in melanocytes which may eventually lead to cell death. These results provide novel information with regard to melanogenesis and its regulation.

- Zhang X, Yan G, Ji J, Wu J, Sun X, Shen J, Jiang H, Wang H.

PDE5 inhibitor promotes melanin synthesis through the PKG pathway in B16 melanoma cells. *J Cell Biochem.* 2012 Aug;113(8):2738-43. doi: 10.1002/jcb.24147.

PDE inhibitors could increase cellular cGMP levels and are used to treat erectile dysfunction as well as pulmonary arterial hypertension. cGMP production was reported to be necessary for UVB-induced melanin synthesis, however, the effect of PDE5 inhibitor on melanin synthesis has not been examined. We found that PDE5 inhibitor (sildenafil or vardenafil) and the cGMP analog 8-CPT-cGMP stimulated CREB phosphorylation, leading to increased tyrosinase expression and melanin synthesis, which was counteracted by KT5823, a selective cGMP-dependent protein kinase (PKG) inhibitor. However, KT5823 did not affect cAMP-elevating agent-mediated melanin synthesis, indicating that KT5823 selectively inhibited cGMP-induced melanin synthesis. This

is the first study to find that PDE5 inhibitor can promote melanin synthesis and reveal that PKG-dependent CREB phosphorylation and tyrosinase expression is involved in cGMP-induced melanin synthesis. Our results suggest that PDE5 inhibitor may be beneficial for the treatment of hypopigmentation diseases.

8. Melanosomes

(Pr J. Borovansky)

Reviews

Sitaram and Marks put together an elegant review describing current models of protein trafficking required for melanosome biogenesis in mammalian melanocytes. Pleasure of reading is amplified by colour illustrations.

Kauwar devoted his review to the treatment of melasma. It emphasizes an advantage of targeting melanosomes by using low-fluence q-switched neodymium-doped yttrium aluminium garnet lasers. The QS lasers induce high local temperature gradients between the melanosome and its surrounding structures, causing the melanosome to fracture. High-pressure acoustic waves from this interaction lead to melanocyte or phagocyte cell death.

Melanosomes

Properties of melanosomes

Fossil feathers, hair and eyes are regularly preserved as carbonized traces comprised of masses of micrometre-sized bodies that are spherical, oblate or elongate in shape. This demonstrates the unusual stability of melanin/melanosome structure. /See also *Carney et al. Nature Communications 3, article No.637,2012*/. It has been shown that certain trace elements occur in fossils as organometallic compounds that can be used as biomarkers for melanin. This time *Lindgren et al.* expanded the knowledge by comparing negative ion ToF SIMS spectra of synthetic melanin with that of microbodies isolated from an argentinoid fish eye from early Eocene of Denmark. The melanosome-like microbodies contained molecularly preserved melanin.

Melanosome biogenesis

Han et al used knockout mice to analyze the cutaneous functions of two type II receptors — Bmpr2 (a known receptor for bone morphogenetic protein) and Acvr2a (a known receptor for Bmps and activins). The lack of Bmpr2 and Acvr2a led to a failure of melanosomes to grow to their normal size, which in turn resulted in the production of small quantities of melanin followed by a hypopigmentation of hair shafts and a gray colour. The results showed that Bmpr2 and Acvr2a are necessary for a proper melanosome biogenesis.

Melanosome transfer

Using a coculture system of primary human melanocytes and keratinocytes *Tang et al* demonstrated that low concentration of hydrogen peroxide (<0.3mM) can increase melanin synthesis and melanosome transfer. *Kim et al* reported that an upregulation of PDZ domain protein kidney 1 (PDZK1) is clinically correlated with melasma. An overexpression of PDZK1 increased tyrosinase expression and melanosome transfer to keratinocytes. *Wu et al.* suggested that melanoregulin, a product of the *dilute suppressor gene*, is a negative regulator of melanosome transfer: *Dilute mice* that simultaneously lack melanoregulin, exhibit a nearly complete restoration of their coat colour. However, melanosomes remain concentrated in the center of melanocytes, from where their shedding can start.

Melanosomal proteins

Chiaverini et al extended the list of melanosomal proteins by cystinosin. It is coded by the CTNS gene; its silencing reduces melanogenesis due to a degradation of tyrosinase by lysosomal proteases.

Antibody drug conjugate (ADC) therapy targets internalizing cell surface proteins with an antibody covalently linked to a potent cytotoxic compound. Upon internalization and a subsequent digestion in the protease-enriched vesicles, the cytotoxic compound is released and kills the cell. *Chen et al* prepared antibody against PMEL17 and conjugated it with monomethylauristatin E. Since the PMEL17 protein is transiently presented at the cell surface before its entry to melanosomes, it proved to be an excellent and specific target. The ADC therapy was successful both in several melanoma lines in vitro and in SK-Mel-23 melanoma bearing mice in vivo.

In an effort to find natural polyphenols with the capacity to inhibit melanin synthesis, *Diwakar et al.* identified a novel combination consisting of *Larix sibirica* extract (containing taxifolin) and *Punica granatum* extract (containing punigalacins). The mixture reduced melanin biosynthesis in Melan A cells without a corresponding effect on cell viability; the expression of Mitf and tyrosinase genes and of melanosomal structural genes (Pmel 17, Mart 1) was inhibited.

Melanosomes and colour of birds.

Eliason and Shawkey showed that speculum (= wing patch) colour is produced by a photonic heterostructure consisting of both a single thin-film of keratin and a two-dimensional hexagonal lattice of melanosomes in feather barbules.

Melanosomes in non-pigment tissues.

Not only melanomas but also some other tumours can produce melanosomes, e.g. PECOMAs (perivascular epitheloid cell tumours). *Finzi et al* reported an HMB45 immunoreactivity of melanosomes and premelanosomes at the ultrastructural level in a pancreatic PECOMA. /see also *ESPCR Bull. No.61, 1813, 2008 and No.65, 1983, 2009*./

People engaged in melanosome research

All melanosome lovers are familiar with brilliant studies of Graca Raposo devoted to the biogenesis of melanosomes as exemplars of lysosome-related organelles. Now an interview by *Caitlin Sedwick* disclosed details of the life journey and achievements of G.Raposo in a friendly non-official human way.

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9. Melanoma experimental, cell culture

(Dr R. Morandini)

RAF inhibitors treatment resistance are not well understood. To understand this behaviour many studies have focused on cell-autonomous mechanisms of drug resistance. But is it only the right way?

In a recent paper Straussman et al. make a relation between resistance to RAF inhibitors and tumour-environment. He proposes that the tumour micro-environment confers innate resistance to therapy. The key protein seems to be HGF: proteomic analysis showed that stromal cell secretion of hepatocyte growth factor resulted in immediate resistance to RAF inhibition. More generally, this study indicates that the systematic dissection of interactions between tumours and their micro-environment can uncover important mechanisms underlying drug resistance.

Cell fusion and the subsequent aneuploidy, commonly observed in melanoma, are associated with poor prognosis but the pathological consequences of cell fusion in melanoma remain unknown. Mi et al. have developed an efficient cell fusion method: an improved phytohemagglutinin-polyethylene glycol fusion to obtain stable melanoma tumor-tumor cell fusion hybrids. This method offers a good opportunity to study influence of cell fusion on drug resistance and metastatic properties of melanoma cells.

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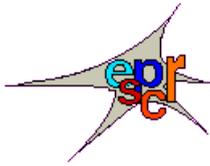
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ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2012 XVIIth Meeting of the ESPCR

September 11-14, Geneva, Switzerland

Contact: Web: www.espcr.org/ESPCR2012

2012 42nd Annual ESDR Meeting

September 19-22, Venice, Italy

Contact: Web: www.esdr2012.org/

2012 PASPCR Meeting

September 22-25, Salt Lake City, Utah

Contact: Web: <http://paspcr.med.umn.edu/>

2012 5th Asian Society for Pigment Cell Research

November 3-4, New Delhi, India

Contact: Web: www.aspcr2012.com

2012 24th Annual Meeting of the Japanese Society for Pigment Cell Research

November 24-25, Nagahama, Shiga-pref, JAPAN

Contact: Prof. Hiroaki Yamamoto: h_yamamoto@nagahama-i-bio.ac.jp

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

2013 XVIIIth Meeting of the ESPCR

Lisbon, Portugal

Contact: Miguel Seabra and Graça Raposo

2014 XXIInd IPCC Meeting

September 4-7, Singapore

Contact: Web: www.ipcc2014.org

2015 45th Annual ESDR Meeting

September 9-12, Rotterdam, The Netherlands