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CONTENT

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Pr A. Napolitano)

Several papers related to the properties of natural and synthetic melanins have appeared in this period. John Simon's group reported an innovative method for determination of the absorption coefficient of melanosomes from photoemission electron microscopy images. This was applied to irides of different colors which have been shown to contain both eumelanin and pheomelanin at various ratios by chemical degradation analysis (Peles and Simon *J. Phys Chem. B*.) as well as to newborn and adult melanosomes (Peles and Simon *Photochem Photobiol*) containing melanins at different ratio of DHICA and DHI based again on chemical analysis. The unexpected water solubility of DHI melanin prepared in buffer solutions in the presence of low percentage of polyvinyl alcohol allowed detection of chromophores in the early stages of polymer formation without confounding scattering effects (Pezzella et al *Photochem Photobiol*) Peculiar electrochromic properties were exhibited from a melanin like polymer obtained from 5,6-dimethoxyindole-2-carboxylic acid which exhibited high crystallinity degree which is not usually observed in melanins (Povlich et al *Macromolecules*).
A very interesting study (Jiang et al *Free Rad Biol Med*) allowed to assess the potential of DHICA pathway and the properties of the pigments thereof by use of Dct-/- knockout mice in comparison with wild-type C57BL/6 mice. Dct inactivation resulted in lower skin photoprotection and higher levels of reactive oxygen species on UVA irradiation indicating that the function attributed to eumelanins are mostly due to their DHICA content. This view was also confirmed by in vitro experiments in which the superior scavenging ability of synthetic DHICA melanin with respect to DHI melanin was demonstrated by esr measurements.
Among the newly discovered compounds /extracts with antimelanogenic activity are a group of lignans isolated from the Tuber-barks of Colocasia antiquorum (Kim et al *J Agr Food Chem*).

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2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

The increased melanin production in response to UV irradiation is mediated by several intracellular signaling pathways among which, a cAMP-dependent pathway seems crucial. Heat shock proteins (HSPs) are known to be induced in response to various stressors with the aim to confer cellular protection. Several HSPs are normally expressed in the skin and result up-modulated in response to stressors such as heat and UV. Particularly, HSP70 protects both keratinocytes and melanocytes against UV. **Hoshino *et al.*** investigated the effect of HSP70 expression on the production of melanin both *in vivo* and *in vitro*. The authors demonstrate that the induction of melanin synthesis mediated by the treatment with the cAMP-elevating agent IBMX resulted inhibited in the cells overexpressing HSP70. The authors analyzed also the expression of tyrosinase (activity, mRNA and protein levels) showing that overexpression of HSP70 suppress tyrosinase expression at the transcription level, whereas it did not affect the level of MITF, which plays a positive role in the UV-mediated induction of tyrosinase. Experiments of immunoprecipitation and doublestaining demonstrated that HSP70 can physically interact and co-localize with MITF in the nucleus. In addition, HSP70 inhibits the tyrosinase gene transcription in nuclear extracts suggesting that HSP70 binds to MITF in the nucleus and inhibits its binding to the promoter region of the tyrosinase gene. Finally, UV-irradiation on transgenic mice expressing HSP70 showed less melanin content compared to wilde-type animals. Since HSP70 have a protective effect from UV-mediated cellular damages, the authors suggest that non-toxic HSP70 inducers could be pharmaceutically and cosmetically beneficial as hypopigmenting agents, suppressing melanin synthesis and simultaneously protecting the skin against UV damages. To test the hypothesis that different mechanisms are involved in the pigmentary responses of the skin to different types of UV, **Choi W *and co-workers*** used immunohistochemistry and whole human genome microarray analysis of human skin in situ to characterize how melanocyte-specific proteins and melanogenic signaling pathways are regulated by repetitive exposure to different types of UV (UVA and/or UVB). SSR or UVB increased the expression of many known melanocyte-specific genes, such as TYR, DCT, and MART-1, whereas UVA did not induce such upregulation of pigment cell-specific genes, suggesting that UVA elicits the tanning of skin via a distinct mechanism. The authors demonstrated that the longer term tans elicited by repetitive UVA exposure are similar in mechanism to immediate pigment darkening and persistent pigment darkening and reflect changes in pre-existing melanin and/or melanogenic intermediates. **Choi T *et al*** investigated whether NAD(P)H:quinone oxidoreductase-1 (NQO1) affects the melanogenesis. The authors treated the cultured normal human melanocytes and zebrafish with NQO1 inhibitors, ES936 and dicoumarol, showing that melanogenesis was significantly decreased by the addition of NQO1 inhibitors in both NHMC and zebrafish models. By using oligonucleotide microarray and 2D MALDI-TOFMS methods, the researchers have identified NQO1 as a new pigmentation regulatory enzyme that increases tyrosinase activity, probably by suppressing its degradation. Several papers focused on the role of lipid oxidation by-products on melanogenesis. **Gledhill *and co-workers*** tried to clarify whether epidermal melanocytes (EM) -produced prostaglandin-E_2 (PGE_2) contributes to UVR-induced skin inflammation, and whether this is correlated with melanogenesis. The authors assessed whether EM can produce PGE2 under baseline (or arachidonic acid-stimulated) conditions and whether this production is altered after UVR-exposure. In a second phase of the study they evaluated which components of the PGE_2 synthetic pathway are expressed by normal human adult primary EM. Finally, the authors investigated whether there is a correlation between PGE_2 production by EM in response to UVB and their capacity for melanogenesis. Their results confirmed that EM have the capacity to produce PGE_2, and show that the production of this pro-inflammatory eicosanoid in normal human EM can be increased by UVB exposure., even if this response does not appear to occur in a melanogenesis-dependent manner. Moreover the researchers described the multiple enzymatic routes by which PGE_2 can be produced in EM, highlighting the importance of this eicosanoid to melanocyte function. The ability of melanocytes to release PGE2 after UV exposure has been investigated by another research team. **Starner *et al.*** examined the synthesis of PGE_2 in melanocytes in response to repetitive low doses of UVR, the effect of PGE_2 on tyrosinase activation and melanocyte proliferation, and the effect of PGE_2 on the: cyclic AM-protein kinase A (cAMP-PKA) pathway, and the receptors that mediate this response. The authors showed that PGE_2 is synthesized by melanocytes in response to UVR, and that PGE_2 stimulates cAMP release, tyrosinase activity and proliferation in human melanocytes. Moreover, PGE_2 was found to activate EP_3 and EP_4 , two G-protein coupled receptors, in melanocytes, which has opposing effects on cAMP production. PGE_2 stimulates EP_4 receptor signalling in melanocytes, resulting in cAMP production. Conversely, PGE_2 also stimulated the EP_3 receptor in melanocytes, resulting in lowered basal cAMP levels. These results suggest that the relative levels of expression and/or signalling of EP_3 and EP_4 receptors may control tyrosinase activation in melanocytes in response to PGE_2. **Masoodi *and co-workers*** checked whether the production of prostaglandins is related to that of melanin in pigment-producing cells and whether α-MSH is involved in this process. PGD_2 and PGE_2 were the major prostaglandins identified in human epidermal melanocytes and FM55 melanoma cells western blotting analysis revealed that both cell types expressed the constitutive isoform of cyclooxygenase (COX-1) but not the inducible isoform COX-2. α-MSH had no effect on melanin production in FM55 cells and stimulated PGD_2 production. The authors suggest that α-MSH acts independently of cAMP and specifically regulates PGD_2 synthesis at the level of the activity of lipocalin-type PGD synthase (L-PGDS). This α-MSH-mediated effect may be associated with its role as an immune modulator. Another study investigated the function of L-PGDS in melanin producing cells. **Takeda *et al.*** explored the hitherto unknown role of L-PGDS in the biological actions of retinoic acid (RA) and provided the evidence for the link between L-PGDS and the RA signaling. The authors showed that treatment with RA decreased the proliferation of L-PGDS-expressing cells by 20%, but not mock-transfected cell lines lacking L-PGDS expression. Moreover, RA increased the transient expression of a reporter

gene carrying the RA-responsive elements in L-PGDS-expressing cell, suggesting that L-PGDS may increase the sensitivity to RA. L-PGDS was found to be able to restrict the proliferation of melanocytes by modulating the RA signaling. Considering the evolutionary conserved RA signaling and the conserved transporter function of L-PGDS, the authors suggest that L-PGDS may fine-tune the action of RA, which contributes to the regulation of epidermal pigmentation.

Melanocytes differentiate from melanoblasts originating from the neural crest cells and migrate to the epidermis. The presence of melanocyte stem cells (MSCs) in the bulge area of the hair follicle has been shown. However, the detailed features of these cells remain not completely clear because of the difficulty to isolate and culture these cells. In order to better charachterize these stem cells, **Yamada *et al.*** evaluated specific cell-surface markers expressed on MSCs. Using hair follicle of mouse back skin, the Authors classified the hair follicles into four areas (hair bulb, hair bulb to bulge-lower bulge-, bulge, epidermis to bulge-upper bulge) and analyzed the gene expression profile of each area. The results demonstrated that the expression levels of the receptors for Wnt molecules Frizzled (Fzd)-4, Fzd-7, low density lipoprotein receptor-related protein-5 (Lrp5) and Lrp6 were higher in the bulge compared to other areas. Co-staining by FACS analysis of Fzd4, Fzd7 and Kit, a surface marker expressed on melanoblasts, demonstrated that cells positive for Fzd4 and Fzd7 were different from those positive for Kit. Moreover, Fzd4 and Fzd7 positive cells isolated by FACS analysis required a longer culture time for differentiation into mature melanocyte than Kit positive cells, suggesting that Fzd4, Fzd7 positive cells are more immature cells than melanoblasts and that both Fzd4, Fzd7 can be useful and specific markers expressed on MSCs.

The neural stem cell marker nestin is expressed in the hair follicle stem cells. Recently, it has been suggested that some cutaneous neoplasms comprised melanoma originate from the epidermal and hair follicle stem cells. Positive and increased reactivity for nestin was previously found in melanomas and it was significantly correlated with the more advanced lesions. By immunoperoxidase and immunofluorescence staining techniques, **Kanoh *et al.*** analyzed the expression of nestin in melanoma (27 samples: 15 nodular melanomas consisting of 5 amelanotic and 10 melanotic type and 12 superficial spreading melanomas) and correlated its immunoreactivity with the type of melanoma and the expression to other diagnostic markers used for HMB45 negative melanoma.

Based on the finding that positive reactivity for nestin was especially observed in melanoma cells in the dermal parts of HMB-45 negative amelanotic and melanotic nodular melanomas, the authors suggest the utility to use nestin as a marker for the diagnosis of HMB-45-negative melanoma lesions.

Medic and Ziman analyzed the expression of the transcription factor PAX3 in normal skin melanocytes, naevi and melanoma lesions. PAX3 is a key regulator of melanocyte development. In addition, it is frequently expressed in melanomas and naevi and has been considered as an important marker for melanoma staging. Although several studies reported no expression of PAX3 in melanocytes of normal skin, the authors conclusively show the presence of PAX3 in follicular and epidermal melanocytes, co-expressing with melanocyte markers MITF and Melan-A. Particularly, the study shows that PAX3 seems to correlate with melanocytes in an undifferentiated (characterized by the co-expression of PAX3, HES1 and MITF) and more differentiated (characterized by the co-expression of PAX3, MITF and Melan-A) status and decreases in mature terminally differentiated cells (Melan-A-positive and PAX3-negative). The study supports also the role of PAX3 as a cell survival regulator, showing positive staining for the antiapoptotic factor BCL2-like 1 in the majority of cells expressing PAX3 in both normal skin, naevi and melanoma samples, hypothesizing that normal melanocytes might utilize the same survival mechanism of melanoma cells. The cell adhesion molecule MCAM, which is associated to melanoma progression and metastasis, resulted to be co-expressed with PAX3 in melanomas, naevi but not in normal epidermal melanocytes, suggesting a possible role for PAX3 in regulating migration of melanoma cells. The possible connections between signaling pathways involved in the melanocyte differentiation and signal trasduction mechanisms activated by lipid metabolites have been explored. The relevance of Wnt/β-catenin signaling in the melanocyte differentiation and melanoma development is well known, whereas the effect of β-catenin in normal human epidermal melanocytes has not been completely identified. By using adenoviral gene delivery system **Kim *et al.*** investigated the effect of β-catenin in cultured human epidermal melanocytes. The authors demonstrated that β-catenin has a potential for reducing the melanocyte dendricity, via the regulation of intracellular signaling molecules such as Rho family GTPases, MAPKs, and PKC isoforms. These results suggest a role of β-catenin in support of the morphological changes of melanocytes, which may be related with cell's propensity toward the melanoma initiation and/or promotion. The CD44 molecules are glycosylated adhesion proteins with several biological functions including the involvement in tumor progression and metastasis. It has been shown that the formation of a complex between the variant CD44v6 and the receptor for hepatocyte growth factor (HGF) c-Met is necessary for the activation of the receptor. Based on the fact that HGF is endogenously expressed in melanoma cells and that it increases the expression of CD44v6 in mouse melanoma cells, **Damm *et al.*** analyzed the effect of HGF on the expression of CD44v6 in human melanocytes and human melanoma cells and evaluated whether CD44v6 expression correlates with the progression in melanocytic lesions; in addition, the authors analyzed the intracellular signals and transcription factors involved in the regulation of its expression. The authors found a weak basal expression of CD44v6 in normal human melanocytes, which was strongly up-regulated in response to HGF treatment. Melanoma cells expressed a higher basal expression, which was not affected by HGF, probably because of a constititive autocrine loop. Parallel analysis of melanocytic lesions by immunohistochemistry demonstrated a high membranous expression of CD44v6 in primary melanomas, cutaneous and lymph node metastasis. The evaluation of the possible intracellular pathways involved in the HGF-dependent up-modulation of the molecule in melanocytes demonstrated an NF-kB-mediated transcriptional regulation involving the transcription fators Egr-1, C/EBP-□. Finally, the authors demonstrate that a CD44 blocking antibody reduces the HGF-mediated phosphorylation of c-Met and dereases cell

motility, revealing that the motility of primary human melanocytes induced by HGF depends on c-Met-CD44v6 interaction. Taken together, these results suggest a key role of the adhesion molecule CD44v6 in the early phases of melanomagenesis.
Vitiligo is a chronic disorder characterized by skin, hair and mucous membranes depigmented patches due to progressive loss of melanocytes. **Zhao *et al.*** explored genomic and gene specific DNA methylation levels in peripheral blood mononuclear cells (PBMCs) of twenty patients with vitiligo and twenty age and sex matched healthy controls. DNA methylation is an epigenetic mechanism controlling several biological functions including gene transcription, genomic imprinting, chromatin remodelling. The DNA methylation status is regulated by specific DNA methyltranferases (DNMTs) and methyl-DNA binding domain proteins (MBDs). In their study the authors found that genomic DNA-methylation in vitiligo PBMCs was higher than that of controls together with a significant up-modulation of the DNMT1, MBD1, MBD3, MBD4 and MeCP2 mRNA expression. The authors demonstrate also a positive correlation between global DNA methylation and the expression of MBD1 and MBD3 in vitiligo, suggesting that an increase of these two methyl-DNA binding domain proteins may contribute to the development of the disease. The analysis of the expression and of the methylation status of specific genes revealed a lower mRNA level of IL-10 and a higher methylation level of its enhancer region in vitiligo PBMCs compared to that of healthy controls. IL-10 is known to be sensitive to modification in its methylation status and is known to be involved in autoimmunity. Taken together, these results suggest the involvement of DNA methylation in regulating the expression of genes related to autoimmune reactivity, such as IL-10, in PBMCs of vitiligo subjects, possibly contributing to the loss of melanocytes occurring in this disease. The evidence that vitiligo susceptibility is associated to genetic alterations has been confirmed by a multi-centric study coordinated by **Spritz.** Based on previous results of a gene-wide association (GWA) study of individuals with generalized vitiligo, their families and controls, identifying ten different loci that contribute to generalized vitiligo risk, the researchers tested additional loci that showed suggestive association in the GWA, using two replication cohorts of European descent. The authors observed replicated association of generalized vitiligo with variants of ten genes, in particular FOXP1, encoding proteins with roles in the immune system contribute to susceptibility to generalized vitiligo.

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3. MSH, MCH, other hormones, differentiation

(Pr M. Böhm)

Alpha-MSH, POMC and MC-Rs – what's new?

No evidence for autoantibodies against MC-1R in patients with vitiligo?
There is compelling evidence for immune dysregulation such as autoreactive T cells and/or autoantibodies in at least a subset of patients with vitiligo. It is therefore of particular interest as to whether patients with vitiligo have circulating antibodies against the MC-1R which is expressed by normal melanocytes and which acts as a key regulator of pigmentation and cytoprotection. In a recent paper (Agretti *et al.* Endocrinol. Invest. 2010, Epub ahead of print) the authors prepared immunoglobulins G (IgGs) from 41 vitiligo patients with associated or not with thyroid autoimmune diseases or other autoimmune pathologies. The IgGs were incubated with HBL20 melanoma cells in the presence of a submaximal dose of alpha-MSH. IgGs from 30 normal individuals served as negative controls. None of the examined IgGs from patients with vitiligo inhibited alpha-MSH-mediated cAMP production in HBL20 cells suggesting that autoantibodies against MC-1R are rare or absent in these individuals. Limitations of this interesting study are the small sample size and the utilized melanoma cell line in which alpha-MSH signaling may be deviated.

KdPT – an alpha-MSH-related anti-inflammatory tripeptide that does not bind MC-1R Although α-MSH is well known for its anti-inflammatory effects its clinical usefulness for inflammatory disorders is limited due to its lipid- and pigment-inducing effects via activation of melanocortin receptors (MC-Rs). In a recent paper from the research group of M. Böhm, Germany (Mastrofrancesco *et al.,* J. Immunol. 2010, Epub ahead of print), the anti-inflammatory potential and the molecular mechanism of action of KdPT, a tripeptide derivative of the C-terminal end of α-MSH, was examined in detail. Using SZ95 sebocytes as an in vitro model for acne, the most common inflammatory skin disorder, it could be demonstrated that KdPT dose-dependently suppressed IL-1β-induced IL-6 and IL-8 expression. The tripeptide decreased IL-1β-mediated IκBα degradation, reduced nuclear accumulation of p65 and attenuated DNA binding of NF-κB. Moreover, KdPT reduced IL-1β-mediated generation of intracellular reactive oxygen species, which contributed to IL-1β-mediated cytokine induction. KdPT also reduced cell surface binding of fluorochrome-labelled IL-1β in SZ95 sebocytes. Analysis of the crystal structure of the complex between IL-1β / IL-1 receptor type I (IL-1RI) followed by computer modelling of KdPT and subsequent modelling of the peptide receptor complex with the crystal structure of IL-1-RI via manual docking further predicted that the tripeptide through several H-bonds and one hydrophobic bond interacts with the IL-1-RI. Importantly, KdPT did not bind to MC-1R as demonstrated by blocking experiments with a peptide analogue of Agouti signaling protein and by binding assays using MC-1R-expressing B16 melanoma cells. Accordingly, KdPT failed to induce melanogenesis. These data reveal a promising anti-inflammatory potential of KdPT and point towards novel future directions in the treatment of acne but also of various other IL-1-mediated inflammatory diseases with this small molecule. Further work on melanocytes and melanoma cells is currently in progress by M. Böhm´s group in order to investigate modulatory effects of KdPT in pigment cells.

4. Photobiology

(Dr N. Smit)

Cho et al have previously reported on the role of Er-beta in Immunosuppression and because photoimmune suppression is an important risk factor for skin cancer role they studied Er-beta signaling in Skh:hr-1 mice by using an Er antagonist and Er knockout mice and how this affected growth of transplantable tumor cell lines. For B16/F10 melanoma cells growth was significantly faster in the knockout mice compared to wildtype and accelerated by SSUV. This may demonstrate the contribution of immunosuppression in the process of melanomagenesis. Part of the review by Garibayan and Fisher also deals with the role of UVR on immunosuppression and inflammation and how this contributes to initiation, progression and metastasis of melanoma.
Choi investigated the effects of UVA and UVB on gene expression patterns in human skin in situ after repetitive exposures. Whereas UVB stongly influenced the genes involved in the pigmentary responses this was not found for the UVA treatments used. The distinct responses found for UVA and UVB may identify new factors involved in UV-induced responses. This might offer other interesting possibilities such as the comparison between UVA or UVB with the full UV spectrum of UVA + B in solar simulated lamps. Kadekaro et al also used microarrays to study responses to alpha-melanocortin and ultraviolet in melanocyte cultures expressing different melanocortin receptor variants. DNA repair and antioxidant genes were identified to be modulated by alpha- melanocortin. Tomita et al already published effects of PGE2 on melanocytes in vitro in 1987 in JID. Now both Gledhill et al and Starner et al describe the production of PGE2 by human melanocytes. The first suggest that the PGE2 production by EM may contribute to UVR-induced erythema and shows no correlation with pigmentation status. The other paper does indicate that PGE2 acts as an autocrine factor that stimulates tyrosinase.
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  ... little is known about the stress response of melanocytes to UV and the regulation of DNA repair pathways in melanocytes. Unraveling these mechanisms might lead to strategies to prevent melanoma, as well as non-melanoma skin cancer

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  Since alpha-MSH has shown promising anti-inflammatory and antifibrotic properties in numerous preclinical studies, it will be most interesting to evaluate these effects in further clinical pilot studies with NDP-alpha-MSH. In addition to alpha-MSH analogues, truncated tripeptides such as KDPT which do not bind to MC-1R but have sustained anti-inflammatory properties are currently emerging as another novel therapeutic strategy in dermatology

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5. Neuromelanins

(Pr M. d'Ischia)

The possibility that aminochromes generated during neuromelanin synthesis by oxidation of catecholamines induce toxic effects in dopaminergic pigmented neurones has long been considered. Lozano et al. (2010) now report new data indicating that DT-diaphorase plays a protective role against aminochrome toxicity in cell culture. Treatment of cells with a decreased expression of DT-diaphorase with as high as 100 micromolar aminochrome resulted consistently in higher levels of cell death. Attention is called however to the fact that aminochromes are relatively unstable and may undergo decomposition during prolonged incubation experiments, so toxicity data may actually refer at least in part to other products. The application of quantitative ion beam microscopy for detection of iron in parkinsonian substantia nigra is also reported by Barapatre et al. (2010).

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6. Genetics, molecular and developmental biology

(Dr F. Beermann)

Even though it is not a paper on pigmentation, the recent findings of the group of Michael O. Hengartner may shed new light on alternative functions of tyrosinase or its related proteins, in particular for TRP-2/Dct, which is known to be expressed in mouse brain. The work in C.elegans by Sendael et al., (Nature 2010) explored the connection between hypoxia (high levels of HIF) and cell death vs. survival. In their findings, the effector molecule of HIF is - surprisingly - the worm equivalent of TRP-2 (called TYR-2), For example, germ line expression of TYR-2 prevents radiation-induced cell death in C.elegans. This might be the link/explanation to the capacity of human TRP-2 to prevent cisplatin-induced apoptosis in human melanoma cell lines. (See also the News&Views by Powell-Coffman & Coffman, Nature 465, 554-555))

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Abstract: BACKGROUND: Generalized vitiligo is an autoimmune disease characterized by melanocyte loss, which results in patchy depigmentation of skin and hair, and is associated with an elevated risk of other autoimmune diseases.

METHODS: To identify generalized vitiligo susceptibility loci, we conducted a genomewide association study. We genotyped 579,146 single-nucleotide polymorphisms (SNPs) in 1514 patients with generalized vitiligo who were of European-derived white (CEU) ancestry and compared the genotypes with publicly available control genotypes from 2813 CEU persons. We then tested 50 SNPs in two replication sets, one comprising 677 independent CEU patients and 1106 CEU controls and the other comprising 183 CEU simplex trios with generalized vitiligo and 332 CEU multiplex families. RESULTS: We detected significant associations between generalized vitiligo and SNPs at several loci previously associated with other autoimmune diseases. These included genes encoding major-histocompatibility-complex class I molecules (P=9.05x10(-23)) and class II molecules (P=4.50x10(-34)), PTPN22 (P=1.31x10(-7)), LPP (P=1.01x10(-11)), IL2RA (P=2.78x10(-9)), UBASH3A (P=1.26x10(-9)), and C1QTNF6 (P=2.21x10(-16)). We also detected associations between generalized vitiligo and SNPs in two additional immune-related loci, RERE (P=7.07x10(-15)) and GZMB (P=3.44x10(-8)), and in a locus containing TYR (P=1.60x10(-18)), encoding tyrosinase. CONCLUSIONS: We observed associations between generalized vitiligo and markers implicating multiple genes, some associated with other autoimmune diseases and one (TYR) that may mediate target-cell specificity and indicate a mutually exclusive relationship between susceptibility to vitiligo and susceptibility to melanoma.

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Abstract: We conducted a genome-wide association study of generalized vitiligo in the Chinese Han population by genotyping 1,117 cases and 1,429 controls. The 34 most promising SNPs were carried forward for replication in samples from individuals of the Chinese Han (5,910 cases and 9,916 controls) and Chinese Uygur (713 cases and 824 controls) populations. We identified two independent association signals within the major histocompatibility complex (MHC) region (rs11966200, Pcombined=1.48x10(-48), OR=1.90; rs9468925, Pcombined=2.21x10(-33), OR=0.74). Further analyses suggested that the strong association at rs11966200 might reflect the reported association of the HLA-A*3001, HLA-B*1302, HLA-C*0602 and HLA-DRB1*0701 alleles and that the association at rs9468925 might represent a previously unknown HLA susceptibility allele. We also identified one previously undescribed risk locus at 6q27 (rs2236313, Pcombined=9.72x10(-17), OR=1.20), which contains three genes: RNASET2, FGFR1OP and CCR6. Our study provides new insights into the genetic basis of vitiligo.

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Abstract: Induction of senescence permanently restricts cellular proliferation after oncogenic stimulation thereby acting as a potent barrier to tumor development. The relevant effector proteins may therefore be fundamental to cancer development. A recent study identified IGFBP7 as a secreted factor mediating melanocyte senescence induced by oncogenic B-RAF, which is found commonly in cutaneous nevi. In contrast to the previous report, we demonstrate that B-RAF signaling does not induce IGFBP7 expression, nor the expression of the IGFBP7 targets, BNIP3L, SMARCB1, or PEA15, in human melanocytes or fibroblasts. We also found no correlation between B-RAF mutational status and IGFBP7 protein expression levels in 22 melanoma cell lines, 90 melanomas, and 46 benign nevi. Furthermore, using a lentiviral silencing strategy we show that B-RAF induces senescence in melanocytes and fibroblasts, irrespective of the presence of IGFBP7. Therefore, we conclude that the secreted protein IGFBP7 is dispensable for B-RAF(V600E)-induced senescence in human melanocytes.

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TYR-2 in the ASJ sensory neurons. TYR-2 is secreted by ASJ sensory neurons to antagonize CEP-1-dependent germline apoptosis. Knock down of the TYR-2 homologue TRP2 (also called DCT) in human melanoma cells similarly increases apoptosis, indicating an evolutionarily conserved function. Our findings identify a novel link between hypoxia and programmed cell death, and provide a paradigm for HIF-1 dictating apoptotic cell fate at a distance.

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- Wen B, Chen Y, Li H, Wang J, Shen J, Ma A, Qu J, Bismuth K, Debbache J, Arnheiter H, Hou L.
Allele-specific genetic interactions between Mitf and Kit affect melanocyte development. Pigment Cell Melanoma Res 23: 441-447.

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A new transgenic mouse line for tetracycline inducible transgene expression in mature melanocytes and the melanocyte stem cells using the Dopachrome tautomerase promoter. Transgenic Res 2010 (Epub, ahead of print).

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Mutation in archain 1, a subunit of COPI coatomer complex, causes diluted coat color and Purkinje cell degeneration. PLoS Genet 6: e1000956.
Abstract: Intracellular trafficking is critical for delivering molecules and organelles to their proper destinations to carry out normal cellular functions. Disruption of intracellular trafficking has been implicated in the pathogenesis of various neurodegenerative disorders. In addition, a number of genes involved in vesicle/organelle trafficking are also essential for pigmentation, and loss of those genes is often associated with mouse coat-color dilution and human hypopigmentary disorders. Hence, we postulated that screening for mouse mutants with both neurological defects and coat-color dilution will help identify additional factors associated with intracellular trafficking in neuronal cells. In this study, we characterized a mouse mutant with a unique N-ethyl-N-nitrosourea (ENU)-induced mutation, named nur17. nur17 mutant mice exhibit both coat-color dilution and ataxia due to Purkinje cell degeneration in the cerebellum. By positional cloning, we identified that the nur17 mouse carries a T-to-C missense mutation in archain 1 (Arcn1) gene which encodes the delta subunit of the coat protein I (COPI) complex required for intracellular trafficking. Consistent with this function, we found that intracellular trafficking is disrupted in nur17 melanocytes. Moreover, the nur17 mutation leads to common characteristics of neurodegenerative disorders such as abnormal protein accumulation, ER stress, and neurofibrillary tangles. Our study documents for the first time the physiological consequences of the impairment of the ARCN1 function in the whole animal and demonstrates a direct association between ARCN1 and neurodegeneration.

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Melanocyte stem cells express receptors for canonical Wnt-signaling pathway on their surface. Biochem Biophys Res Commun 396: 837-842.

- Yang J, Splittgerber R, Yull FE, Kantrow S, Ayers GD, Karin M, Richmond A.
Conditional ablation of Ikkb inhibits melanoma tumor development in mice. J Clin Invest 120: 2563-2574.

7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borron)

- Cabrera J, Negrín G, Estévez F, Loro J, Reiter RJ, Quintana J.
  Melatonin decreases cell proliferation and induces melanogenesis in human melanoma SK-MEL-1 cells. J Pineal Res. 2010 Apr 12. [Epub ahead of print]
  Melatonin is an indoleamine synthesized in the pineal gland, and after its release into the blood, it has an extensive repertoire of biological activities, including antitumoral properties. In this study, we found that melatonin reduced the growth of the human melanoma cells SK-MEL-1. The antiproliferative effect was associated with an alteration in the progression of the phases of the cell cycle and also with an increase in tyrosinase activity, the key regulatory enzyme of melanogenesis. Antagonists for melatonin membrane receptors (luzindole and 4-P-PDOT) and the general G-coupled receptor inhibitor, pertussis toxin, did not prevent the melatonin-induced cell growth arrest; this suggests a mechanism independent of G-coupled membrane receptors. In contrast, p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway seems to play a significant role in cell growth inhibition by melatonin. The indoleamine-induced phosphorylation of p38 MAPK and the effect on cell proliferation were abrogated by the specific inhibitor SB203580. Furthermore, comparative studies with known antioxidants such as N-acetyl-l-cysteine and trolox indicate that the growth of SK-MEL-1 cells is highly sensitive to antioxidants.

- Fairhead M, Thöny-Meyer L.
  Role of the C-terminal extension in a bacterial tyrosinase. FEBS J. 2010 May;277(9):2083-95. Epub 2010 Mar 22.
  The well studied bacterial tyrosinases from the Streptomyces sp. bacteria are distinguishable from their eukaryotic counterparts by the absence of a C-terminal extension. In the present study, we report that the tyrosinase from the bacterium Verrucomicrobium spinosum also has such a C-terminal extension, thus making it distinct from the Streptomyces enzymes. The entire tyrosinase gene from V. spinosum codes for a 57 kDa protein (full-length unprocessed form), which has a twin arginine translocase type signal peptide, the two copper-binding motifs typical of the tyrosinase protein family and the aforementioned C-terminal extension. We expressed various mutants of the recombinant enzyme in Escherichia coli and found that removal of the C-terminal extension by genetic engineering or limited trypsin digest of the pro-form results in a more active enzyme (i.e. 30-100-fold increase in monophenolase and diphenolase activities). Further studies also revealed the importance of a phenylalanine residue in this C-terminal domain. These results demonstrate that the V. spinosum tyrosinase is a new example of this interesting family of enzymes. In addition, we show that this enzyme can be readily overproduced and purified and that it will prove useful in furthering the understanding of these enzymes, as well as their biotechnological application.

- Guan C, Lin F, Zhou M, Hong W, Fu L, Xu W, Liu D, Wan Y, Xu A.
  The role of VIT1/FBXO11 in the regulation of apoptosis and tyrosinase export from endoplasmic reticulum in cultured melanocytes. Int J Mol Med. 2010 Jul;26(1):57-65.
  Our previous study has shown that VIT1 gene in Chinese vitiligo patients is de facto the FBXO11 gene, and the silencing of that gene has an impact on the ultrastructure of melanocytes. In this study, we further identified the role of the FBXO11 gene in melanocytes and the relationship between dilated endoplasmic reticulum (ER) and tyrosinase by inhibition and overexpression of FBXO11 gene. Cell proliferation, apoptosis, cycle and migration of melanocytes were examined when the FBXO11 gene was silenced or overexpressed. The results showed that FBXO11 gene promoted cell proliferation and suppressed cell apoptosis, and yet had little effect on cell migration. Obvious swelling of ER was found in the cells transfected with siRNA of FBXO11 gene. Interestingly, protein level of tyrosinase was extraordinarily high following inhibition of FBXO11 gene. Further examination revealed that tyrosinase and calreticulin were co-localized in ER of transfected cells following siRNA of FBXO11 gene, suggesting that tyrosinase could not be exported from ER effectively. Collectively, our results support the notion that FBXO11 plays an important role in regulating proliferation and apoptosis of melanocytes, and functional export of tyrosinase from ER in vitiligo melanocytes.

- Guo YJ, Pan ZZ, Chen CQ, Hu YH, Liu FJ, Shi Y, Yan JH, Chen QX.
  Inhibitory Effects of Fatty Acids on the Activity of Mushroom Tyrosinase. Appl Biochem Biotechnol. 2010 Jun 11.
  The effects of fatty acids, octanoic acid, (2E, 4E)-hexa-2,4-dienoic acid, hexanoic acid, (2E)-but-2-enoic acid, and butyric acid on the activities of mushroom tyrosinase have been investigated. The results showed that the fatty acids can potently inhibit both monophenolase activity and diphenolase activity of tyrosinase, and that the unsaturated fatty acids exhibited stronger inhibitory effect against tyrosinase than the corresponding saturated fatty acids, and the inhibitory effects were enhanced with the extendability of the fatty acid chain. For the monophenolase activity, the fatty acids could not only lengthen the lag period, but also decrease the steady-state activities. For the diphenolase activity, fatty acids displayed reversible inhibition. Kinetic analyses showed that octanoic acid and hexanoic acid were mixed-type inhibitors and (2E,4E)-hexa-2,4-dienoic acid and (2E)-but-2-enoic acid were noncompetitive inhibitors. The inhibition constants have been determined and compared.

- Hoshino T, Matsuda M, Yamashita Y, Takehara M, Fukuya M, Mineda K, Maji D, Ihn H, Adachi H, Sobue G, Funasaka Y, Mizushima T.
  Suppression of melanin production by expression of HSP70. J Biol Chem. 2010 Apr 23;285(17):13254-63. Epub 2010 Feb 22.
  Skin hyperpigmentation disorders due to abnormal melanin production induced by ultraviolet (UV) irradiation are both a clinical and cosmetic problem. UV irradiation stimulates melanin production in melanocytes by increasing intracellular cAMP. Expression of heat shock proteins (HSPs), especially HSP70, is induced by various stressors, including UV irradiation, to provide cellular resistance to such stressors. In this study we examined the effect of expression of HSP70 on melanin production both in vitro and in vivo. 3-Isobutyl-1-methylxanthine (IBMX), a cAMP-elevating agent, stimulated melanin production in cultured mouse melanoma cells, and this stimulation was suppressed in cells overexpressing HSP70. IBMX-dependent transcriptional activation of the tyrosinase gene was also suppressed in HSP70-overexpressing cells. Expression of microphthalmia-associated transcription factor (MITF), which positively regulates transcription of the tyrosinase gene, was up-regulated by IBMX; however, this up-regulation was not suppressed in HSP70-overexpressing cells. On the other hand, immunoprecipitation and immunostaining analyses revealed a physical interaction between and co-localization of MITF and HSP70, respectively. Furthermore, the transcription of tyrosinase gene in nuclear extract was inhibited by HSP70. In vivo, UV irradiation of wild-type mice increased the amount of melanin in the basal layer of the epidermis, and this increase was suppressed in transgenic mice expressing HSP70. This study provides the first evidence of an inhibitory effect of HSP70 on melanin production both in vitro and in vivo. This effect seems to be mediated by modulation of MITF activity through a direct interaction between HSP70 and MITF.

- Jiang S, Liu XM, Dai X, Zhou Q, Lei TC, Beermann F, Wakamatsu K, Xu SZ.
  Regulation of DHICA-mediated antioxidation by dopachrome tautomerase: implication for skin photoprotection against UVA radiation. Free Radic Biol Med. 2010 May 1;48(9):1144-51. Epub 2010 Feb 1.
  Dopachrome tautomerase (Dct) is a critical enzyme in the melanogenesis pathway that isomerizes the intermediate dopachrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and influences the proportion of DHICA monomer incorporated into the 5,6-dihydroxyindole (DHI) polymer in eumelanin. To investigate whether Dct inactivation affects skin photoprotection against ultraviolet radiation, we examined levels of reactive oxygen species (ROS), sunburn cell formation, epidermal cell apoptosis, and melanin composition in skins of Dct(-/-) knockout mice compared with skins of wild-type C57BL/6 mice under UVA-induced oxidative stress. The results demonstrate that Dct inactivation elevates the level of ROS, increases the numbers of sunburn cells and apoptotic cells, and decreases the amount of eumelanin in the epidermis upon exposure to chronic UVA radiation. Moreover, we determined the effects of DHICA-melanin, DHI-melanin, and a mixture of both on hydroxyl radical generation in the Fenton reaction utilizing an electron spin resonance assay. DHICA-melanin exhibits a potent hydroxyl radical-scavenging activity, whereas DHI-melanin does not. Thus, this study suggests that DHICA monomers are required to incorporate into the DHI polymer backbone of eumelanin, which highlights the important role of Dct in the regulation of DHICA-mediated antioxidation.

- Kholmanskikh O, van Baren N, Brasseur F, Ottaviani S, Vanacker J, Arts N, van der Bruggen P, Coulie P, De Plaen E.
  Interleukins 1alpha and 1beta secreted by some melanoma cell lines strongly reduce expression of MITF-M and melanocyte differentiation antigens. Int J Cancer. 2010 Jan 22. [Epub ahead of print]
  We report that melanoma cell lines expressing the interleukin-1 receptor exhibit 4- to 10-fold lower levels of mRNA of microphthalmia-associated transcription factor (MITF-M) when treated with interleukin-1beta. This effect is NF-kappaB and JNK-dependent. MITF-M regulates the expression of melanocyte differentiation genes such as MLANA, tyrosinase and gp100, which encode antigens recognized on melanoma cells by autologous cytolytic T lymphocytes. Accordingly, treating some melanoma cells with IL-1beta reduced by 40-100% their ability to activate such antimelanoma cytolytic T lymphocytes. Finally, we observed large amounts of biologically active IL-1alpha or IL-1beta secreted by two melanoma cell lines that did not express MITF-M, suggesting an autocrine MITF-M downregulation. We estimate that approximately 13% of melanoma cell lines are MITF-M-negative and secrete IL-1 cytokines. These results indicate that the repression of melanocyte-differentiation genes by IL-1 produced by stromal cells or by tumor cells themselves may represent an additional mechanism of melanoma immune escape.

- Lee JH, Jang JY, Park C, Kim BW, Choi YH, Choi BT.
  Curcumin suppresses alpha-melanocyte stimulating hormone-stimulated melanogenesis in B16F10 cells.
  Int J Mol Med. 2010 Jul;26(1):101-6.
  The present study was designed to assess the potential inhibitory activity of curcumin on the alpha-melanocyte stimulating hormone (alpha-MSH)-stimulated melanogenesis signal pathway in B16F10 melanoma cells. The molecular mechanism of curcumin-induced inhibitory activity on the alpha-MSH-stimulated melanogenesis signal pathway, including expression of melanogenesis-related proteins and activation of melanogenesis-regulating proteins, was examined in B16F10 cells. Curcumin suppressed the cellular melanin contents and the tyrosinase activity in alpha-MSH-stimulated B16F10 cells. In addition, the expression of melanogenesis-related proteins such as microphthalmia-associated transcription factor (MITF), tyrosinase, and tyrosinase-related protein 1 and 2 was suppressed by curcumin in the alpha-MSH-stimulated B16F10 cells. Notably, a melanogenesis-regulating signal such as mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) or phosphatidylinositol 3-kinase (PI3K)/Akt was

activated by curcumin in the B16F10 cells treated with or without alpha-MSH. The suppressive activity of curcumin on alpha-MSH-induced melanogenesis was down-regulated by PD98059 and by LY294002. Our results suggest that the suppressive activity of curcumin on alpha-MSH-stimulated melanogenesis may involve the down-regulation of MITF and its downstream signal pathway through the activation of MEK/ERK or PI3K/Akt.

- Li X, Guo L, Sun Y, Zhou J, Gu Y, Li Y.
  Baicalein inhibits melanogenesis through activation of the ERK signaling pathway. Int J Mol Med. 2010 Jun;25(6):923-7.
  Baicalein is one of the major flavonoids in Scutellaria baicalensis. However, the effects of baicalein on melanogenesis are unknown. The objective of this study was to evaluate the depigmenting capacity of baicalein and to elucidate its mechanism of action. B16F10 mouse melanoma cells were used to examine the effect of baicalein on melanogenesis by measurement of melanin content and tyrosinase activity after treatment. To ascertain the baicalein activity, the effect on two protein kinases, ERK and Akt and downstream microphthalmia-associated transcription factor (MITF) were examined by Western blotting and RT-PCR. Baicalein significantly inhibited melanin synthesis in a concentration-dependent manner without cytotoxicity. Tyrosinase activity was also reduced. Baicalein decreased MITF and tyrosinase levels but did not decrease MITF mRNA. Western blotting showed that baicalein induced ERK activation. Using the specific ERK phosphorylation inhibitor, PD98059, we blocked the hypopigmentation effect, and also abrogated the baicalein-mediated activation of ERK. However, baicalein did not induce Akt activation. These results suggest that the ERK pathway is involved in the melanogenic signaling cascade, and that ERK activation by baicalein reduces melanin synthesis via MITF downregulation and is subsequent to the inhibition of tyrosinase synthesis.

- Manga P, Bis S, Knoll K, Perez B, Orlow SJ.
  The unfolded protein response in melanocytes: activation in response to chemical stressors of the endoplasmic reticulum and tyrosinase misfolding. Pigment Cell Melanoma Res. 2010 Apr 23. [Epub ahead of print]
  Accumulation of proteins in the endoplasmic reticulum (ER) triggers the unfolded protein response (UPR), comprising three signaling pathways initiated by Ire1, Perk and Atf6 respectively. UPR activation was compared in chemically stressed murine wildtype melanocytes and mutant melanocytes that retain tyrosinase in the ER. Thapsigargin, an ER stressor, activated all pathways in wildtype melanocytes, triggering Caspase 12-mediated apoptosis at toxic doses. Albino melanocytes expressing mutant tyrosinase showed evidence of ER stress with increased Ire1 expression; but the downstream effector, Xbp1, was not activated even following thapsigargin treatment. Attenuation of Ire1 signaling was recapitulated in wildtype melanocytes treated with thapsigargin for eight days, with diminished Xbp1 activation observed after four days. Atf6 was also activated in albino melanocytes, with no response to thapsigargin, while the Perk pathway was not activated and thapsigargin treatment elicited robust expression of the downstream effector CHOP. Thus, melanocytes adapt to ER stress by attenuating two UPR pathways.

- Muñoz-Muñoz JL, Garcia-Molina F, Varon R, Garcia-Ruíz PA, Tudela J, Garcia-Cánovas F, Rodríguez-López JN.
  Suicide inactivation of the diphenolase and monophenolase activities of tyrosinase. IUBMB Life. 2010 Jul;62(7):539-47.
  The suicide inactivation mechanism of tyrosinase acting on its phenolic substrates has been studied. Kinetic analysis of the proposed mechanism during the transition phase provides explicit analytical expressions for the concentrations of o-quinone versus time. The electronic, steric, and hydrophobic effects of the phenolic substrates influence the enzymatic reaction, increasing the catalytic speed by three orders of magnitude and the inactivation by one order of magnitude. To explain this suicide inactivation, we propose a mechanism in which the enzymatic form oxy-tyrosinase is responsible for the inactivation. In this mechanism, the rate constant of the reaction would be directly related with the strength of the nucleophilic attack of the C-1 hydroxyl group, which depends on the chemical shift of the carbon C-1 (delta(1)) obtained by (13)C-NMR. The suicide inactivation would occur if the C-2 hydroxyl group transferred the proton to the protonated peroxide, which would again act as a general base. In this case, the coplanarity between the copper atom, the oxygen of the C-1 and the ring would only permit the oxidation/reduction of one copper atom, giving rise to copper (0), hydrogen peroxide, and an o-quinone, which would be released, thus inactivating the enzyme. One possible application of this property could be the use of these suicide substrates as skin depigmenting agents.

- Sendoel A, Kohler I, Fellmann C, Lowe SW, Hengartner MO.
  HIF-1 antagonizes p53-mediated apoptosis through a secreted neuronal tyrosinase. Nature. 2010 Jun 3;465(7298):577-83.
  Hypoxia-inducible factor (HIF) is a transcription factor that regulates fundamental cellular processes in response to changes in oxygen concentration. HIFalpha protein levels are increased in most solid tumours and correlate with patient prognosis. The link between HIF and apoptosis, a major determinant of cancer progression and treatment outcome, is poorly understood. Here we show that Caenorhabditis elegans HIF-1 protects against DNA-damage-induced germ cell apoptosis by antagonizing the function of CEP-1, the homologue of the tumour suppressor p53. The antiapoptotic property of HIF-1 is mediated by means of transcriptional upregulation of the tyrosinase family member TYR-2 in the ASJ sensory neurons. TYR-2 is secreted by ASJ sensory neurons to antagonize CEP-1-dependent germline apoptosis. Knock down of the TYR-2 homologue TRP2 (also called DCT) in human melanoma cells similarly increases apoptosis,

indicating an evolutionarily conserved function. Our findings identify a novel link between hypoxia and programmed cell death, and provide a paradigm for HIF-1 dictating apoptotic cell fate at a distance.

- Starner RJ, McClelland L, Abdel-Malek Z, Fricke A, Scott G.
PGE(2) is a UVR-inducible autocrine factor for human melanocytes that stimulates tyrosinase activation. Exp Dermatol. 2010 May 25. [Epub ahead of print]
Prostaglandins activate signalling pathways involved in growth, differentiation and apoptosis. Prostaglandin E(2) (PGE(2)) is released by keratinocytes following ultraviolet irradiation (UVR) and stimulates the formation of dendrites in melanocytes. We show that multiple irradiations of human melanocytes with UVR-activated cPLA(2), the rate-limiting enzyme in eicosanoid synthesis and stimulated PGE(2) secretion. PGE(2) increased cAMP production, tyrosinase activity and proliferation in melanocytes. PGE(2) binds to four distinct G-protein coupled receptors (EP(1-4)). We show that PGE(2) stimulates EP(4) receptor signalling in melanocytes, resulting in cAMP production. Conversely, PGE(2) also stimulated the EP(3) receptor in melanocytes, resulting in lowered basal cAMP levels. These data suggest that relative levels or activity of these receptors controls effects of PGE(2) on cAMP in melanocytes. The data are the first to identify PGE(2) as an UVR-inducible autocrine factor for melanocytes. These data also show that PGE(2) activates EP(3) and EP(4) receptor signalling, resulting in opposing effects on cAMP production, a critical signalling pathway that regulates proliferation and melanogenesis in melanocytes.

- Vavricka CJ, Ray KW, Christensen BM, Li J.
Purification and N-glycosylation analysis of melanoma antigen dopachrome tautomerase. Protein J. 2010 Apr;29(3):204-12.
Dopachrome tautomerase (DCT) plays a critical role in lowering the oxidative stress resulting from melanogenesis. Levels of DCT are elevated in melanoma cell lines that are especially resistant to chemotherapy and radiation. DCT is processed as a melanoma antigen and is a potential target for immunotherapy. In order to establish a more complete understanding of the role that DCT may play in the etiology and treatment of melanoma skin cancer, isolation of highly pure and properly processed protein is necessary. Purification of native DCT has been problematic due to a hydrophobic transmembrane anchor and interactions with melanin. In this study, DCT was expressed, without its carboxy-terminal transmembrane region using an Sf9 insect cell protein expression system and its recombinant protein was purified by various chromatographic techniques. Analysis of DCT tryptic peptides by MALDI-TOF/TOF determined N-glycosylation as a primary post-translational modification. Our success in the expression of soluble mammalian DCT and the characterization of N-glycosylation sites is a useful reference toward the comprehensive understanding of the structure/function relationship of mammalian DCT.

- Yamada T, Akamatsu H, Hasegawa S, Inoue Y, Date Y, Mizutani H, Yamamoto N, Matsunaga K, Nakata S.
Melanocyte stem cells express receptors for canonical Wnt-signaling pathway on their surface. Biochem Biophys Res Commun. 2010 Jun 11;396(4):837-42.
It has been reported that melanocytes play important roles in skin and hair pigmentation and are differentiated from melanocyte stem cells (MSCs) residing in the bulge area of hair follicles. Recently, interest has been growing in MSCs because regulation of the upstream of differentiated melanocytes is essential for the determination of skin and hair pigmentation; however, their precise characteristics remain to be elucidated. The aim of this study is to explore cell-surface markers expressed on MSCs in order to understand their characteristics. To explore genes specifically expressed in the bulge region, we classified a hair follicle into four areas, hair bulb, hair bulb to bulge (lower bulge), bulge, and epidermis to bulge (upper bulge), and collected these areas from back skin sections of C57BL/6 mice by laser microdissection. Real-time RT-PCR performed on these areas revealed that Frizzled (Fzd)-4, Fzd7, low density lipoprotein receptor-related protein 5 (Lrp5), and Lrp6, receptors for Wnt molecules, were expressed higher in the bulge area than other areas. Furthermore, FACS analysis showed that populations of Fzd4(+) cells and Fzd7(+) cells were different from those of Kit(+) cells (precursor of melanocytes: melanoblasts). Fzd4(+) and Fzd7(+) cells isolated by FACS required a longer culture period to differentiate into mature melanocytes than Kit(+) cells. Up-regulation of mRNA expressions of melanocyte markers (dopa chrometautomerase: Dct, tyrosinase: Tyr, tyrosinase-related protein 1: Tyrp1) was observed in Fzd4(+) and Fzd7(+) cells following Kit(+) cells during differentiation. These results suggested that Fzd4(+) and Fzd7(+) cells were more immature than melanoblasts, therefore raising the possibility that Fzd4(+) and Fzd7(+) cells are MSCs.

8. Melanosomes

(Pr J. Borovansky)

Reviews: Review devoted to melanosome biogenesis, physiological and pathological phenotypes associated to melanosome was published by *Schiaffino*. It emphasizes that the MC1R and OA1-mediated pathways provide extremely innovative examples of signal transduction systems. Thus they play fundamental roles in the biology and pathology of pigmentation and bring crucial insights into the regulation of melanosome biogenesis and transport and also into development of cancer and albinism. *Brenner and Berking* wrote (in German) a brief review of biochemistry and regulation of melanogenesis suited for dermatologists but not for scientists involved in research. Colour illustrations were taken over from research papers of other authors.

Quantitative parameters in relation to melanosomes: The first direct measurement of the absorption coefficient of intact melanosomes isolated from RPE cells was achieved by means of photoemission electron microscopy by *Peles and Simon*. Their results demonstrate a direct relationship between the absorption coefficient and the relative DHI/DHICA content; the UV absorption coefficient was associated with increasing DHICA content, as for eumelanosomes. Hyperspectral imagery was used by *Nunez et al* to estimate the amount of melanosomes contained within pixels identified as skin, which gives an estimate of skin colour. Having exploited a previously described semiempirical model for diffuse reflectance of two-layered media, *Yudovsky and Pilon* developed a method for the estimation of parameters listed in the title. *Michihara et al*. concluded that δ-tocotrienol, used as a therapeutic and/or preventive drug for hyperpigmentation and as a component of whitening cosmetics, does not show serious side effects: neither lysosomes nor melanosomes were released from the treated cells, since β-glucuronidase (melanosome and lysosome marker), melanin content and tyrosinase did not rise in the culture medium.

Melanosomal proteins: GPNMB (glycoprotein nonmetastatic melanoma protein b), localized in melanosomes, is a membrane bound glycoprotein that shows high homology with the melanosomal structural protein PMel17/gp100. *Hoashi et al* reported that the GPNMB is heavily glycosylated and unlike the PMell7/gp100 it is in a higher concentation in the stage III and IV melanosomes. *Watt et al* defined two domains within the Pmel17 (an N-terminal domain and a downstream domain with a homology to a polycystic kidney -1 repeat) that promote an amyloid fibril formation. They also suggested that the fibril formation might be physically linked with the multivesicular body sorting. Using a series of deletion and missense mutants of PMel17 *Leonhart et al* showed that the integrity of the junction between the N-terminal region and the polycystic kidney-like domain is crucial for endoplasmic reticulum export, subcellular targetting and the fibril formation by the PMel17 and thus for establishing functional melanosomes.
Chou and Shen have introduced „EUK-mPLOC 2.0“**,** a new predictor of subcellular localization of eukaryotic proteins. The introduction requires a knowledge of higher mathematics. The predictor can identify eukaryotic proteins among 22 cellular locations (including melanosomes with 47 identified proteins). EUK-mPLOC 2.0 is said to be user-friendly and is freely accesible at http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/.

Melanogenesis: Melanin synthesis in adenoviral-transduced tyrosinase-gene-expressing amelanotic ARPE19 cells was analyzed as in regard to its localization by *Biesemeier et al*. Active tyrosinase was found in small granule-like vesicles in the cytoplasm, in the endoplasmic reticulum and in the nuclear membrane (for the nuclear localization of tyrosinase see also e.g. *Lerche W, Wulle KG/* Z.Zellforsch.Mikroskop. Anat. 76: 452-457, 1967 and *McCurdy HM*/ Pigment Cell 3:46-52, 1976) and in multivesicular and multilamellar organelles. (For the deposition of melanin in the absence of melanosomal matrix proteins see also *Borovanský et al/* Arch Derm Res. 289 : 145–150, 1997). PMel1, TRP-1 and premelanosomes were not detected.

Melanosome transport: A requirement for filopodia in the transport of melanosomes both from melanocytes to keratinocytes and between keratinocytes was demonstrated by *Singh et al* in experiments using filopodial markers (MyoX/Cdc-42) and the filopodial disrupter (low dose cytochalasin B). A knockdown of keratinocyte MyoX protein resulted in the inhibition of melanosome uptake by keratinocytes.

Exploitation of melanosome properties for therapy of malignant melanoma: Melanosomes exhibit affinity for aromatic and polycyclic compounds. *Gardette et al* in a preclinical *in vitro* evaluation of ^{125}I-labelled acridine and acridone compounds observed a significant radiotoxicity to B16F10 melanoma cells. (See also *Miot-Noirault E*/Cancer Biother Radiopharm. 24(5):629-36, 2009.)
The cellular uptake was similar for both compounds, the acridine derivative showed a higher nuclear accumulation and hence it appeared as a better candidate for an application in targetted radionuclide therapy. The acridone compound with stronger affinity to melanin,was scavenged in melanosomes and its access to the nucleus was restricted. (Similar phenomenon was mentioned also by *Chen et al*./PNAS 103, 9903-9907, 2006.)

Melanosomes in melanomas: A case report by *Zerfas et al* of amelanotic melanoma in the white rabbit. A facial mass of 0.5 ccm on histopathology consisted of anisocytotic and anisokaryotic polygonal to spinoloid cells. Fontana-Masson staining was negative for melanin. Positive staining for MART-1 with TEM-observable type II melanosome confirmed the

diagnosis of amelanotic melanoma. *Nakahara et al*. established two novel cell lines from a malignant melanoma in oral mucosa. Amelanotic NM78AMcells produced no melanin, NM78MM cells produced melanosomes that were present at various stages of development in the cytoplasm and were secreted into the medium. The oxygen concentration in the medium was monitored to investigate the susceptibility of both cell lines to various anticancer drugs.

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  The classical pathway of melanogenesis is not essential for melanin synthesis in the adult retinal pigment epithelium. Cell Tissue Res. 339(3): 551-60, 2010.

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  Principles of skin pigmentation : Biochemistry and regulation of melanogenesis.[In German.] Hautarzt. 2010 Jun 13. [Epub ahead of print]

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  A new method for predicting the subcellular localization of eukaryotic proteins with both single and multiple sites: Euk-mPLoc 2.0. PLoS One. 5(4): e9931, 2010.

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  Evaluation of new iodinated acridine derivatives for targeted radionuclide therapy of melanoma using (125)I, an Auger electron emitter. Invest New Drugs. 2010 Jun 22.

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  Glycoprotein nonmetastatic melanoma protein b, a melanocytic cell marker, is a melanosome-specific and proteolytically released protein. FASEB J 24(5): 1616-629, 2010.

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  Visualization of the melanosome transfer-inhibition in a mouse epidermal cell co-culture model. Int J Mol Med. 25(2): 249-53, 2010.

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  Endoplasmic reticulum export, subcellular distribution, and fibril formation by Pmel17 require an intact N-terminal domain junction. J Biol Chem. 285(21): 16166-16183, 2010.

- Michihara A, Shitamani M, Morita S, Akasaki K.
  Reduction of mevalonate pyrophosphate decarboxylase in mouse melanoma cells trated with δ-tocotrienol is not associated with reduction of cholesterol content or release of lysosomes and melanosomes. J Health Sci 56(3): 355-360, 2010.

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9. Melanoma experimental, cell culture

(Dr R. Morandini)

It is well known that the human melanoma microenvironment is able to influence tumor growth and progression. Furthermore melanoma is a complex "system" characterized by high genetic and cell signaling heterogeneity. Two of the main activating mutations of critical genes that regulate signal transduction are B-RAF and N-RAS mutation. Among these oncogenic alterations, activating mutations and over expression of c-KIT represent key events in defined subgroups of melanoma. The c-KIT protein is a receptor tyrosine kinase that signals in response to its ligand SCF and activate the phosphoinositide 3-kinase/protein kinase B (AKT) and MAP-kinase cascade pathway. These activated signaling pathways are critical in melanoma progression. Belleudi shows that KGF/FGF7 secreted from keratinocytes present in the microenvironment of the melanoma tumor showed enhanced expression and secretion of SCF. This results of an increasing growth of melanoma cells that can be blocked by the c-kit inhibitor imatinib.
In the same field, Paraiso has studied a combined B-RAF/MEK inhibition strategy to prevent the emergence of drug resistance in B-RAF/V600E mutated melanoma.

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D. Other tools and cell culture

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E. Melanoma Experimental

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ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of Events

2010 XVIth Meeting of the ESPCR
September, 4-7, Hinxton-Cambridge, UK
Contact: Dr Robert Kelsh
Web: ESPCR Meeting Hinxton-Cambridge

2010 40th Annual ESDR Meeting
September, 8-11, Helsinski, Finland
Contact: Aira RAUDASOJA
Kalevankatu 12, 3rd floor, 00100 Helsinki, Finland
Tel : +358 9 4542 190 Fax : +358 9 4542 1930
Web : http://www.esdr.org

2010 Perspectives in Melanoma XIV
September, 17-18, 2010
Contact: Web : Perspectives in melanoma XIV

2010 16th Annual Meeting Pan American Society for Pigment Cell Research
The Pigmentation and Melanoma Research Congress
Sep 30-Oct 2, The BC Cancer Agency, Vancouver, Canada
Contact: Dr. Youwen ZHOU, MD, PhD (Co-Chair)
Web: www.paspcr2010.org

2010 19th Congress of the European Academy Of Dermatology and Venereology (EADV)
October, 6-10, GOTHENBURG, Sweden
Contact: c/o MCI Deutschland GmbH
Astrid SCHÖRNIG
Markgrafenstr. 56, 10117 Berlin, Germany
Tel: +49 (0)30 20 45 40 83 Fax: +49 (0)30 20 45 40 85
Email: president(at)EADVGothenburg2010.org
Web : http://www.eadvgothenburg2010.org/

2010 The 23rd Meeting of the JSPCR

November, 27-28,
Contact: NAKAGAWA, Hidemi, MD
(Department of Dermatology The Jikei University School of Medicine)
Web: http://www.gakkai-net.jp/shikiso23/

2011 41st Annual ESDR Meeting

September, 7-10 Barcelona, Spain
Contact: Web: www.esdr.org

2011 XXIth IPCC

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

2012 XVIIth Meeting of the ESPCR

September, Geneva, Switzerland
Contact: Dr Bernhard WEHRLE-HALLER

NEW MEMBERS

The ESPCR is delighted to welcome the following colleagues to membership and hope they will play a full and active part in the Society

ARMARO Marzia
EPFL
CH- Lausanne

BONET Caroline
INSERM U895 C3M
F- Nice

BRAIG Simone
University of Regensburg Medical Hospital
G- Regensburg

CAMPAGNE Cécile
INRA - Ecole National de Vétérinaire d\'Alfort
F- Maisons-Alfort

CHENG Phil
University Hospital of Zurich
CH- Zurich

COLANESI Sarah
University of Bath
UK- Bath

EICHHOFF Ossia
University Hospital of Zürich
CH- Zürich

FEELEY Natasha
Curtin University of Technology
AUS- Bentley

FLORI Enrica
Cutaneous Physiopathology Laboratory
I- Rome

GRABACKA Maja
University of Agriculture
PL- Krakow

JOHANSEN Peter
Faculty of Health Sciences
University of Copenhagen
DK- Copenhagen O

KOVACS Daniela
Cutaneous Physiopathology Laboratory
I- Rome

MARESCA Vittoria
Instituto Dermatologico San Gallicano
F- Orsay Cedex

OLIVARES SÁNCHEZ Maria Concepción
University of Murcia
Espinardo
I- Murcia

PASCAL Michel
Centre Laser Espace Saint-Honore
F- PARIS

RAHOMA Sherif
University of Sheffield
UK- Sheffield

SARODE Bhushan
Ecole Polytechnique Fédérale de Lausanne
CH- Lausanne

SCHIFFNER Susanne
University Hospital of Regensburg
D- Regensburg

VIBERT Laura
University of Bath
UK- Bath

WIDMER Daniel
University Hospital of Zürich
CH- Zürich