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BULLETIN

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Minutes of the ESPCR 2011 Board of Officers Meeting, Palais des Congrès de Bordeaux, France, Tuesday 20 September 2011, 5.00-6.00 p.m.

1. Opening of the meeting: L. Larue, President, opened the meeting and welcomed the Board members: Marie-Dominique Galibert, Ghanem Ghanem (Bulletin Editor), Robert Kelsh, Rosalie Luiten, Lluis Montoliu (Treasurer and Webmaster), Alessandra Napolitano, M. Picardo (ex-officio), Alain Taïeb (Secretary) and two guests Bernhard Wehrle Haller and Graça Raposo

2. Apologies for absence were received from Anja Bosserhof, Miguel Seabra and Jo Lambert

3. Approval of the Minutes of the 2010 Board meeting: the minutes of the 2010 board meeting were approved and signed by the President

4. 2010 Hinxton meeting final report (R Kelsh)
The 2010 meeting featured 41 invited speakers and 180 registrants. The £ 11, 867.92 loss of the meeting was covered as stipulated per contract by the Welcome Trust.

Expenses	
Conference Centre costs, Hire of venue etc	£77,480.67
WTSC e.g. Coaches, external accommodation, abstract book, advertising	£15,502.02
Speaker Travel	£10,627.86
Queens College Dinner	£8,645.61
	£112,256.16
Received Funds	
Registration Fees	£70,453.00
Sponsorship	£6,160.45
WT Contribution	£20,000.00
Other income (sponsorship of travel, committee meetings etc.)	£3,774.79
	£100,388.24
Balance	- £11,867.92

The President thanked Robert Kelsh for the excellence of the scientific content of the meeting and perfect local organization.

5. President's report (L. Larue)
5a) ESPCR at ESDR meeting.
The theme of the workshop was "DNA repair and melanoma ». Despite excellent talks delivered by ESPCR invited speakers, the number of attendees was limited (about 40) because of poor

advertisement and a concurrent EADO session on melanoma.
Another session is planned at the tricontinental international investigative dermatology (IID) meeting in Edinburgh in 2013. The President proposed to invite officially the ESDR to the Lisbon 2013 ESPCR meeting to organize a kind of reciprocal workshop. Possible topics include « interaction of the keratinocyte with melanocyte » « importance of the stroma on melanocyte behaviour » or “skin inflammation and pigment cells”.
5b) The President thanked Prof. Marco d'Ischia for his initiative in creating EuMelanet, an ESPCR Interest Group which attracts new ESPCR members.
The President’s report was approved by the board

6. Secretary’s report (A. Taieb)
The Secretary mentioned that no elections of board members are scheduled next year, but that potential candidates should be informed and approached for the year after.
The Fritz Anders Lecture for next year (Geneva) should be discussed as soon as possible between the Board and the Organiser. The selection of a high profile scientist of the organizing country is welcome.
The Secretary’s report was approved by the board

7. Awards committee report (A. Napolitano, M.D. Galibert)
A. Napolitano commented on behalf of the ESPCR Awards Committee for the Raper Medal: (Robert Kelsh, Miguel Seabra, Alessandra Napolitano) the nomination of Marco d’Ischia for 2011 which fits perfectly the aims of the Medal as an international recognition of outstanding contributions to the biochemistry of pigmentation.
MD Galibert described the selection process used to select the 42 IPCC travel awards (30 IPCC, 9 IFPCS, 3ESPCR). She represented the ESPCR among the PCS international committee (C Le Poole PASPCR, T Kunisada JSPCR, P Kumarasinghe ASPCR). This experience was discussed by the board and MD Galibert was asked to produce a short memo emphasizing possible improvements which may help future selection committees.

8. Treasurer’s report (L. Montoliu)
The ESPCR had 144 members on 17/09/2011, including 108 regular members (75%), 21 student Members (15%), 13 Honorary Members (9%) and 2 Supporting Members (1%). Only 17 Members subscribed to printed PCMR issues. Only 20% of members accessed the Journal via the IFPCS website, and the majority opted for a 1 year membership (88%).

ESPCR Treasurer Report 2011 (from 01-09-2010 to 17-09-2011)

ESPCR AISBL Bank account at BNP-Paribas-Fortis

Balance on 01/09/10	22.399,09 €
Balance on 17/09/11	**28.802,61 €**

INCOME

ESPCR memberships through PAYPAL account	**7.315,00 €**
ESPCR memberships through Bank Wire Transfer	**4.375,00 €**
ESPCR memberships through cheques	**323,57 €**
Donation GALDERMA (for ESPCR2010 related activities)	**729,00 €**
Donation ARIV ONLUS (for VETF-vitiligo related activities)	**8.813,19 €**
Donation GIULIANI (for IPCC related activities)	**15.000,00 €**
Bank Interest	**43,39 €**
TOTAL INCOME	**36.599,15 €**
PENDING INCOME (7 ESPCR Memberships unpaid, 5 regular, 2 stud.)	**665,00 €**

OUTGOINGS

ESPCR 2010 vitiligo-related activities (Galderma funds)	**-720,93 €**
IPCC 2011 related activities (Giuliani funds)	**-15.000,00 €**
ESPCR delegates attending ESDR 2011 in Barcelona (registration)	**-2.100,00 €**
ALTA SRLU (vitiligo projects-Ariv Onlus funds)	**-1.800,00 €**
Raper Medal	**-180,00 €**
PAYPAL fees	**-262,79 €**
BNP-Paribas-Fortis Bank Fees and other	**-121,9 €**
IFPCS fees for ESPCR 2010 (12.509,00 USD)	**-9.535,03 €**
espcr.eu web domain (4ARM)	**-24,20 €**
espcr.eu web domain (x3 years)	**-67,54 €**
IFPCS web hosting (x3 years) – to be invoiced to IFPCS	**-383,24 €**
TOTAL OUTGOINGS	**-30.195,63 €**
PENDING OUTGOINGS (2011 IFPCS fee, PCMR) estimated	**-8.133,00 €**
PENDING OUTGOINGS (2011 ESPCR travel awards, 3x 500€) estimated	**-1.500,00 €**

TOTAL INCOME	**36.599,15 €**
TOTAL OUTGOINGS	**-30.195,63 €**
BALANCE ON 17-09-11	**28.802,61 €**

A 5,000 € grant from the ARIVONLUS donation to help the organization of the IPCC Vitiligo Global Issues Consensus Conference was discussed and approved by the Board.
The Treasurer's report was approved by the board.

9. Webmaster's report (L. Montoliu)

The webmaster presented the statistics of the website, indicating that the site seems to be receiving more visitors who browse fewer pages.ESPCR members have increased their access through the members-only pages. He mentioned several updates and improvements (EUMELANET,VETF, ESPCR Bulletin, books advertisement, promotion of meetings, IPCC 2011 announcements ,Colour Genes, announcements posted at ESPCR blog and distributed through the ESPCR list, ESPCR membership)

The President thanked the webmaster for his dedication to this strategic position.

10. Bulletin report (G. Ghanem)
G. Ghanem indicated a change in the editorial Board of the Bulletin. F. Beermann and N. Smit wished to step down after about 18 years of constant support and input. F. Beermann was replaced by Lluis Montoliu. The Photobiology section covered by Nico Smit is still open. A proposal was to contact Marie-Dominique Galibert for this position.
We would like to thank Drs Beerman and Smit for their contributions to the Bulletin all these years and to acknowledge their invaluable long-term contribution to the success of the Bulletin.

11. Upcoming meetings

11.a IPCC 2011 (A. Taïeb)

The IPCC had some difficulties to balance its budget, obliging us to cut some costs and attract new emergency sponsors to cover a foreseeable loss. The final budget should be balanced. 500 delegates of 37 countries preregistered, a number close to that of Sapporo, but the number of IFPCS members seems lower than expected.

11.b ESPCR 2012 Geneva (B. Werhle-Haller):

Dates: 11-14th of September 2012. Venue: Centre Médical Universitaire, University of Geneva. B Werhle-Haller is preparing an attractive program and is looking for help for organization and fund raising. The ESPCR board is pleased to offer help on these matters.

11.c 2013 and 2015 meetings (M. Seabra, L. Larue)

ESPCR 2013 will be held in Lisbon. The date is not yet decided: 9-13th or 16-20th September 2013. The venue will be the Faculdade de Ciências Mèdicas at Lisbon University Organiser: Miguel Seabra/Co-Organiser: Graça Raposo. Very good conditions to host the meeting are expected based on the slide presentation of G Raposo.
For 2015, the President has approached Luisa Lanfrancone in Milan (IFOM) as a possible organizer.

12. Honorary member nomination (L. Larue)
The nomination of Paco Solano, proposed by Lluís Montoliu and Lionel Larue was unanimously approved.

13. Other businesses
The board discussed the nomination process for the next Editor-in-Chief of PCMR, which should take place in January 2012, with an effective appointment one year later. There are so far no declared candidates on the side of the IFPCS and a special IFPCS council meeting during IPCC will discuss this issue. The President favours an increase of section editors to help the EIC, for a more effective handling of manuscripts. The editor search by Wiley will take into consideration both the IFPCS and SMR candidates, but the final choice remains in their hands. The IFPCS candidate should be nominated by the end of the year, preferably before the SMR Tampa meeting.

14. Close of the Board Meeting (L. Larue)

Minutes of the ESPCR 2011 General Assembly Meeting,
Palais des Congrès de Bordeaux, France,
Thursday 22 September 2011, 6.00-7.00 p.m, Hall B

1. Opening of the GA: L. Larue, President, opened the meeting and welcomed the ESPCR members present which met the required quorum.

2. Approval of the Minutes of the 2010 GA meeting (Hinxton): the minutes of the 2010 GA meeting were approved and signed by the President.

3. President's report (L. Larue)
5a) ESPCR at ESDR meeting.
The theme of the workshop was "DNA repair and melanoma ». Despite excellent talks delivered by ESPCR invited speakers, the number of attendees was limited (about 40) because of poor advertisement and a concurrent EADO session on melanoma.
Another session is planned at the tricontinental international investigative dermatology (IID) meeting in Edinburgh in 2013. The President proposed we invite officially the ESDR to the Lisbon 2013 ESPCR meeting to organize a kind of reciprocal workshop.
5b) The President thanked Prof. Marcho d'Ischia, for his initiative creating EuMelanet, an ESPCR Interest Group which attracts new ESPCR members.
The President's report was approved by the GA

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The Secretary mentioned that no elections of board members are scheduled next year, but that potential candidates should be informed and approached for the year after.
The Fritz Anders Lecture for next year (Geneva) should be discussed as soon as possible between the Board and the Organiser. The selection of a high profile scientist from the organizing country would be desirable.
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A 5,000 € grant from the ARIVONLUS donation to help the organization of the IPCC Vitiligo Global Issues Consensus Conference was discussed and approved by the Board.
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Lluis Montoliu presented the statistics of the website, indicating that the site seems to be receiving more visitors who browse fewer pages.ESPCR members have increased their access through the members-only pages. He mentioned several updates and improvements (EUMELANET,VETF, ESPCR Bulletin, books advertisement, promotion of meetings, IPCC 2011 announcements ,Colour Genes, announcements posted at ESPCR blog and distributed through the ESPCR_list, ESPCR membership)
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10. Close of the GA Meeting (L. Larue)

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Pr A. Napolitano)

The structure of synthetic eumelanins has been investigated by different approaches. In one study (Reale et al J. Mass Spectrom.) by use of mass spectrometry in the matrix assisted laser desorption ionization mode the oxidation of the eumelanin precursor 5,6-dihydroxyindole (DHI) and the N-methyl derivative (DHI-N-Me) was monitored obtaining for the first time evidence for the formation of regular collections of oligomers of increasing masses, spanning the entire m/z ranges up to 5000 Da (>30-mer) for DHI and 8000 Da (> 50-mer) for DHI-NMe.
Thioflavin T, an extrinsic probe known to report on sheet structure, was used to investigate the structure of synthetic eumelanin generated by oxidation of DOPA thus overcoming the problem of the intrinsic fluorescence of the pigment (Sutter et al Appl. Phys Letts). The results suggest formation of a protomolecule and its assembly into a stacked sheet structure rather than a randomized heteropolymer formed by sequential monomer addition.
P. Meredith and his associates offer an interpretation of the electrical and photo-conductivity properties of melanins in the solid-state alternative to the well established amorphous semiconductor model. It is shown that the hydrated dielectric model, is an artifact related to measurement geometry and non-equilibrium behaviour, as it is often the case in the electric measurements on low conducibility disordered organic materials.
An interesting report on the ability of both eumelanin and pheomelanin of promoting DNA strand breaks in the dark appeared on Free Rad Biol Med (Suzukawa et al), with eumelanin being more active than pheomelanin. The extent of damage was enhanced by the ability of the pigment to bind to DNA minor groove, but was significantly reduced after irradiation or exposure to singlet oxygen possibly because of significant structural modifications. These results would be in line with previous data showing the light independent prooxidant property of pheomelanin.

Structure, Reactivity and Properties

- Bernardus Mostert A, Powell BJ, Gentle IR, Meredith P.
  On the origin of electrical conductivity in the bio-electronic material melanin. Applied Physics Letters, 100 (9) 93701/1-093701/3.

- Greco G,Panzella L,Napolitano A, d'Ischia M.
  The fundamental building blocks of red human hair pheomelanin are isoquinoline-containing dimers. Pigment Cell & Melanoma Research (2012),25(1),110-112.

- Martin-Sanchez P M, Sanchez-Cortes S, Lopez-Tobar E, Jurado V, Bastian F, Alabouvette C, Saiz-Jimenez C.
  The nature of black stains in Lascaux Cave, France, as revealed by surface-enhanced Raman spectroscopy. Journal of Raman Spectroscopy (2012),43(3),464-467.

- Reale S, Crucianelli M, Pezzela A, d'Ischia M, De Angelis F.
  Exploring the frontiers of synthetic eumelanin polymers by high-resolution matrix-assisted laser/desorption ionization mass spectrometry. Journal of Mass Spectrometry (2012) 47(1): 49-53.

- Sutter JU, Bidlakova T, Karolin J, Birch DJS.
  Eumelanin kinetics and sheet structure. Applied Physics Letters (2012), 100 (11): 113701/1-113701/4.

– Suzukawa AA, Vieira A, Winnischofer SMB, Scalfo AC, Di Mascio P, Ferreira da Costa AM, Ravanat J-L, Martins de Luna D, Rocha Merlin ME, Martinez GR.
Novel properties of melanins include promotion of DNA strand breaks, impairment of repair and reduced ability to damage DNA after quenching of singlet oxygen. Free radical biology & medicine (2012). Asap.

– Swalwell H, Latimer J, Haywood RM, Birch-Machin MA.
Investigating the role of melanin in UVA/UVB- and hydrogen peroxide-induced cellular and mitochondrial ROS production and mitochondrial DNA damage in human melanoma cells. Free Radical Biology & Medicine (2012) 52 (3): 626-634.

Melanin-analysis

– Commo S, Wakamatsu K, Lozano I, Panhard S, Loussouarn G, Bernard B A, Ito S.
Age-dependent changes in eumelanin composition in hairs of various ethnic origins. International Journal of Cosmetic Science (2012) 34(1): 102-107.

– Dzierzega-Lecznar A, Kurkiewicz S, Stepien K
Detection and quantitation of a pheomelanin component in melanin pigments using pyrolysis-gas chromatography/tandem mass spectrometry system with multiple reaction monitoring mode. Journal of Mass Spectrometry (2012) 47 (2) 242-245.

Melanogenesis and its Modulation

– Ha YM, Park YJ, Kim J-A, Park D, Park JY, Lee HJ, Lee JY, Moon HR, Chung HY.
Design and synthesis of 5-(substituted benzylidene)thiazolidine-2,4-dione derivatives as novel tyrosinase inhibitors. European Journal of Medicinal Chemistry (2012), 49, 245-252.

– Han Y K, Park YJ, Ha YM, Park D, Lee JY, Lee N, Yoon JH, Moon HR, Chung HY
Characterization of a novel tyrosinase inhibitor, (2RS,4R)-2-(2,4-dihydroxyphenyl)thiazolidine-4-carboxylic acid (MHY384). Biochimica et Biophysica Acta, General Subjects (2012), 1820(4), 542-549.

– Hosoya T, Nakata A, Yamasaki F, Abas F, Shaari K, Lajis NHj, Morita H.
Curcumin-like diarylpentanoid analogues as melanogenesis inhibitors. Journal of Natural Medicines (2012), 66(1), 166-176.

– Huang Y-C, Liu K-C, Chiou Y-L.
Melanogenesis of murine melanoma cells induced by hesperetin, a Citrus hydrolysate-derived flavonoid. Food and Chemical Toxicology (2012) , 50(3-4) : 653-659.

– Kang S-M, Heo S-J, Kim K-N, Lee S-H, Yang H-M, Kim A-D, Jeon Y-J.
Molecular docking studies of a phlorotannin, dieckol isolated from Ecklonia cava with tyrosinase inhibitory activity. Bioorganic & Medicinal Chemistry (2012), 20(1), 311-316.

– Masuda M, Itoh K, Murata K, Naruto S, Uwaya A, Isami F, Matsuda H.
Inhibitory effects of Morinda citrifolia extract and its constituents on melanogenesis in murine B16 melanoma cells. Biological & Pharmaceutical Bulletin (2012), 35(1), 78-83.

– Ohgidani M, Komizu Y, Goto K, Ueoka R.
Antimelanogenic and antioxidative effects of residual powders from Shochu distillation remnants. Food Chemistry (2012) 132 (4): 2140-2143.

– Satooka H, Kubo I.
Resveratrol as a kcat type inhibitor for tyrosinase: Potentiated melanogenesis inhibitor. Bioorganic & Medicinal Chemistry (2012), 20(2), 1090-1099.

– Tu C-X, Lin M, Lu S-S, Qi X-Y, Zhang R-X, Zhang Y-Y.
Curcumin Inhibits Melanogenesis in Human Melanocytes. Phytotherapy Research (2012), 26(2), 174-179.

– Yang Z, Zhang Y, Sun L, Wang Y, Gao X, Cheng Y.
An ultrafiltration high-performance liquid chromatography coupled with diode array detector and mass spectrometry approach for screening and characterising tyrosinase inhibitors from mulberry leaves. Analytica chimica acta (2012), 719, 87-95.

Plant and fungal pigments

– Araujo M, Xavier JR, Nunes CD, Vaz PD, Humanes M.
Marine sponge melanin: a new source of an old biopolymer. Structural Chemistry Volume23 (1) s115-122.

– Casadevall A, Nakouzi A, Crippa PR, Eisner M.
Fungal melanins differ in planar stacking distances. PLoS One (2012), 7(2), e30299.

– Dong C, Yao Y.
Isolation, characterization of melanin derived from Ophiocordyceps sinensis, an entomogenous fungus endemic to the Tibetan Plateau. Journal of bioscience and bioengineering (2012), 113(4): 474-9.

– Eisenman, Helene C.; Casadevall, Arturo.
Synthesis and assembly of fungal melanin. Applied Microbiology and Biotechnology (2012), 93(3), 931-940.

– Jalmi P, Bodke P, Wahidullah S, Raghukumar S.
The fungus Gliocephalotrichum simplex as a source of abundant, extracellular melanin for biotechnological applications. World Journal of Microbiology & Biotechnology (2012), 28(2), 505-512.

2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

Vitiligo is an acquired idiopathic pigmentary disorder of skin and hair characterized by well-circumscribed asymptomatic white macules. Vitiligo affects both melanocytes and keratinocytes causing degenerative changes. Psoralen ultraviolet A (PUVA) is an important modality in treating vitiligo but its effect on melanocytes and keratinocytes is not sufficiently studied. In this work, Anbar et al investigated 30 cases of non-segmental vitiligo regarding the changes of melanocytes and keratinocytes in both vitiliginous and nearby areas before and after PUVA therapy, using 3,4-dihydroxyphenylalanine (DOPA) reaction, immuno-histochemical stain and electron microscope. The authors find that PUVA increases the number of active epidermal melanocytes and recovers the melanocyte and keratinocyte degeneration in both the leucodermic and the apparently normal perilesional skin. The reversal of degeneration in all the tested areas after PUVA points towards the role of this modality in both repigmentation and protection against further depigmentation.

Recently, a new mechanism for the transfer of melanosomes was reported in which pigment globules containing multiple melanosomes are released into the extracellular space from melanocytes and are then ingested by keratinocytes In the study by Ando et al., the authors expand knowledge about a new pathway of melanosome transfer, in which pigment globules are released into the extracellular space from various areas of melanocyte dendrites. The melanosomes incorporated within these pigment globules are then dispersed into the cytosol of keratinocytes within a few days following the gradual degradation of the membrane that surrounds each melanosome cluster. Finally, the authors show that this process has a role in the distribution of melanosomes around the perinuclear areas of keratinocytes.

The melanocortin-1 receptor (MC1R) expressed by melanocytes is a key determinant of skin and hair colour phenotypes. The β-defensin 3 peptide was recently shown to be a novel MC1R ligand and regulator of pigment-type switching in both mice and dogs. Although there is as yet no demonstration of an equivalent role in human pigmentation, the work by Beaumont et al., provides in vitro evidence for MC1R-dependent β-defensin 3 (HBD3) activation of both MAPK and cAMP signalling pathways. Given that HBD3 is present at high levels in human skin, the authors propose that HBD3 may be a hitherto overlooked regulator of human melanocyte responses.

Pigmentation is induced by production of melanin in specialized organelles termed melanosomes and by transfer of these organelles from melanocytes to surrounding keratinocytes. The chemical basis of melanogenesis is relatively well known but the mechanism of melanosome transfer is not well studied. Various pigmentary disorders and cosmetic applications require the use of depigmenting agents. Most hypopigmenting agents are tyrosinase inhibitors and only a few agents are known to inhibit transfer of melanosomes. However, standardized methods to study melanosome transfer are not yet established. Choi et al. describe a simple method to study melanosome transfer by using flow cytometry.

The basic-helix-loop-helix-leucine zipper transcription factor MITF plays major roles in the development and physiology of vertebrate melanocytes and melanoma cells. It is regulated by post-translational modifications, including phosphorylation at serine-73, which based on in vitro experiments imparts on MITF an increased transcriptional activity paired with a decreased stability. Serine-73 is encoded by the alternatively spliced exon 2B, which is preferentially skipped in mice carrying a targeted serine-73-toalanine mutation. In a new study Debbache et al. measured the relative abundance of exon 2B+ and exon 2BRNAs in freshly isolated and FACS-sorted wild-type melanoblasts and melanocytes, and generated a series of knock-in mice allowing forced incorporation of either alanine, aspartate, or wild-type serine at position 73. None of these knock-in alleles, however, creates a striking pigmentation phenotype on its own, but differences between them can be revealed either by a general reduction of Mitf transcript levels or in heteroallelic combinations with extant Mitf mutations. In fact, compared with straight serine-73 knockin mice with their relative reduction of 2B+ Mitf, forced incorporation of alanine-73 leads to greater increases in MITF protein levels, melanoblast and melanocyte numbers, and extent of pigmentation in particular allelic combinations. These results underscore, in vivo, the importance of the link between alternative splicing and post-translational modifications and may bear on the recent observation that exon 2B skipping can be found in metastatic melanoma.

The repigmentation of adult skin after wound healing, or loss of melanocytes (e.g. vitiligo), poses a particular and unique challenge to the invading population of melanocytes. The melanocytes, or their precursors, resident in adjacent skin or in nearby niches, must be activated to proliferate, and then negotiate their way through a tightly held epidermal barrier. While there have been many studies on migratory cells during embryonic development and in particular physiological processes, the mechanisms whereby melanocytes negotiate their way through the epidermis to achieve this repigmentation have not been described in any detail. Further knowledge of these processes will certainly contribute towards elucidating the mechanisms of repigmentation in pigmentary disorders. In this study, through the use of both lateral and vertical/ transwell migration assays, Keswell et al investigate ways in which melanocytes might move through layers of keratinocytes. They propose a possible model that may describe the route of melanocyte migration. Upon stimulation by keratinocyte factors, the melanocyte dendrite extends into the spaces between the keratinocytes. The dendrite functions as an anchor by adhering to the keratinocytes via various adhesion molecules e.g. E-cadherin. Anchorage of the melanocyte dendrite to the keratinocytes allows for the melanocytes to be pulled through the small spaces between the keratinocytes. Once the entire cell has migrated to its destination, new intercellular connections can be established and pigment production and transfer can resume.

Melanin is produced in melanocytes and stored in melanosomes. In spite of its beneficial sun- protective effect, abnormal accumulation of melanin results in estheticproblems. Hydroquinone, competing with tyrosine, is a major ingredient in topical pharmacological agents. However, frequent adverse reactions are amongst its major limitation. To solve this problem, several alternatives such as arbutin, kojic acid, aloesin, and 4-n-butyl resorcinol have been developed. Kim et al. classify hypopigmenting agents according to their mechanism of action; a) regulation of enzyme, which is subdivided into three categories, i) regulation of transcription and maturation of tyrosinase, ii) inhibition of tyrosinase activity, and iii) post-transcriptional control of tyrosinase; b) inhibition of melanosome transfer, and c) additional mechanisms such as regulation of the melanocyte environment and antioxidant agents.

Melanocytes and keratinocytes are involved in skin photoprotection against the damaging effects of ultraviolet radiation (UV), through a complex cross-talk involving several cytokines and growth factors, produced both in the epidermal and in the dermal compartment. UV-induced melanogenesis is one of the most perceptible feature of melanocyte differentiation, with the production of melanins as photoprotective end products. Two types of melanin have been identified in the skin. Eumelanin pigments, prevalent in the skin of darkly pigmented individuals, act as a filter against UV and possess scavenger properties. Pheomelanins, predominantly present in subjects with pale skin and red or fair hair, fuction apparently as photosensitizers, increasing the production of reactive oxygen species (ROS) following UV. Although the protective role exerted by melanocytes is traditionally attributed to the ability to synthesize the eumelanin end products, several studies indicate an important role also for the pigment intermediates, formed along the entire biochemical pathway. In their study, Kovacs et al demonstrate the contribution of the eumelanin precursor DHICA to the photoprotection of the overall skin through its ability to act, not only as an eumelanin intermediate but also as a chemical messenger for the neighbouring keratinocytes, via paracrine interactions and melanosome transfer, aimed to increase the defences of the entire skin. In particular, the authors show the ability of DHICA to induce the antioxidant defence mechanisms and differentiation in keratinocytes, accompanied by a decrease in the damages and apoptosis following UV exposure. They propose that DHICA may have not only the function of a eumelanin protective building block but also that of a diffusible chemical mediator involved in the protective defences of the overall epidermis, especially under conditions characterized by increased melanogenesis such as inflammatory reactions and UV exposure. This work not only brings further insights into the complex paracrine interactions among epidermal cells but may also provide a possible explanation of the abnormal susceptibility of red-haired fair-complexioned individuals to sunburn and skin cancer, also due to the inability to produce and release DHICA as a crucial messenger involved in skin defences and protection.

Ultraviolet radiation from sunlight is an environmental factor that has a variety of physiological and biological effects, including immune suppression, cellular aging, DNA damage and initiation of apoptosis. In addition, UV radiation promotes the generation of reactive oxygen species (ROS) that can cause oxidative damage, and ultimately lead to tumor formation. Solar UV radiation is also a very prominent environmental toxic agent and it is known to be one of the main causes of human skin cancers, such as cutaneous, malignant melanomas and non-melanoma tumors. The review by López-Camarillo et al reviewed the specific signal transduction pathways and transcription factors involved in the cellular

response to UV-irradiation. In particular the authors discussed the role of p38, MAPK, JNK, ERK1/2, and ATM kinases and the participation of NF-κB, AP-1, and NRF2 transcription factors in the response network to UV exposure. Moreover the authors focused on the review of data emerging from the use of DNA microarray technology to determine changes in global gene expression in keratinocytes and melanocytes in response to UV treatment. Deciphering the complex interplay between signalling kinases and transcription factors effectors in the control of gene expression after UV radiation is imperative to develop novel therapeutic strategies to overcome skin damage. Progress in understanding the multitude of mechanisms induced by UV-exposure could lead to the identification and potential development of specific inhibitors for the prevention and control of skin photoaging and carcinogenesis.

A distinguished characteristic of human epidermis is the absence of vasculature that results in a constitutive low level of tissue oxygenation. A recent evaluation of the O2 tension in human skin showed that while the dermis is well oxygenated and vascularized displaying 10% O2, in the epidermis the O2 tension ranges from mildly hypoxic (1–5% O2) to severely hypoxic (< 1% O2) in certain skin appendages. This mild hypoxia is now seen as a new host microenvironmental factor capable of regulating key physiopathological processes of the epidermal cells. In this work, Nys et al investigated how reduced oxygen availability of the epidermis modulates the response of keratinocytes (NHKs) and melanocytes (NHEMs)to noxious ultraviolet B radiation (UVB). The authors demonstrate that the hypoxic microenvironment favors survival and reduces apoptosis of UVB-irradiated NHEMs and their malignant counterparts (melanoma cells). In contrast, the authors unravels that NHKs are sensitized to UVB-induced apoptosis when embedded in their natural mild hypoxic environment, through a redox mechanism enhancing the basal level of ROS in these cells. Under hypoxia, UVB-mediated p38MAPK/JNK activation is more sustained and promotes mitochondrial apoptosis through the engagement of Noxa and Bim proteins. This ROS mediated effect is specific for benign NHKs, thus suggesting that mild hypoxia may play a dual role in the epidermis: it functions to prevent photocarcinogenesis by enhancing the apoptotic removal of UVB-damaged keratinocytes while it may facilitate carcinogenesis when malignant transformation has occurred.

It is believed that the inherent differentiation program of melanocytes duringembryogenesis predisposes melanoma cells to high frequency of metastasis. Sox10, a transcription factor expressed in neural crest stem cells and a subset of progeny lineages, plays a key role in the development of melanocytes. Seong et al. show that B16F10 melanoma cells transfected with siRNAs specific for Sox10 display reduced migratory activity which in turn indicated that a subset of transcriptional regulatory target genes of Sox10 is likely to be involved in migration and metastasis of melanoma cells. We carried out a microarray-based gene expression profiling using a Sox10-specific siRNA to identify relevant regulatory targets and found that multiple genes including melanocortin-1 receptor (Mc1r) partake in the regulation of migration. The authors provide evidences that the effect of Sox10 on migration is mediated in large part by Mitf, a transcription factor downstream to Sox10. Among the mouse melanoma cell lines examined, however, only B16F10 showed robust down-regulation of Sox10 and inhibition of cell migration indicating that further dissection of dosage effects and/or cell line-specific regulatory networks is necessary. The involvement of Mc1r in migration was studied in detail in vivo using a murine metastasis model. Specifically, B16F10 melanoma cells treated with a specific siRNA showed reduced tendency in metastasizing to and colonizing the lung after being injected in the tail vein. These data reveal a cadre of novel regulators and mediators involved in migration and metastasis of melanoma cells that represents potential targets of therapeutic intervention.

The Melanocortin 1 receptor (MC1R) represents one out of a group of five G-protein coupled receptors (MC1R-MC5R) ubiquitously expressed in all cells of the skin (keratinocytes, melanocytes, fibroblasts, cells of the immune system). The MC1R exhibits several variants in form of single nucleotide polymorphisms (SNPs) that are known to differentially regulate melanocyte function. In fibroblasts, the MC1R has been shown to regulate fibroblast function by affecting the synthesis and degradation of collagen. In contrast to melanocytes, the impact of MC1R polymorphisms on receptor function in fibroblasts has not been investigated so far. The aim of the study by Stanisz et al was therefore to investigate the functional relevance of MC1Rwt compared with MC1R polymorphisms in dermal fibroblasts, and to relate the findings to melanocytes, which are well characterized concerning MC1R, as positive control. The authors find that fibroblasts, as well as melanocytes, show differences in MC1R function depending on the MC1R genotype. MC1R stimulation with α-MSH in wild type (MC1Rwt) melanocytes results in an increase of intracellular cAMP and cellular proliferation. In contrast, MC1Rwt fibroblasts react with a decrease of intracellular cAMP and proliferation. In MC1R polymorphic fibroblasts

(R163Q, R151C and V60L) both effects are significantly alleviated. Similar, but inverse effects could be found in MC1R polymorphic melanocytes (R142H and V92M) with a significantly lower cAMP increase and proliferation rate compared to MC1Rwt melanocytes. These results indicate that the MC1R displays reciprocal growth responses in melanocytes and fibroblasts, depending on the MC1R genotype. Thus, the MC1R seems to be not solely important for the skin pigmentary system, but also for the fibroblast function, and might influence different processes of the dermal compartment like wound healing, fibrosis and keloid formation.

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3. MSH, MCH, other hormones, differentiation

(Pr M. Böhm)

Human beta-defensin 3 - an emerging player in human skin pigmentation?

Melanin pigmentation of the skin is crucially regulated by the melanocortin-1 receptor (MC1R) and its ligand α-melanocyte-stimulating hormone (α-MSH). It was previously shown that a mutation in the canine *CBD103* gene, coding for β-defensin 103, results in black coat color of domestic dogs and the grey wolf (Anderson *et al.* Science 2009; 323: 1339-1343; Candille *et al.* Science 2007; 318: 1418-1423). CBD103 was shown to bind to canine and murine Mc1r but did not increase cAMP in mouse melanocytes. Until recently, the role of the human ortholog of CBD103, human β-defensin 3 (HBD3), remained unknown. In a recent study by Beaumont *et al.* (Pigm. Cell Melanoma Res. 2012, in press) the effect of HBD3 on intracellular cAMP and kinase signalling was investigated in detail employing a heterologous expression system of human embryonic kidney cells (HEK293) stably expressing MC1R or the vector control. Surprisingly, HBD3 exerted weak *agonistic* effects on intracellular cAMP signalling at 100 and 300 nM compared with NDP-α-MSH. On the other hand, HBD3 was a potent competitive *antagonist* to NDP-α-MSH although weaker compared with a synthetic peptide corresponding to the natural MC1R antagonist agouti signalling protein (ASIP). Interestingly, HBD3-induced activation of extracellular signal-regulated kinase-1/2 was maintained in HEK293 cells expressing a number of different MC1R variants. In summary, these findings suggest that HBD3 could be a novel player in human melanin pigmentation. It will be very interesting to investigate if normal human melanocytes expressing wild-type and loss of functional MC1R alleles respond to HBD3 in a similar fashion as demonstrated in this artificially gene-engineered heterologous system of HEK293 cells.

Chicken, colors and the role of agouti signaling protein

Feather pigmentation by chicken is regulated by the chicken *Extended black* locus which encodes the MC1R. Mutations of the MC1R are responsible for various pigmentation phenotypes in wild birds. It was recently shown by Yoshihara *et al.* (Gen. Comp. Endocrinol. 2011; 171: 46-51) that – in apparent anaology to the lower vertebrate and human hair follicle – chicken feather hair follicles express a localized MC1R agonist production machinery that includes adrenocorticotropin and α-MSH. In a recent follow-up paper by the same group (Yoshihara *et al.* Gen. Comp. Endocrinol. 2012, 175: 495-499) the presence of ASIP in chicken feather follicles was investigated by a detailed molecular biology and immunohistochemical approach. Using rapid amplification of cDNA 5´end (5´RACE) and 3´RACE it could be demonstrated that various ASIP mRNA variants generated by alternative splicing and promoters are expressed in chicken feather follicles. These mRNAs were expressed mainly in the feather pulp of the follicles along with ASIP immunostaining. Interestingly, ASIP immunoreactivity colocalized with pheomelanin but not with eumelanin in pulp areas that face developing barbs. These observations indicate that ASIP is present in the coat of chicken where it appears to regulate the elaborate color pattern of individual feathers.

Melanoma, obesity and the potential role of the melanocortin system

In a recent theoretical paper by Morpurgo *et al*. (Med. Hypotheses 2012; 78: 533-535) an interesting hypothesis was presented which is based on epidemiological observations pointing towards an increased incidence of melanoma in obese patients. In this context it is interesting that the central melanocortin system plays a crucial role in energy expenditure and metabolism (Cone. Nat. Neurosci. 2005; 8: 571-578). In the hypothalamus, α-MSH acts on MC4R expressing neurons to promote an anorexigenic response. This pathway can be antagonized by ASIP and the related agouti-related protein (AGRP), both natural MCR antagonists expressed within the brain. Importantly, this central melanocortin effector pathway is closely linked to hormonal signals derived from various peripheral organs and tissues including the stomach and adipose tissue. The latter produces a panel of hormone-like substances called adipokines including leptin, and possibly, ASIP as well (Klein *et al.*, Exp. Dermatol. 2007; 16: 45-70). Of note, patients with obesity do not have increased peripheral blood levels of leptin as expected due to leptin receptor insensitivity. In the cited paper by Morpurgo *et al*. it is suggested that obesity promotes an impaired functional state of the melanocyte α-MSH-MC1R/MC4R system due to increased secretion of some natural α-MSH antagonists and/or decreased receptor expression for α-MSH. This scenario may lead to reduced melanogenesis and impaired DNA repair upon sun exposure enhancing the overall risk for melanoma development. All in all, this viewpoint is certainly fun to read, with perhaps some ideas worth to be proven by appropriate experimental set-ups.

4. Photobiology

(Pr M-D Galibert)

- Giles N, Pavey S, Pinder A, Gabrielli B.
  Multiple melanoma susceptibility factors function in an ultraviolet radiation response pathway in skin. Br J Dermatol. 2012 Feb;166(2):362-71. doi: 10.1111/j.1365-2133.2011.10635.x. Epub 2011 Dec 5.
  Abstract :
  BACKGROUND:
  Exposure to ultraviolet radiation (UVR) and the familial melanoma susceptibility gene p16 (CDKN2A) are among the major risk factors which have been identified to contribute to the development of melanoma, and also significantly contribute to squamous cell carcinoma. We have previously shown that UVR induces p16(CDKN2A) expression in melanoma and keratinocyte cell lines and human skin, but the regulatory mechanisms controlling this expression are unknown.
  OBJECTIVES:
  To determine the mechanism by which UVR induces p16(CDKN2A) expression in melanocytes and keratinocytes in the epidermis.
  METHODS:
  We have used an in vitro cell lines model of the UVR response in skin to assess the changes in p16(CDKN2A) expression and the signalling pathways regulating these changes, and validated these findings in whole human skin cultures.
  RESULTS:
  We show that UVR-induced ERK signalling, mediated by BRAF, regulates p16(CDKN2A) expression at the transcriptional, and possibly translational level.
  CONCLUSIONS:
  This study demonstrates the biological connection between the known melanoma genes p16 (CDKN2A) and BRAF in a normal physiological response to UVR in the skin, and highlights the importance of defects in this biological pathway to melanoma and squamous cell carcinoma development.

- Ham SA, Hwang JS, Yoo T, Lee H, Kang ES, Park C, Oh JW, Lee HT, Min G, Kim JH, Seo HG.
  Ligand-activated PPARδ inhibits ultraviolet B-induced senescence of human keratinocytes via PTEN-mediated inhibition of superoxide production. Biochem J. 2012 Feb 16. [Epub ahead of print]
  Abstract : Ultraviolet (UV) radiation-mediated photodamage to the skin has been implicated in premature aging and photoaging-related skin cancer and melanoma. Little is known about the cellular events that underlie premature senescence, or how to impede these events. Here, we demonstrate that peroxisome proliferator-activated receptor (PPAR) δ regulates UVB-induced premature senescence of normal keratinocytes. Activation of PPARδ by GW501516, a specific ligand of PPARδ, significantly attenuated UVB-mediated generation of reactive oxygen species (ROS) and suppressed senescence of human keratinocytes. Ligand-activated PPARδ up-regulated the expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and suppressed the phosphatidylinositol-3 kinase (PI3K)/Akt pathway. Concomitantly, translocation of Rac1 to the plasma membrane, which leads to the activation of NADPH oxidases and generation of ROS, was significantly attenuated. siRNA-mediated knockdown of PTEN abrogated the effects of PPARδ on cellular senescence, on PI3K/Akt/Rac1 signaling, and on generation of ROS in keratinocytes exposed to UVB. Finally, when HR-1 hairless mice were treated with GW501516 before exposure to UVB, the number of senescent cells in the skin was significantly reduced. Thus, ligand-activated PPARδ confers resistance to UVB-induced cellular senescence by up-regulating PTEN and thereby modulating PI3K/Akt/Rac1 signaling to reduce ROS generation in keratinocytes.

- Jarrett SG, Novak M, Dabernat S, Daniel JY, Mellon I, Zhang Q, Harris N, Ciesielski MJ, Fenstermaker RA, Kovacic D, Slominski A, Kaetzel DM.
  Metastasis suppressor NM23-H1 promotes repair of UV-induced DNA damage and suppresses UV-induced melanomagenesis. Cancer Res. 2012 Jan 1;72(1):133-43. Epub 2011 Nov 11.

Abstract : Reduced expression of the metastasis suppressor NM23-H1 is associated with aggressive forms of multiple cancers. Here, we establish that NM23-H1 (termed H1 isoform in human, M1 in mouse) and two of its attendant enzymatic activities, the 3'-5' exonuclease and nucleoside diphosphate kinase, are novel participants in the cellular response to UV radiation (UVR)-induced DNA damage. NM23-H1 deficiency compromised the kinetics of repair for total DNA polymerase-blocking lesions and nucleotide excision repair of (6-4) photoproducts in vitro. Kinase activity of NM23-H1 was critical for rapid repair of both polychromatic UVB/UVA-induced (290-400 nm) and UVC-induced (254 nm) DNA damage, whereas its 3'-5' exonuclease activity was dominant in the suppression of UVR-induced mutagenesis. Consistent with its role in DNA repair, NM23-H1 rapidly translocated to sites of UVR-induced (6-4) photoproduct DNA damage in the nucleus. In addition, transgenic mice hemizygous-null for nm23-m1 and nm23-m2 exhibited UVR-induced melanoma and follicular infundibular cyst formation, and tumor-associated melanocytes displayed invasion into adjacent dermis, consistent with loss of invasion-suppressing activity of NM23 in vivo. Taken together, our data show a critical role for NM23 isoforms in limiting mutagenesis and suppressing UVR-induced melanomagenesis.

- Mouret S, Forestier A, Douki T.
  The specificity of UVA-induced DNA damage in human melanocytes. Photochem Photobiol Sci. 2012 Jan;11(1):155-62. Epub 2011 Oct 10.
  Abstract : Exposure to solar UV radiation is the origin of most skin cancers, including deadly melanomas. Melanomas are quite different from keratinocyte-derived tumours and exhibit a different mutation spectrum in the activated oncogenes, possibly arising from a different class of DNA damage. In addition, some data suggest a role for UVA radiation in melanomagenesis. To get further insight into the molecular mechanisms underlying induction of melanoma, we quantified a series of UV-induced DNA damage in primary cultures of normal human melanocytes. The results were compared with those obtained in keratinocytes from the same donors. In the UVB range, the frequency and the distribution of pyrimidine dimers was the same in melanocytes and keratinocytes. UVA was also found to produce thymine cyclobutane dimer as the major DNA lesion with an equal efficiency in both cell types. In contrast, following UVA-irradiation a large difference was found for the yield of 8-oxo-7,8-dihydroguanine; the level of this product was 2.2-fold higher in melanocytes than in keratinocytes. The comet assay showed that the induction of strand breaks was equally efficient in both cell types but that the yield of Fpg-sensitive sites was larger in melanocytes. Our data show that, upon UVA irradiation, oxidative lesions contribute to a larger extent to DNA damage in melanocytes than in keratinocytes. We also observed that the basal level of oxidative lesions was higher in the melanocytes, in agreement with a higher oxidative stress that may be due to the production of melanin. The bulk of these results, combined with qPCR and cell survival data, may explain some of the differences in mutation spectrum and target genes between melanomas and carcinomas arising from keratinocytes.

- Perez C, Parker-Thornburg J, Mikulec C, Kusewitt DF, Fischer SM, Digiovanni J, Conti CJ, Benavides F.
  SKHIN/Sprd, a new genetically defined inbred hairless mouse strain for UV-induced skin carcinogenesis studies. Exp Dermatol. 2012 Mar;21(3):217-20. doi: 10.1111/j.1600-0625.2011.01430.x.
  Abstract : Strains of mice vary in their susceptibility to ultra-violet (UV) radiation-induced skin tumors. Some strains of hairless mice (homozygous for the spontaneous Hr(hr) mutation) are particularly susceptible to these tumors. The skin tumors that develop in hairless mice resemble, both at the morphologic and molecular levels, UV-induced squamous cell carcinomas (SCC) and their precursors in human. The most commonly employed hairless mice belong to the SKH1 stock. However, these mice are outbred and their genetic background is not characterized, which makes them a poor model for genetic studies. We have developed a new inbred strain from outbred SKH1 mice that we named SKHIN/Sprd (now at generation F31). In order to characterize the genetic background of this new strain, we genotyped a cohort of mice at F30 with 92 microsatellites and 140 single nucleotide polymorphisms (SNP) evenly distributed throughout the mouse genome. We also exposed SKHIN/Sprd mice to chronic UV irradiation and showed that they are as susceptible to UV-induced skin carcinogenesis as outbred SKH1 mice. In addition, we proved that, albeit with low efficiency, inbred SKHIN/Sprd mice are suitable for transgenic production by classical pronuclear microinjection. This new inbred strain will be useful for the development of transgenic and congenic strains on a hairless inbred background as well as the establishment of syngeneic tumor cell lines. These new tools

can potentially help elucidate a number of features of the cutaneous response to UV irradiation in humans, including the effect of genetic background and modifier genes

- Sollberger G, Strittmatter GE, Kistowska M, French LE, Beer HD.
  Caspase-4 is required for activation of inflammasomes. J Immunol. 2012 Feb 15;188(4):1992-2000. Epub 2012 Jan 13.
  Abstract : IL-1β and IL-18 are crucial regulators of inflammation and immunity. Both cytokines are initially expressed as inactive precursors, which require processing by the protease caspase-1 for biological activity. Caspase-1 itself is activated in different innate immune complexes called inflammasomes. In addition, caspase-1 activity regulates unconventional protein secretion of many other proteins involved in inflammation and repair. Human caspase-4 is a poorly characterized member of the caspase family, which is supposed to be involved in endoplasmic reticulum stress-induced apoptosis. However, its gene is located on the same locus as the caspase-1 gene, which raises the possibility that caspase-4 plays a role in inflammation. In this study, we show that caspase-4 expression is required for UVB-induced activation of proIL-1β and for unconventional protein secretion by skin-derived keratinocytes. These processes require expression of the nucleotide-binding domain leucine-rich repeat containing, Pyrin domain containing-3 inflammasome, and caspase-4 physically interacts with its central molecule caspase-1. As the active site of caspase-4 is required for activation of caspase-1, the latter most likely represents a substrate of caspase-4. Caspase-4 expression is also essential for efficient nucleotide-binding domain leucine-rich repeat containing, Pyrin domain containing-3 and for absent in melanoma 2 inflammasome-dependent proIL-1β activation in macrophages. These results demonstrate an important role of caspase-4 in inflammation and innate immunity through activation of caspase-1. Therefore, caspase-4 represents a novel target for the treatment of (auto)inflammatory diseases.

- 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 Feb 9. doi: 10.1038/jid.2011.478. [Epub ahead of print]
  Abstract: 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.Journal of Investigative Dermatology advance online publication, 9 February 2012; doi:10.1038/jid.2011.478.

5. Neuromelanins

(Pr M. d'Ischia)

A most useful basic treatise illustrating current knowledge on Neuromelanin, its biological role and relation with Parkinson's disease has been provided by Double et al. (2011) in the book on Melanins and Melanosomes edited by Borovansky and Riley.
An additional link between neuromelanin and oxidative cell damage is provided by Elstner et al. (2011) who first provided evidence for higher levels of mitochondrial DNA deletions in pigmented dopaminergic neurons of the Substantia nigra (SN) relative to all other cells investigated, suggesting a possible key to the exceptional vulnerability of the nigro-striatal system in Parkinson Disease (PD) and aging. A role of autoimmune-based mechanisms in the etiopathogenesis of PD is proposed by Oberlaender et al. (2011) who showed that neuromelanin is recognized by dendritic cells inducing a T-cell response. It is noteworthy that the effect is elicited by human SN neuromelanin but not by synthetic dopamine melanin, an observation that raises issues concerning the validity of dopamine melanin as a biological model for human NM (see also conclusions by Li et al., 2011). On this basis NM is suggested to serve as an initial trigger for an autoimmune response in susceptible individuals. The potential of antibodies against NM as biomarker candidates for PD is suggested by Gerlach et al. (2012) as a focus for future studies.

- Double Kay L, Maruyama Wakako, Naoi Makoko, Gerlach Manfred, Riederer Peter.
  Biological role of neuromelanin in the human brain and its importance in Parkinson's disease.
  Melanins and Melanosomes 225-246, 2011.

- Elstner Matthias, Mueller Sarina K, Leidolt Lars, Laub Christoph, Krieg Lena, Schlaudraff Falk, Liss Birgit, Morris Chris, Turnbull Douglass M, Masliah Eliezer et al.
  Neuromelanin, neurotransmitter status and brainstem location determine the differential vulnerability of catecholaminergic neurons to mitochondrial DNA deletions. Molecular Brain 4, 43, 2011.
  Background: Deletions of the mitochondrial DNA (mtDNA) accumulate to high levels in dopaminergic neurons of the substantia nigra pars compacta (SNc) in normal aging and in patients with Parkinson's disease (PD). Human nigral neurons characteristically contain the pigment neuromelanin (NM), which is believed to alter the cellular redox-status. The impact of neuronal pigmentation, neurotransmitter status and brainstem location on the susceptibility to mtDNA damage remains unclear. We quantified mtDNA deletions (ΔmtDNA) in single pigmented and non-pigmented catecholaminergic, as well as non-catecholaminergic neurons of the human SNc, the ventral tegmental area (VTA) and the locus coeruleus (LC), using laser capture microdissection and single-cell real-time PCR. Results: In healthy aged individuals, ΔmtDNA levels were highest in pigmented catecholaminergic neurons (25.2 ± 14.9 %), followed by non-pigmented catecholaminergic (18.0 ± 11.2 %) and non-catecholaminergic neurons (12.3 ± 12.3 %; $p < 0.001$). Within the catecholaminergic population, ΔmtDNA levels were highest in dopaminergic neurons of the SNc (33.9 ± 21.6 %) followed by dopaminergic neurons of the VTA (21.9 ± 12.3 %) and noradrenergic neurons of the LC (11.1 ± 11.4 %; $p < 0.001$). In PD patients, there was a trend to an elevated mutation load in surviving non-pigmented nigral neurons (27.13 ± 16.73) compared to age-matched controls (19.15 ± 11.06; $p = 0.052$), but levels where similar in pigmented nigral neurons of PD patients (41.62 ± 19.61) and controls (41.80 ± 22.62). Conclusions: Catecholaminergic brainstem neurons are differentially susceptible to mtDNA damage. Pigmented dopaminergic neurons of the SNc show the highest ΔmtDNA levels, possibly explaining the exceptional vulnerability of the nigro-striatal system in PD and aging. Although loss of pigmented noradrenergic LC neurons also is an early feature of PD pathol., mtDNA levels are not elevated in this nucleus in healthy controls. Thus, ΔmtDNA are neither an inevitable consequence of catecholamine metab. nor a universal explanation for the regional vulnerability seen in PD.

- Gerlach Manfred, Maetzler Walter, Broich Karl, Hampel Harald, Rems Lucas, Reum Torsten, Riederer Peter, Stoeffler Albrecht, Streffer Johannes, Berg Daniela.
  Biomarker candidates of neurodegeneration in Parkinson's disease for the evaluation of disease-modifying therapeutics. From Journal of Neural Transmission 119(1), 39-52, 2012.
  Reliable biomarkers that can be used for early diagnosis and tracking disease progression are the cornerstone of the development of disease-modifying treatments for Parkinson's disease (PD). The German Society of Exptl. and Clin. Neurotherapeutics (GESENT) has convened a Working Group to review the current status of proposed biomarkers of neurodegeneration according to the following criteria and to

develop a consensus statement on biomarker candidates for evaluation of disease-modifying therapeutics in PD. The criteria proposed are that the biomarker should be linked to fundamental features of PD neuropathol. and mechanisms underlying neurodegeneration in PD, should be correlated to disease progression assessed by clin. rating scales, should monitor the actual disease status, should be pre-clin. validated, and confirmed by at least two independent studies conducted by qualified investigators with the results published in peer-reviewed journals. To date, available data have not yet revealed one reliable biomarker to detect early neurodegeneration in PD and to detect and monitor effects of drug candidates on the disease process, but some promising biomarker candidates, such as antibodies against neuromelanin, pathol. forms of α-synuclein, DJ-1, and patterns of gene expression, metabolomic and protein profiling exist. Almost all of the biomarker candidates were not investigated in relation to effects of treatment, validated in exptl. models of PD and confirmed in independent studies.

- Li Jie, Zhao Peng, Yang Junfeng, Zhang Renyun, Li Shen, Liu Dan.
  Oxidative stress regulated heme-oxygenase-1 and glutathione S-transferase-m1 gene expression changes in cell lines exposed to melanins. Neural Regeneration Research 6(34),2661-2665, 2011.
  To investigate the effects of oxidative stress on substantia nigra neuronal degeneration and death in patients with Parkinson's disease, we treated neuroblastoma cells (SK-N-SH) and glioma cells with Fenton's reagent, iron chelating agent, neuromelanin and dopamine melanin. We investigated the changes in expression of nine oxidative stress-related genes and proteins. The levels of mRNAs for heme-oxygenase-1 and glutathione S-transferase-m1 were significantly reduced in SK-N-SH cells exposed to oxidative stress, and increased in glial cells treated with deferoxamine. These results revealed that SK-N-SH neurons react sensitively to oxidative stress, which implies different outcomes between these two types of cells in the substantia nigra. Moreover, the influences of neuromelanin and dopamine melanin on cell function are varied, and dopamine melanin is not a good model for neuromelanin.

- Oberlaender Uwe, Pletinckx Katrien, Doehler Anja, Mueller Nora, Lutz Manfred B, Arzberger Thomas, Riederer Peter, Gerlach Manfred, Koutsilieri Eleni, Scheller Carsten.
  Neuromelanin is an immune stimulator for dendritic cells in vitro. BMC Neuroscience 12,116, 2011.
  Background: Parkinson's disease (PD) is characterized at the cellular level by a destruction of neuromelanin (NM)-contg. dopaminergic cells and a profound redn. in striatal dopamine. It has been shown recently that anti-melanin antibodies are increased in sera of Parkinson patients, suggesting that NM may act as an autoantigen. In this study we tested whether NM is being recognized by dendritic cells (DCs), the major cell type for inducing T- and B-cell responses in vivo. This recognition of NM by DCs is a prerequisite to trigger an adaptive autoimmune response directed against NM-assocd. structures. Results: Murine DCs were treated with NM of substantia nigra (SN) from human subjects or with synthetic dopamine melanin (DAM). DCs effectively phagocytized NM and subsequently developed a mature phenotype (CD86high/MHCIIhigh). NM-activated DCs secreted the proinflammatory cytokines IL-6 and TNF-α. In addn., they potently triggered T cell proliferation in a mixed lymphocyte reaction, showing that DC activation was functional to induce a primary T cell response. In contrast, DAM, which lacks the protein and lipid components of NM but mimics the dopamine-melanin backbone of NM, had only very little effect on DC phenotype and function. Conclusions: NM is recognized by DCs in vitro and triggers their maturation. If operative in vivo, this would allow the DC-mediated transport and presentation of SN antigens to the adaptive immune system, leading to autoimmmunity in susceptible individuals. Our data provide a rationale for an autoimmune-based pathomechanism of PD with NM as the initial trigger.

6. Genetics, molecular and developmental biology

(Dr. L. Montoliu)

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General strategy to analyse coat colour phenotypes in mice.
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Wnt3a promotes melanin synthesis of mouse hair follicle melanocytes.
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Mutation spectrum of the TYR and SLC45A2 genes in patients with oculocutaneous albinism.
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Regulation of the human tyrosinase gene in retinal pigment epithelium cells: the significance of transcription factor orthodenticle homeobox 2 and its polymorphic binding site.
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7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borron)

- Chen GH, Chen WM, Huang YC, Jiang ST.
  Expression of Recombinant Mature Human Tyrosinase from Escherichia coli and Exhibition of Its Activity without Phosphorylation or Glycosylation. J Agric Food Chem. 2012 Mar 21;60(11):2838-43. Epub 2012 Mar 1.
  A cDNA encoding mature human tyrosinase was cloned into pET-23a(+) and transformed into E. coli BL21(DE3). Three major recombinant proteins, mature human tyrosinase (RHT(20-531)), N-terminal truncated human tyrosinase (RHT(168-531)), and β-lactamase, were overexpressed as inclusion bodies in E. coli after 12 h of induction with 1.0 mM isopropyl-β-d-thiogalactopyranoside at 37 °C. After sonication and centrifugation, the inclusion body was harvested, solubilized, dialyzed, and refolded into the active form with monophenolase and diphenolase activities. It was purified to homogeneity by DEAE-Sepharose FF and Sephadex G-75. The molecular mass and N-terminal sequence were 57.0 kDa and GHFPRAC, respectively, and corresponded to those of mature human tyrosinase. The RHT was active in a broad range of temperature and pH, and with optimum activity at 70 °C and pH 8.5.

- Chen W, Wang H, Dong B, Dong Z, Zhou F, Fu Y, Zeng Y.
  Molecular cloning and expression analysis of tyrosinase gene in the skin of Jining gray goat (Capra hircus). Mol Cell Biochem. 2012 Mar 10.
  Tyrosinase is the key regulatory enzyme of melanogenesis and plays a major role in mammal coat color. For the first time, we have sequenced and characterized the tyrosinase (TYR) of Jining Gray Goat (Capra hircus), which is the world-famous fur-bearing animal with its special color and pattern. The full-length cDNA was cloned by a reverse-transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA end (RACE) method. As a result, one 2131-bp nucleotide sequence representing the full-length cDNA of TYR was obtained. The entire open reading frame (ORF) of the TYR is 1593 bp and encodes for 530 amino acids, which is well conserved compared with TYR of various species with higher degree of sequence similarity with other mammalian (74-99 %) than amphibian, aves, and fishes (56-73 %). The deduced amino acids contained one signal peptide, one transmembrane domain, five N-linked glycosylation sites, and two copper binding sites. The result of real-time quantitative PCR showed that the expression level of TYR was the highest in the dark-gray goats and the lowest in the light-gray ones, while the goats of dark-gray individuals have more than 50 % black fiber and light-gray ones less than 30 %. During the whole life of Jining gray goat, TYR expression level changes with certain regularity and their coat color will change correspondingly by investigating the expression level in ten development stages. After comparing the result and the coat phenotype, we presume that it seems to have a positive relationship between the color depth of coat and the expression level of TYR.

- Citek C, Lyons CT, Wasinger EC, Stack TD.
  Self-assembly of the oxy-tyrosinase core and the fundamental components of phenolic hydroxylation. Nat Chem. 2012 Mar 4;4(4):317-22. doi: 10.1038/nchem.1284.
  The enzyme tyrosinase contains two Cu(I) centres, trigonally coordinated by imidazole nitrogens of six conserved histidine residues. The enzyme activates O(2) to form a μ-η(2):η(2)-peroxo-dicopper(II) core, which hydroxylates tyrosine to a catechol in the first committed step of melanin biosynthesis. Here, we report a family of synthetic peroxo complexes, with spectroscopic and chemical features consistent with those of oxygenated tyrosinase, formed through the self-assembly of monodentate imidazole ligands, Cu(I) and O(2) at -125 °C. An extensively studied complex reproduces the enzymatic electrophilic oxidation of exogenous phenolic substrates to catechols in good stoichiometric yields. The self-assembly and subsequent reactivity support the intrinsic stability of the Cu(2)O(2) core with imidazole ligation, in the absence of a polypeptide framework, and the innate capacity to effect hydroxylation of phenolic substrates. These observations suggest that a foundational role of the protein matrix is to facilitate expression of properties native to the core by bearing the entropic costs of assembly and precluding undesired oxidative degradation pathways.

- Dong D, Jiang M, Xu X, Guan M, Wu J, Chen Q, Xiang L.

The effects of NB-UVB on the hair follicle-derived neural crest stem cells differentiating into melanocyte lineage in vitro. J Dermatol Sci. 2012 Apr;66(1):20-8. Epub 2012 Feb 16.
BACKGROUND: Narrow-band UVB (NB-UVB) is an effective therapeutic option in the treatment of vitiligo. Despite the apparent clinical efficacy, the underlying mechanism of how topical NB-UVB induces repigmentation in vitiligo has not been clearly elucidated. OBJECTIVES: To investigate the effects of NB-UVB on the maturation of melanocyte lineage differentiated from hair follicle-derived neural crest stem cells (HF-NCSCs) in vitro. METHODS: HF-NCSCs were isolated from mouse whisker follicles. The isolated cells were multipotent and expressed embryonic NCSC biomarkers. The effects of NB-UVB on development and differentiation of HF-NCSCs were evaluated. We assessed cell viability, melanogenesis and migration of melanocytes derived from HF-NCSCs after NB-UVB radiation. Tyrosinase, Tyrp1, Dct, Kit, Mc1R, Fzd4, NT3R, Ednra, EP1, TGFβR, Sox10, Mitf, Lef1 and Pax3 gene expression was measured by quantitative RT-PCR, while Tyrosinase, Sox10 and Mitf protein expression were measured by Western blot analysis. Cell migration was measured by Boyden chamber transwell assay. RESULTS: NB-UVB increased the expression of tyrosinase during melanocytic differentiation from mouse HF-NCSCs, however, NB-UVB inhibited proliferation of melanocytes derived from HF-NCSCs. Mechanistically, increased melanocyte maturation after NB-UVB treatment was resulted from increased expression of several key melanogenic factors, including Sox10, Kit and Mc1R, which play a critical role to promote tyrosinase expression. Furthermore, the migration of the HF-NCSCs-derived melanocytes was downregulated as NB-UVB doses increased. However, the migration of HF-NCSCs was upregulated under 0.4J NB-UVB radiation. CONCLUSIONS: Those data provide in vitro evidence demonstrating some direct effects of NB-UVB on pigmentation of melanocyte lineage differentiated from HF-NCSCs, and may provide a possible mechanism for the effect of NB-UVB in vitiligo.

- Fujieda N, Murata M, Yabuta S, Ikeda T, Shimokawa C, Nakamura Y, Hata Y, Itoh S.
Multifunctions of MelB, a fungal tyrosinase from Aspergillus oryzae. Chembiochem. 2012 Jan 23;13(2):193-201. doi: 10.1002/cbic.201100609. Epub 2011 Dec 30.
The pro form of melB tyrosinase from the melB gene of Aspergillus oryzae was over-produced from E. coli and formed a homodimer that exhibited the spectral features of met-tyrosinase. In the presence of NH(2)OH (reductant), the proenzyme bound dioxygen to give a stable (μ-η(2):η(2) -peroxo)dicopper(II) species (oxy form), thus indicating that the pro form tyrosinase can function as an oxygen carrier or storage protein like hemocyanin. The pro form tyrosinase itself showed no catalytic activity toward external substrates, but proteolytic digestion with trypsin activated it to induce tyrosinase activity. Mass spectroscopy analyses, mutagenesis experiments, and colorimetry assays have demonstrated that the tryptic digestion induced cleavage of the C-terminal domain (Glu458-Ala616), although the dimeric structure of the enzyme was retained. The structural changes induced by proteolytic digestion might open the entrance to the enzyme active site for substrate incorporation.

- Gasparetti C, Nordlund E, Jänis J, Buchert J, Kruus K.
Extracellular tyrosinase from the fungus Trichoderma reesei shows product inhibition and different inhibition mechanism from the intracellular tyrosinase from Agaricus bisporus. Biochim Biophys Acta. 2012 Apr;1824(4):598-607. Epub 2012 Jan 14.
Tyrosinase (EC 1.14.18.1) is a widely distributed type 3 copper enzyme participating in essential biological functions. Tyrosinases are potential biotools as biosensors or protein crosslinkers. Understanding the reaction mechanism of tyrosinases is fundamental for developing tyrosinase-based applications. The reaction mechanisms of tyrosinases from Trichoderma reesei (TrT) and Agaricus bisporus (AbT) were analyzed using three diphenolic substrates: caffeic acid, L-DOPA (3,4-dihydroxy-l-phenylalanine), and catechol. With caffeic acid the oxidation rates of TrT and AbT were comparable; whereas with L-DOPA or catechol a fast decrease in the oxidation rates was observed in the TrT-catalyzed reactions only, suggesting end product inhibition of TrT. Dopachrome was the only reaction end product formed by TrT- or AbT-catalyzed oxidation of L-DOPA. We produced dopachrome by AbT-catalyzed oxidation of L-DOPA and analyzed the TrT end product (i.e. dopachrome) inhibition by oxygen consumption measurement. In the presence of 1.5mM dopachrome the oxygen consumption rate of TrT on 8mM L-DOPA was halved. The type of inhibition of potential inhibitors for TrT was studied using p-coumaric acid (monophenol) and caffeic acid (diphenol) as substrates. The strongest inhibitors were potassium cyanide for the TrT-monophenolase activity, and kojic acid for the TrT-diphenolase activity. The lag period related to the TrT-catalyzed oxidation of monophenol was prolonged by kojic acid, sodium azide and arbutin; contrary it was reduced by potassium cyanide.

Furthermore, sodium azide slowed down the initial oxidation rate of TrT- and AbT-catalyzed oxidation of L-DOPA or catechol, but it also formed adducts with the reaction end products, i.e., dopachrome and o-benzoquinone.

– González AG, Ureña AG, Lewis RJ, van der Zwan G.
Spectroscopy and Kinetics of Tyrosinase Catalyzed trans-Resveratrol Oxidation. J Phys Chem B. 2012 Mar 1;116(8):2553-60. Epub 2012 Feb 17.
The spectroscopy and kinetics of the tyrosinase catalyzed trans-resveratrol oxidation were investigated by measuring both UV-vis absorption spectra over the 200-500 nm range and Raman spectra over the 600-1800 cm(-1) region. Room temperature UV-vis absorption spectra, as a function of time, showed the presence of two isosbestic points located at λ(1) = 270 nm and λ(2) = 345.5 nm delimiting two different regions: the reactant region around 300 nm, where the absorption decreased with time, and the product region over the low wavelength (λ < 260 nm) and high wavelength (λ > 390 nm) wavelength zone in which the absorption increased with time until, in both cases, constant values were achieved. A first-order kinetics was deduced with a rate coefficient of k(1) = (0.10 ± 0.001) min(-1), which turned out to be independent of substrate concentration over the 50-5 μM range; a feature that was rationalized by invoking the limiting case of the Michaelis-Menten scheme appropriate for substrate concentration much lower than the respective Michaelis constant. The observation of the distinct resonance enhanced Raman lines, specifically those peaking at 830 cm(-1), 753 cm(-1), and 642 cm(-1) together with their time evolution, permitted us to gain insight into some crucial features and steps of the catalytic reaction. Namely, that the formation of the so-called trans-resveratrol and tyrosinase (S)P complex with its O-O bridge plays a crucial role in the first steps of this enzymatic reaction and that the hydroxylation of the ortho C-H bond of the trans-resveratrol OH group occurs after O-O bond cleavage in the tyrosinase active site. The present study makes clear that a class of potential inhibitors of tyrosinase can be found in compounds able to bind the two Cu (II) ions of the enzyme bidentate form.

– Khan MT.
Novel Tyrosinase Inhibitors From Natural Resources - Their Computational Studies. Curr Med Chem. 2012 Mar 13. [Epub ahead of print]
Tyrosinase is a multi-copper enzyme widely distributed in different organisms, including plants & mammals, etc., which is responsible for pigmentations, undesired browning of fruits and vegetables. This is the key enzyme in the melanogenesis in human and molting process of insects. Therefore the inhibitors of the enzyme may lead to novel skin whitening agents, anti-browning substances or compounds for insect control. A large numbers of moderate to potent tyrosinase inhibitors have been reported during the last decade. From our group, we reported a number of potent inhibitors from synthetic, semi-synthetic and natural origins. The compounds are from several chemical classes, like phenolics, terpenes, steroids, chalcones, flavonoids, alkaloids, long-chain fatty acids, coumarins, sildenafil analogs, bipiperidines, biscoumarins, oxadiazole, tetraketones, etc. More recently, the crystal structure of mushroom and couple of other tyrosinases has been published and more recently the crystal structure of mushroom tyrosinase complexes with a highly potent inhibitor tropolone has been reported. Yet there is a lack of information of inhibitor-tyrosinase intermolecular interactions. To overcome such issues, some researchers started utilizing in silico tools, like molecular docking simulations, for such purposes. There are also few papers published about the successful utilization of computational tools like QSAR-based and ligand-based virtual screening to identify novel and potent inhibitors of the enzyme. In our group, we are using all possible computational tools, like ligand-based as well structure-based approaches, to identify new inhibitors. In this review, some of such examples are briefly described.

– 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 Mar 6. [Epub ahead of print]
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 dopachrome tautomerase (DPI) and was insensitive to antagonists of other ROS-producing enzyme systems including mitochondrial enzymes, cycloxygenase, 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.

- Marini A, Farwick M, Grether-Beck S, Brenden H, Felsner I, Jaenicke T, Weber M, Schild J, Maczkiewitz U, Köhler T, Bonfigli A, Pagani V, Krutmann J.
  Modulation of skin pigmentation by the tetrapeptide PKEK: in vitro and in vivo evidence for skin whitening effects. Exp Dermatol. 2012 Feb;21(2):140-6. doi: 10.1111/j.1600-0625.2011.01415.x. Epub 2011 Dec 6.
  Uneven skin pigmentation is a significant cosmetic concern, and the identification of topically applicable molecules to address this issue is of general interest. We report that the tetrapeptide PKEK (Pro-Lys-Glu-Lys) can exert skin whitening effects based on one in vitro and four double-blinded vehicle-controlled in vivo studies. (i) Treatment of human keratinocytes with PKEK significantly reduced UVB-stimulated mRNA expression of interleukin (IL)-6, IL-8 and TNF-α and, most importantly, proopiomelanocorticotropin (POMC), i.e. a gene encoding the pigmentation-inducing soluble mediator α- (α-MSH). (ii) PKEK treatment significantly inhibited UVB-induced upregulation of genes encoding for IL-1α, IL-6, IL-8, TNF-α as well as POMC and tyrosinase in 10 healthy volunteers pretreated with PKEK for 4 weeks once daily. (iii) In a study enrolling 39 Caucasian women, facial pigment spots significantly faded after 6 weeks when PKEK was combined with the skin whitener sodium ascorbyl phosphate (SAP), whereas PKEK or SAP alone led to less pronounced fading of the pigment spots. (iv) Addition of PKEK enhanced the skin whitening potency of a SAP-containing preparation if applied for 8 weeks to the back of hands of 19 Caucasians. (v) 27 Japanese women were treated on their faces twice daily with an SAP only or a PKEK+SAP-containing formulation for 8 weeks. Application of PKEK+SAP significantly reduced skin pigmentation by 26% and by 18% according to SCINEXA score. We demonstrate that PKEK has the capacity to reduce UVB-induced skin pigmentation and may be suited to serve as a skin tone-modulating agent in cosmetic products.

- Muñoz-Muñoz JL, Garcia-Molina F, Berna J, Garcia-Ruiz PA, Varon R, Tudela J, Rodriguez-Lopez JN, Garcia-Canovas F.
  Kinetic characterisation of o-aminophenols and aromatic o-diamines as suicide substrates of tyrosinase. Biochim Biophys Acta. 2012 Apr;1824(4):647-55. Epub 2012 Feb 10.
  We study the suicide inactivation of tyrosinase acting on o-aminophenols and aromatic o-diamines and compare the results with those obtained for the corresponding o-diphenols. The catalytic constants follow the order aromatic o-diamines<o-aminophenols<o-diphenols, which agrees with the view that the transfer of the proton to the peroxide group of the oxy-tyrosinase form is the slowest step in the catalytic cycle. As regards the apparent inactivation constant, it remains within the same order of magnitude, although slightly lower in the case of the aromatic o-diamines and o-aminophenols than o-diphenols: o-diamines<o-aminophenols<o-diphenols. The efficiency of the second nucleophilic attack of substrate on CuA seems to be the determining factor in the bifurcation of the inactivation and catalytic pathways. This attack is more efficient in o-diamines (where it attacks a nitrogen atom) than in o-aminophenols and o-diphenols (where it attacks an oxygen atom), favouring the catalytic pathway and slowing down the inactivation pathway. The inactivation step is the slowest of the whole process. The values of r, the number of turnovers that 1mol of enzyme carries out before being inactivated, follows the order aromatic o-diamines<o-aminophenols<o-diphenols. As regards the Michaelis constants, that of the o-diamines is slightly lower than that of the o-diphenols, while that of the o-aminophenols is slightly greater than that observed for the o-diphenols. As a consequence of the above,

the inactivation efficiency, λ(max)/K(m)(S), follows this order: o-diphenols>o-aminophenols>aromatic o-diamines.

- Osório RE, Peralta RA, Bortoluzzi AJ, de Almeida VR, Szpoganicz B, Fischer FL, Terenzi H, Mangrich A, Mantovani K, Ferreira D, Rocha WR, Haase W, Tomkowicz Z, dos Anjos A, Neves A.
  Synthesis, magnetostructural correlation, and catalytic promiscuity of unsymmetric dinuclear copper(II) complexes: models for catechol oxidases and hydrolases. Inorg Chem. 2012 Feb 6;51(3):1569-89. Epub 2012 Jan 19.
  Herein, we report the synthesis and characterization, through elemental analysis, electronic spectroscopy, electrochemistry, potentiometric titration, electron paramagnetic resonance, and magnetochemistry, of two dinuclear copper(II) complexes, using the unsymmetrical ligands N',N',N-tris(2-pyridylmethyl)-N-(2-hydroxy-3,5-di-tert-butylbenzyl)-1,3-propanediamin-2-ol (L1) and N',N'-bis(2-pyridylmethyl)-N,N-(2-hydroxybenzyl)(2-hydroxy-3,5-di-tert-butylbenzyl)-1,3-propanediamin-2-ol (L2). The structures of the complexes [Cu(2)(L1)(μ-OAc)](ClO(4))(2)•(CH(3))(2)CHOH (1) and [Cu(2)(L2)(μ-OAc)](ClO(4))•H(2)O•(CH(3))(2)CHOH (2) were determined by X-ray crystallography. The complex [Cu(2)(L3)(μ-OAc)](2+) [3; L3 = N-(2-hydroxybenzyl)-N',N',N-tris(2-pyridylmethyl)-1,3-propanediamin-2-ol] was included in this study for comparison purposes only (Neves et al. Inorg. Chim. Acta2005, 358, 1807-1822). Magnetic data show that the Cu(II) centers in 1 and 2 are antiferromagnetically coupled and that the difference in the exchange coupling J found for these complexes (J = -4.3 cm(-1) for 1 and J = -40.0 cm(-1) for 2) is a function of the Cu-O-Cu bridging angle. In addition, 1 and 2 were tested as catalysts in the oxidation of the model substrate 3,5-di-tert-butylcatechol and can be considered as functional models for catechol oxidase. Because these complexes possess labile sites in their structures and in solution they have a potential nucleophile constituted by a terminal Cu(II)-bound hydroxo group, their activity toward hydrolysis of the model substrate 2,4-bis(dinitrophenyl)phosphate and DNA was also investigated. Double electrophilic activation of the phosphodiester by monodentate coordination to the Cu(II) center that contains the phenol group with tert-butyl substituents and hydrogen bonding of the protonated phenol with the phosphate O atom are proposed to increase the hydrolase activity (K(ass.) and k(cat.)) of 1 and 2 in comparison with that found for complex 3. In fact, complexes 1 and 2 show both oxidoreductase and hydrolase/nuclease activities and can thus be regarded as man-made models for studying catalytic promiscuity.

- Pucci M, Pasquariello N, Battista N, Di Tommaso M, Rapino C, Fezza F, Zuccolo M, Jourdain R, Finazzi-Agro A, Breton L, Maccarrone M.
  Endocannabinoids stimulate human melanogenesis via type-1 cannabinoid receptor. J Biol Chem. 2012 Mar 19. [Epub ahead of print]
  We show that a fully functional endocannabinoid system is present in primary human melanocytes (NHEM cells), including anandamide (AEA), 2-arachidonoylglycerol, the respective target receptors (CB1, CB2 and TRPV1) and their metabolic enzymes. We also show that at higher concentrations AEA induces NHEM cell apoptosis (~3-fold over controls at 5 microM), through a TRPV1-mediated pathway that increases DNA fragmentation and p53 expression. Instead, at lower concentrations AEA and other CB1-binding endocannabinoids dose-dependently stimulate melanin synthesis and enhance tyrosinase gene expression and activity (~3-fold and ~2-fold over controls at 1 microM). This CB1-dependent activity was fully abolished by the selective CB1 antagonist SR141716 or by RNA interference of the receptor. CB1 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 (MITF). Silencing of tyrosinase or MITF further demonstrated the involvement of these proteins in AEA-induced melanogenesis. In addition, CB1 activation did not engage the key-regulator of skin pigmentation cyclic AMP, showing a major difference compared to the regulation of melanogenesis by α-melanocyte-stimulating hormone through melanocortin 1 receptor.

- Shang Y, Duan Z, Huang W, Gao Q, Wang C.
  Improving UV resistance and virulence of Beauveria bassiana by genetic engineering with an exogenous tyrosinase gene. J Invertebr Pathol. 2012 Jan;109(1):105-9. Epub 2011 Oct 15.
  Insect pathogenic fungi like Beauveria bassiana have been developed as environmentally friendly biocontrol agents against arthropod pests. However, restrictive environmental factors, including solar

ultraviolet (UV) radiation frequently lead to inconsistent field performance. To improve resistance to UV damage, we used Agrobacterium-mediated transformation to engineer B. bassiana with an exogenous tyrosinase gene. The results showed that the mitotically stable transformants produced larger amounts of yellowish pigments than the wild-type strain, and these imparted significantly increased UV-resistance. The virulence of the transgenic isolate was also significantly increased against the silkworm Bombyx mori and the mealworm Tenebrio molitor. This study demonstrated that genetic engineering of B. bassiana with a tyrosinase gene is an effective way to improve fungal tolerance against UV damage.

- 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 Feb 9. doi: 10.1038/jid.2011.478. [Epub ahead of print]
  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.

- 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 Mar 22. doi: 10.1002/jcb.24147. [Epub ahead of print]
  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)

Recent **reviews** have covered various facets of melanosome research: regulation of melanogenesis (Otreba et al); formation and localization of melanin granules in the cell wall in fungi (Eisenman & Casadevall); role of RAB family GTPases and their effectors in melanosomal logistics (Ohbayashi & Fukuda); inhibition of melanosome transfer as a mechanism involved in hypopigmentation (Kim et al).

The largest group of research papers deals with the **transfer of melanosomes** from melanocyte to keratinocyte: transfer of melanosomes via shedding the
microvesicle system (Ando et al., Scott); a new assay method exploiting the flow cytometry for a quantitation of melanosome transfer (Choi et al); ebselen as a transfer inhibitor (Kasraee et al).
Melanosome transport: Melanoregulin regulates the retrograde melanosome transport (Ohbayashi et al).

Melanosomal biogenesis, degradation and constituents. Laubéry et al demonstrated that myosinVI does not participate in the actin-dependent melanosome movement but it does so in the biogenesis of melanosomes. The interaction of G□i3 with the Oa1 G-protein coupled receptors controls the size of melanosomes in the RPE. (Young et al). The function of MART-1 was studied in a knockout mouse model lacking the MART-1 by Aydin et al. In Long Evans rats fed a low zinc diet differences in Zn concentration were found between choroid and RPE melanosomes (Biesemeier at al, Julien et al) and giant melanosomes with a considerably increased copper level were noted in the choroid (Biesemeier at al). Physical properties of melanosomes can be influenced by the water content: the bovine choroid melanosomes dehydration decreased their absorption coefficient (Lin et al.). Degradation of melanosomes in the RPE , most probably induced by iron-mediated reactive oxygen species, was characterized by Wolkow et al thus confirming our hypothesis that the melanin moiety of the melanosome should be degraded by the redox rather than by hydrolytic mechanism – Borovanský et al./ Folia Biologica (Praha) 45: 47-52 ,1999.

Functions of melanosomes. The cytoprotective function of RPE melanosomes against the non-photic stress was elegantly proved by Burke et al. Further evidence for sequestration of chemotherapeutics in melanosomes was brought by Huang et al.- see also Chen et a./Proc Natl Acad Sci USA 103: 9903-9907, 2006.

Miscellaneous reports. Statistical analysis of the fossilized melanosomes morphology enabled to predict the colour of Archaeopterix feather (Carney et al). A clinicopathological study of pigmented basal cell carcinomas was performed by Kirzhner and Jakobiec.

- Ando H, Niki Y, Ito M, Akiyama K, Matsui MS, Yarosh DB, Ichihashi M.
  Melanosomes are transferred from melanocytes to keratinocytes through the processes of packaging, release, utake, and dispersion. J Invest Dermatol. 132(4):1222-1229, 2012.
  Melanosomes are transferred from melanocytes to keratinocytes via the shedding vesicle system. This packaging system generates pigment globules containing multiple melanosomes in a unique manner. The authors had previously described the involvement of pigment globules in the melanosome transfer in a separate paper - see Cell Logistics 1(1): 12-20, 2011 reported in ESPCR Bull. No70, p.2190. See also Scott below in the list.

- Aydin IT, Hummler E, Smit NP, Beermann F.
  Coat color dilution in mice because of inactivation of the melanoma antigen MART-1. Pigment Cell Melanoma Res. 25(1):37-46, 2012.
  In order to specify the function of MART-1 (melanoma antigen recognized by T cells 1) a new knockout mouse model lacking MART-1 was developed. The loss leads to a coat colour phenotype with a reduction in total melanin content in the skin and hair. The lack of MART-1 did not affect the localization of melanocyte-specific proteins nor the maturation of Pmel17.

- Biesemeier A, Julien S, Kokkinou D, Schraermeyer U, Eibl O.

A low zinc diet leads to loss of Zn in melanosomes of the RPE but not in melanosomes of the choroidal melanocytes. Metallomics 2012 Feb 13. [Epub ahead of print]
In Long Evans rats fed a low Zn diet differences were found in Zn concentration between the choroid melanosomes (no change) and the RPE melanosomes (decrease of Zn). Zn deficient rats produced giant melanosomes in the choroid (with a 6fold increase in Cu compared to the controls). Changes in the N mole fraction of giant melanosomes suggested changes in the melanosomal skeleton. The analyses were conducted using combined EDX (dispersive X-ray microanalysis) and EELS (electron energy loss spectroscopy).

- Burke JM, Kaczara P, Skumatz CM, Zareba M, Raciti MW, Sarna T.
Dynamic analyses reveal cytoprotection by RPE melanosomes against non-photic stress. Mol Vis.17:2864-2877, 2011.
ARPE-19 cells containing phagocytized melanosomes from RPE porcine eyes , or for a comparison phagocytized latex beads ,were exposed to oxidative stress. Cells containing melanosomes proved to be more resistant than those containing latex beads which directly suggests an antioxidant function of melanosomes within the RPE cells. (see also Zareba et al- Free Rad Biol Med 40: 87-100, 2006.)

- Carney RM, Vinther J, Shawkey MD, D´Alba l, Ackermann J.
New evidence on the colour and nature of the isolated Archaeopteryx feather. Nature Communications 3, article No.637, 2012.
The first evidence of colour from Archaeopteryx based on fossilized colour-imparting melanosomes discovered in this isolated feather specimen was reported by Carney et al. . . Using a phylogenetically diverse database of extant bird feathers, statistical analysis of melanosome morphology predicts that the original colour of this Archaeopteryx feather was black, with a 95% probability.

- Choi HR, Park SH, Choi JW, Kim DS.
A simple assay method for melanosome transfer. Ann Dermatol. 24(1): 90-94, 2012.
A simple method to study melanosome transfer from melanocytes to keratocytes using flow cytometry was developed.

- Eisenman HC, Casadevall A.
Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol. 93(3):931-940, 2012.
A minireview devoted to melanogenesis and melanin granule formation in fungi. The fungal melanin may be synthesized in internal vesicles akin to mammalian melanosomes and transported to the cell wall where melanin granules are likely crosslinked to polysaccharides.

- Huang ZM, Chinen M, Chang PJ, Xie T, Zhong L, Demetriou S, Patel MP, Scherzer R, Sviderskaya EV, Bennett DC, Millhauser GL, Oh DH, Cleaver JE, Wei ML.
Targeting protein-trafficking pathways alters melanoma treatment sensitivity. Proc Natl Acad Sci U S A. 109(2):553-558, 2012.
The sensitivity of melanoma cells to cis-diaminedichloroplatinum II, carboplatin, dacarbazine, or temozolomide was found to be increased by up to 10-fold by targeting genes that regulate both protein trafficking and the formation of melanosomes because the amount of melanosomes available for the sequestration of therapeutic agents was reduced.. Melanoma cells depleted of either of the protein-trafficking regulators vacuolar protein sorting 33A protein (VPS33A) or the cappuccino protein (CNO), have increased the nuclear localization of cDDP.

- Julien S, Biesemeier A, Kokkinou D, Eibl O, Schraermeyer U.
Zinc deficiency leads to lipofuscin accumulation in the retinal pigment epithelium of pigmented rats.
PLoS One.6(12): e29245. 2011. Epub 2011 Dec 22.
In Long Evans rats fed with a zinc deficient diet for 6 months , in addition to the finding in the title, various ultrastructural changes were noted. As for the melanosomes, their number decreased in the RPE cells and an EDX analysis revealed the zinc mole fraction decreases in them.

- Kasraee B, Nikolic DS, Salomon D, Carraux P, Fontao L, Piguet V, Omrani GR, Sorg O, Saurat JH. **Ebselen is a new skin depigmenting agent that inhibits melanin biosynthesis and melanosomal transfer**. Exp Dermatol. 21(1):19-24, 2012.
  Ebselen, a cell permeable glutathione peroxidase mimic, inhibited the melanosome transfer in vitro from B16 melanocytes to keratinocytes in a coculture and in reconstituted epidermis (Melanoderm) as well as in vivo in guinea pig ears. It reduced melanin content in melanocytes in all the 3 systems studied ; inhibition of tyrosinase was not observed.

- Kim H, Choi HR, Kim DS, Park KC.
  Topical hypopigmenting agents for pigmentary disorders and their mechanisms of action. Ann Dermatol. 24(1): 1-6, 2012. A review article dealing with topics mentioned in the title. The inhibition of melanosome transfer is one of the mechanisms involved in inducing hypopigmention.

- Kirzhner M, Jakobiec FA.
  Clinicopathologic and immunohistochemical features of pigmented basal cell carcinomas of the eyelids. Am J Ophthalmol 153(2): 242-252, 2012.
  Microscopic features of 6 pigmented basal cell carcinomas of the eyelid were characterized: The clinical pigmentation was imparted by varying densities and by the distribution of melanocytes with arborizing dendrites, which were present in basal cell carcinomas. Melanophages with phagocytized melanosomes within the stroma and the basaloid cell melanization also contributed to the pigmentation. The MITF highlighted melanocytic nuclei in the tumour lobules, while the MART-1 and HMB-45 revealed the dendritic shapes of the entrapped melanocytes. There was a subtotal blockage of melanosome transfer to the surrounding basaloid cells.

- Lin E, Peles DN, Simon JD.
  The effect of hydration on the UV absorption coefficient of intact melanosomes. Photochem Photobiol Sci. 11(4):687-691, 2012.
  Physical properties of bovine choroid melanosomes were shown to depend on their water content. A dehydration of melanosomes under ultrahigh vacuum manifested itself by a decrease in the absorption coefficient at 244nm to about 60% of its initial value.

- Loubéry S, Delevoye C, Louvard D, Raposo G, Coudrier E.
  Myosin VI regulates actin dynamics and melanosome biogenesis.Traffic. 2012 Feb 9. doi: 10.1111/j.1600-0854.2012.01342.x. [Epub ahead of print]
  Having used an in vitro motility assay in combination with gene silencing and imaging approaches, the authors studied the function of myosin VI associated with melanosomes. They conclude that myosin VI is not involved in an actin-dependent melanosome movement in vitro or in vivo. Myosin VI regulates the biogenesis of melanosomes : It regulates the size of maturing melanosomes and their melanin content by controlling the delivery of Tyrp-1 to them in melanocytes and choroid cells. Another myosin VI isoform functions in the RPE cells.

- Ohbayashi N, Fukuda M.
  Role of Rab family GTPases and their effectors in melanosomal logistics. J Biochem. 2012 Feb 9. [Epub ahead of print] PMID:22323658.
  Current knowledge regarding the melanosomal logistics, i.e melanosome biogenesis and transport, with a particular focus on the roles of Rab type small GTPases and both their regulators and effectors was reviewed..

- Ohbayashi N, Maruta Y, Ishida M, Fukuda M.
  Melanoregulin regulates retrograde melanosome transport through interaction with the RILP•p150Glued complex in melanocytes. J Cell Sci jcs.094185; Advance Online Publication, January 24, 2012.
  Melanoregulin (Mreg), a dilute suppressor gene product, has been implicated in the regulation of the melanosome transport in mammalian epidermal melanocytes. This study demonstrates that Mreg regulates a microtubule-dependent retrograde melanosome transport through the dynein-dynactin motor complex. Mreg interacted with the C-terminal domain of RILP (Rab 7A interacting lysosomal protein)

and formed a complex with RILP and p150(Glued), a component of the dynein-dynactin motor complex.

- Otręba M, Rok J, Buszman E, Wrześniok D.
  Regulation of melanogenesis: the role of cAMP and MITF. (in Polish). Postepy Hig Med Dosw. 66: 33-40, 2012.

- Scott G.
  Demonstration of melanosome transfer by a shedding microvesicle mechanism. J Invest Dermatol. 132(4):1073-1074, 2012.
  Scott welcomes a new mechanism of melanosome transfer suggested by Ando that involves a release of melanosome-containing globules, followed by an uptake by keratinocytes. This model adds further complexity to the process of melanosome transfer in the skin.

- Wolkow N, Song Y, Wu TD, Qian J, Guerquin-Kern JL, Dunaief JL.
  Aceruloplasminemia: retinal histopathologic manifestations and iron-mediated melanosome degradation. Arch Ophthalmol. 129(11):1466-1474, 2011.
  The first microscopic, EM, immunohistochemical and secondary ion mass spectrometry study of the retinal pigment epithelium from a patient with aceruloplasminemia. The changes resemble those found in age-related macular degeneration. A degradation of melanosomes was noted, most probably induced by an iron-mediated reactive oxygen species. The RPE cells and the neural retina contained increased levels of iron.

- Young A, Jiang M, Wang Y, Ahmedli NB, Ramirez J, Reese BE, Birnbaumer L, Farber DB.
  Specific interaction of Gαi3 with the Oa1 G-protein coupled receptor controls the size and density of melanosomes in retinal pigment epithelium. PLoS One. 6(9):2011; e24376. Epub 2011
  By using the genetic mouse models Gαi1-/-, Gαi2-/-, Gαi3-/- and the double knockout Gαi1-/-, Gαi3-/- that lack functional Gαi1, Gαi2, Gαi3, or both Gαi1 and Gαi3 proteins, respectively, the authors demonstrated that Gαi3 is critical for the maintenance of a normal melanosomal phenotype and formulated a fundamental conclusion - see the title.

9. Melanoma experimental, cell culture

(Dr R. Morandini)

Is senescence a new study field leading to increase sensitivity of cancer cells to targeted drugs ?

It is well known that cancer is a multistep disease that evolves acquisition of numerous characteristics enabling escape from the normal constraints of cell growth.
Senescence is the irreversible arrest of proliferation; this is a "collective" phenotype of multiple effectors mechanisms including global chromatin and epigenetic modification, DNA-damage response, secretory pathway and autophagy. In the way leading to immortalisation, cancer cells must overcome this process. Tumorigenesis relies on a balance between senescence and immortalisation, therefore senescence should be considered in the context of a larger signalling complex consisting of overlapping processes of immortalisation and apoptosis. Cairney *et al*. developed this topic in the paper published in "Drug Discovery Today" with clear figures and explanation on how senescence can be integrated in drug targeting in cancer cells.
Another way, developed by *Huang et al*., is the modulation of protein trafficking via cell-surface signaling by binding the melanocortin 1 receptor with the antagonist agouti-signaling protein leading to increase sensitivity to some therapeutic agents (cis-platin, carboplatin, dacarbazine, or temozolomide) by decreasing the formation of mature melanosomes.
This modulation decreases the number of melanosomes available for sequestration of therapeutic agents.

In "pure cell culture field" *Li et al*. give a method for the isolation and cultivation of dermal stem cells that differentiate into functional epidermal melanocytes.

A. Signal transduction and cell culture

- Goundiam O, Nagel MD, Vayssade M.
  Akt and RhoA inhibition promotes anoikis of aggregated B16F10 melanoma cells. Cell Biol Int. 2012 Mar 1;36(3):311-9.

- Helmbold P, Richter AM, Walesch S, Skorokhod A, Marsch WCh, Enk A, Dammann RH.
  RASSF10 promoter hypermethylation is frequent in malignant melanoma of the skin but uncommon in nevus cell nevi. J Invest Dermatol. 2012 Mar;132(3 Pt 1):687-94. doi: 10.1038/jid.2011.380.

- Huang ZM, Chinen M, Chang PJ, Xie T, Zhong L, Demetriou S, Patel MP, Scherzer R, Sviderskaya EV, Bennett DC, Millhauser GL, Oh DH, Cleaver JE, Wei ML.
  Targeting protein-trafficking pathways alters melanoma treatment sensitivity. Proc Natl Acad Sci U S A. 2012 Jan 10;109(2):553-8.

- 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 Feb 25.

- Kushiro K, Núñez NP.
  Ethanol inhibits B16-BL6 melanoma metastasis and cell phenotypes associated with metastasis. In Vivo. 2012 Jan-Feb;26(1):47-58.

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  Modulation of NOXA and MCL-1 as a strategy for sensitizing melanoma cells to the BH3-mimetic ABT-737. Clin Cancer Res. 2012 Feb 1;18(3):783-95. Epub 2011 Dec 15.

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Costimulatory molecule expression on human uveal melanoma cells: Functional analysis of CD40 and B7-H1. Exp Eye Res. 2012 Mar;96(1):98-106.

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  The heat shock protein-90 inhibitor XL888 overcomes BRAF inhibitor resistance mediated through diverse mechanisms. Clin Cancer Res. 2012 Feb 20.

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  (V600E)Braf::Tyr-CreERT2::K14-Kitl mice do not develop superficial spreading-like melanoma: keratinocyte Kit ligand is insufficient to "translocate" (V600E)Braf-driven melanoma to the epidermis. J Invest Dermatol. 2012 Feb;132(2):488-91.

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  MC1R Variant Allele Effects on UVR-Induced Phosphorylation of p38, p53, and DDB2 Repair Protein Responses in Melanocytic Cells in Culture. J Invest Dermatol. 2012 Feb 16. doi:10.1038/jid.2011.473.

B. Melanin and cell culture

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  Light-emitting Diodes at 830 and 850 nm Inhibit Melanin Synthesis In vitro. Acta Derm Venereol. 2012 Feb 15.

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  Cellular and clinical report of new Griscelli syndrome type III cases. Pigment Cell Melanoma Res. 2012 Jan;25(1):47-56.

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C. 3D cell culture and/or skin reconstitution

- Elliott NT, Yuan F.
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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

Calendar of Events

2012 Paris Melanoma Conference
April 27-28, Paris
Contact: web: http://www.primeoncology.org/parismelanoma2012

2012 Dermatology Update 2012
May 11-13, 2012, Singapore
Pre-conference Workshops on 11 May: "Basic Science in Dermatology" and "Paediatric Dermatology"
Mandarin Hotel on Orchard Road, Singapore
Contact: Web: www.nsc.gov.sg/dermupdate2012

2012 4th Melanoma Workshop
June 18-20, 2012, Malmö, Sweden
Contact: Web: www.melanoma2012.org/

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

2014 XXIInd IPCC Meeting

September 4-7, Singapore
Contact: Web: www.ipcc2014.org

2015 45th Annual ESDR Meeting

September 9-12, Rotterdam, The Netherlands