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

Discussion

Vitiligo

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An important part of the XV International Pigment Cell Conference was devoted to the new aspects of vitiligo.

David Norris has presented an excellent lecture on the possible pathogenetic mechanisms of the depigmentation and of the repigmentation of the vitiligo spots. He reviewed, according to the new researchers on the disease, the biochemical alterations in the vitiligo subjects, the alteration in the catecholamine metabolism and the dysfunction in the antioxidant defence mechanisms of the melanocytes. These events may produce an alteration of the antigenic pattern of the cells, the release of auto-antigens and the development of an auto-immune reactions towards pigment cells. He underlined that different factors which have been considered as precipitating causes of the disease may produce an oxidative injury of the melanocytes. In this category he included the high UV exposure, the physical trauma and the release of inflammatory mediators such as some cytokines. He presented interesting data on the possible mechanisms of re-pigmentation of the vitiligo spots spontaneously produced or following the PUVA therapy. He demonstrated that leukotriene C4 (a poster form his group has been presented at the meeting), which is known to be a growth and chemiokinetic factor for neonatal melanocytes, is also chemotactic for the melanocytes. In response to PUVA therapy, the increased intraepidermal concentration of LTC4 may act as a signal for melanocyte migration out of the outer root sheath of the hair follicle into the depigmented areas. Through the expression of integrin receptors on melanocytes, the cell can adhere to the basal membrane of the colonised epidermis.

The immunologist's view point on etiopathogenesis of vitiligo has been presented by P.K. Das and co-workers from Amsterdam. This group has shown that melanocytes, in addition to produce cytokines, are able to constitutively express MHC class II antigens and are able to phagocyte latex particles. Now they have reported that melanocytes are capable of phagocitizing Mycobacterium leprae, processing and presenting antigenic fragments to T-cells. Therefore melanocytes may be involved in the immune reaction of the skin and can became a "stand by

target" for activated T-cells. According to their hypothesis, even if the presence of inflammatory infiltrate is not a common finding in vitiligo skin, melanocytes may act as a target for T-cells sensitised towards auto antigens which produce a damage to melanocytes and go away from the skin (Hit and Run phenomenon).

An interesting discussion was developed during the symposium on Vitiligo. P. Grimes reviewed the immune dysfunction detected in vitiligo subjects.

The Amsterdam group presented the immunophenotyping of infiltrate in inflammatory vitiligo, underlying the presence of CD4+ cells and macrophages in the perilesional skin and the decrease in the number of Langerhans cells in lesional skin. These authors suggested the possibility to separate an entity of inflammatory vitiligo in which the immune mechanism may be involved.

Goudie and co-workers presented a study on the possible identification of a specific T-cell clone in the skin of patients with vitiligo. By the polymerase chain reaction this group tried to identify a T-cell receptor gamma gene rearrangement similar to that recovered in cutaneous T-cell lymphoma. The analysis performed in 32 patients was negative, and this group suggested that the site of melanocytes damage may be dependent on other factors such as the inappropriate adhesion molecule expression or MHC class II antigen expression.

The second part of the workshop was focused on the free radical hypothesis of vitiligo and the modification of the scavenger systems in experimental animals and in man.

B. Salzer and K. Schallreuter presented an investigation on the personality and on the possible triggering events in patients with vitiligo. The study, conducted in more than 100 subjects, showed no specific characteristic personality pattern for patients with vitiligo, but underlined that the major part of the patients associated the onset or the progression of the disease with traumatic situation or stressful events.

Bowers reported that in Barred Plymouth Rock (a spontaneous model of vitiligo in animals) the level of superoxide dismutase in melanocytes is decreased, whereas the level of catalase is normal. A working hypothesis was proposed by this group: a genetic defect may account for the alteration of SOD activity and consequently the premature death of melanocytes, which can be observed both in vivo and in vitro in barred feather, may be due to the increased levels of oxyradicals. The addition of the dominant white gene to these chicken constitute the leghoron chicken and may produce the reduction of another anti-oxidant or the generation of a toxic factor leading to an early damage of melanocytes. A similar radical damage combined with a possible genetic defect of anti-oxidant system may account for the melanocyte death in vitiligo.

The radical hypothesis on the pathogenesis of vitiligo was also proposed by S. Passi e co-workers. The Roman group reported that an increase of catecholamine metabolites, namely homovanillic and vanil mandelic acid, can be detected in the urine of subjects with early or active phase of the disease. This finding were irrespective to the clinical appearance of the disease, i.e. segmental, acrofacial or generalised. This group proposed that the increased release of catecholamine at the nerve endings can induce high level of catecholamines on the skin which can produce a cytotoxic effect trough two mechanisms: a direct one via the spontaneous oxidation of the catecholamines and the generation of free radicals; the second one via the vasoconstriction of dermal vessels, the subsequent ischaemia-hypoxia of the skin and the production of oxyradicals following the hypoxic damage of the cells. This hypothesis may account

for the damage of epidermal cells other than melanocytes, such as keratinocytes and Langerhans cells, which has been described in the vitiligo lesions. These authors, according to their results, proposed the administration of a mixture of anti-oxidants, which include tocopherol acetate, selenium and selenium methionine as possible therapy in subjects with active vitiligo. The clinical pictures of some patients treated showed the stop of the progression of depigmentation and the repigmentation of some spots.

An interesting presentation has been done by Dr. K. Schallreuter. She has performed different experiments on the enzymatic activities on the skin vitiligo subjects. According to her results, the keratinocytes are able to synthesise epinephrine via biopterin dependent tyrosine hydroxylase pathway. In vitiligo subjects an alteration of this enzymatic pathway can be detected with an increase of tyrosine hydroxylase activity and the reduction of phenylethanolamine-N-methyl transferase and ultimately an increase of norepinephrine synthesis. As consequence of these enzymatic modifications, there is an increase of the skin content of tetrahydrobiopterin, a fluorescent substance which may be responsible for the appearance of the vitiligo skin under Wood light. In summary the author suggested that vitiligo may be a primarily defect in biopterin production leading to an upregulated catecholamine biosynthesis in epidermis of these patients, the production of elevated amount of toxic free radicals and the consequent damage of melanocytes which possess low antioxidant enzymatic activities.
At the moment we have not completely clarified all the aspects of the pathogenesis of vitiligo, but certainly we have made some new steps in the comprehension of the disease.

CURRENT LITERATURE

We acknowledge the valuable assistance of
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1. Melanins and other pigments chemistry

(Comments by Prof. M. Peter)

- Bilinska B, Szaflik J, Kochanska-Dziurowicz A.
Infrared spectroscopy of melanins isolated from mammalian eyes. Klin. Oczna 94:236-238, 1992.
Abstract: Comparative IR spectra of melanins isolated from choroid epithelium of human, cattle and pig eyes were analyzed. Characteristic bands of IR melanin spectra are: 1600, about 1700, 2860, 2930 with shoulder 2960, and broad band at about 3400 cm-1. The presence of band groups in the region 1400-1100 cm-1 characteristic for the natural melanins was shown. Anal. of the obtained IR spectra of melanins indicates that these melanins are eumelanins.

- Bilinska B, Kolczynska-Szafraniec U, Buszman E.
The structure of melanin biopolymers from human hair. I. IR spectroscopy studies. Postepy Fiz Med V 26:75-80, 1991.
Commentary: Rather descriptive work.
Abstract: IR spectra of melanin isolated from human hair of several colors show that melanins from fair and red hair with different tints isolated by treatment with concd. HCl can be described as pheomelanins or mixed-type melanins. The method of Bolt used for isolating melanins from hair with distinct colors (dark or black hair) suggests the presence of eumelanins. In a comparative anal. of IR spectra, the role of the isolation method used for melanin prepn. that affects the IR spectrum was ascertained.

- Buszman E, Wiewiora A, Bilinska B.
The structure of melanin biopolymers from human hair. II. An electron spin resonance study. Postepy Fiz Med, V 26:81-86, 1991.
Abstract: Melanins isolated from human hair (black, brown, red, dark and light blond, and gray) were purified and examd. by ESR. Differences were found in the ESR signal parameters showing a dependence on the concn. of both the eumelanin and pheomelanin fraction of melanin biopolymers present in the original hair samples. The differences were higher in native hair samples than in melanins obtained after a multistep isolation and purifn. procedure.

- Chakraborty AK, Chakraborty DP.
The effect of tryptophan on dopa-oxidation by melanosomal tyrosinase. Int J Biochem. 25(9):1277-80, 1993.
Commentary: Important information on regulation of melanogenesis in melanoma.
Abstract: 1. Tryptophan has been shown to inhibit dopa-oxidation by melanosomal tyrosinase. 2. The inhibition is of mixed-type with Ki = 1.6 x 10(-3) M. 3. Tryptophan does not interact with the oxidation product of the dopa-oxidase reaction. 4. Neither oxygen nor hydroxyl radicals are involved in the inhibition found in presence of tryptophan. 5. Tryptophan, like dopa, also inhibits tyrosine hydroxylase and dopa-oxidase activity of melanosomal tyrosinase and its inhibitory mechanism differs from inhibition due to non-substrate type compounds like cysteine, ascorbic acid. 6. These experiments together with previous findings suggest that the status of tryptophan may be similar to that of dopa in relation to regulation of melanogenesis.

- Chirila TV.
Melanized poly(HEMA) hydrogels: basic research and potential use. J Biomater Appl 8(2):106-45, 1993.
Commentary: Interesting technology and application to polymeric materials.

- Crescenzi O., D'Ischia M., Napolitano A., Prota G.
The alleged stability of dopa- melanins revisited. Gazz. Chim. Ital. 123:241-242, 1993.
Abstract: In neutral aq. buffer at 25.degree., enzymically produced dopamelanin undergoes self-oxidn. with a marked increase in the content of carboxyl groups, due to peroxidative cleavage. Tyrosinase is not involved in the peroxidn. process.

- Cuevas AA, Garcia-Castineiras S.
Melanins and lens pigments: a comparative study. P R Health Sci J 12(2):129-35, 1993.
Commentary: Evidence for the presence of phaeomelanin obtained by degradation of lens digests. Relevant for cataract research.
Abstract: Based on previous findings that lens pigments and melanins share many physicochemical properties, human lens pigments and natural (hair) and synthetic melanins were submitted to oxidation with permanganate under strong acidic conditions. This procedure has been utilized for the characterization of melanins and results in the well defined products, thiazole-4,5-dicarboxylic acid (TDCA) and pyrrole-2,3,5- tricarboxylic acid (PTCA), which can be quantitated by high performance liquid chromatography (HPLC). PTCA is regarded as a marker of black eumelanins and was therefore a main component of synthetic DOPA- eumelanin and dark hair. Its identity was established by synthesis from 5-hydroxyindole-2- carboxylic acid. TDCA derives from pheomelanins and was therefore an important component of red hair and synthetic GSH- pheomelanin . TDCA was identified by its retention time relative to PTCA. The analysis of a series of cataract digests of increasing pigmentation (type I < type IV < type V) and a purified fraction of lens pigments (DE52 pigment) revealed the presence in these preparations of both PTCA and TDCA. The concentration of TDCA significantly increased with the degree of pigmentation of the digests and reached a maximum in the DE52 pigment. The TDCA/PTCA ratio was high in the lens preparations and comparable to that given by hair pheomelanin . These findings support that pheomelanin is an integral part of lens pigments. By comparing the yields of TDCA in GSH- pheomelanin and in the purified lens pigment, a 9% contribution of pheomelanin to the lens pigment was estimated.

- Enochs WS, Nilges MJ, Swartz HM.
A standardized test for the identification and characterization of melanins using electron paramagnetic resonance (EPR) spectroscopy. Pigment Cell Res, 6:91-9, 1993.
Commentary: Use of autoxidized DOPA Melanin is questionable.

- Enochs WS, Nilges MJ, Swartz HM.
The minocycline-induced thyroid pigment and several synthetic models: identification and characterization by electron paramagnetic resonance spectroscopy. J Pharmacol Exp Ther 266 :1164-76, 1993.

- Jacobson ES, Tinnell SB.
Antioxidant function of fungal melanin. J Bacteriol 175(21):7102-4, 1993.

- Kaliszan R, Kaliszan A, Waine IW.
Prediction of drug binding to melanin using a melanin-based high-performance liquid chromatographic stationary phase and chemometric analysis of the chromatographic data. J Chromatogr 615:281-8, 1993.

- Komiyama K, Takamatsu S, Takahashi Y, Shinose M, Hayashi M, Tanaka H, Iwai Y, Omura S, Imokawa G.
New inhibitors of melanogenesis, OH-3984 K1 and K2. I. Taxonomy, fermentation, isolation and biological characteristics. J Antibiot (Tokyo) 46(10):1520-5, 1993.
Commentary: Isolation and structure of novel melanogenesis inhibitors. Unexpected, interesting structures.
Abstract: Melanogenesis inhibitors, OH-3984 K1 and K2 were isolated from fermentation broth of Streptomyces sp. OH-3984. OH-3984 K1 and K2 inhibited the melanogenesis of B16 melanoma cells at concentrations of 7.5 and 3.8 micrograms/ml, respectively, whereas inhibition of tyrosinase activity has not been observed. The microbial metabolites showed no antimicrobiological activities against Gram-positive and Gram-negative bacteria, fungi or yeast at a concentration of 1,000 micrograms/ml.

- Kurechi T, Inoue Y.
Staining endogenous. Kensa to Gijutsu V 21:485-91, 1993.
Abstract: A review with 6 refs. on endogenous pigments such as hemosiderin, bilirubin, melanin, lipofuscin, and ceroid, and staining method with these pigments.

- Losi A, Bedotti R, Brancaleon L, Viappiani C.
Porphyrin-melanin interaction: effect on fluorescence and non-radiative relaxations. J Photochem Photobiol, B

21(1):69-76, 1993.
Abstract: Optical techniques and pulsed-laser, time-resolved photoacoustics (PA) were employed to obtain information on the mechanism of interaction between cationic zinc tetrabenzylpyridylporphyrin (ZnTBzPyP) and synthetic L-Dopa melanins. Synthetic eumelanin and pheomelanin strongly quench the fluorescence of ZnTBzPyP, but Stern-Volmer plots suggest a mechanism of interaction quite different for the 2 pigments. This diversity was confirmed by PA: for eumelanin no thermal relaxation was obsd. other than prompt heat, whereas for the complexed form of ZnTBzPyP with pheomelanin the authors were able to detect a heat-emitting species with a non-radiative lifetime in the microsecond range. The involvement of oxygen in the photophysics of the complexes formed between the cationic porphyrin and the 2 pigments was demonstrated, but its role has yet to be described.

- Masakatsu Komuro, Chizuru Komiya, Wataru Hori, Ryozo Ishida, Hideo Ohkubo.
Studies on high performance liquid chromatographic measurement of norfloxacin in melanin-containing ocular tissues. Atarashii Ganka 10:1755-9, 1993.
Commentary: Valuable data on drug binding to melanin in ocular tissues.
Abstract: Norfloxacin (NFLX) content was detd. in pigmented ocular tissues by HPLC. [14C]-NFLX was administered to rabbits and melanin-contg. ocular tissues were isolated and analyzed by HPLC or radioassay for comparison. For HPLC, tissues were homogenized with 6 N KOH. After standing overnight at room temp., the homogenate was centrifuged. The supernatant was extd. by a solid-phase extn. procedure. Greater than 95% of NFLX was extd. into the supernatant as detd. by HPLC as well as by radioassay. The calibration curve was linear in the range 0.05-10 .mu.g/mL. The NFLX concn. in pigmented ocular tissues was considerably higher than that in non-pigmented tissues, indicating a high affinity of NFLX for melanin.

- Nagata A., Mishima HK, Kiuchi Y, Hirota A, Kurokawa T, Ishibashi S.
Binding of antiglaucomatous drugs to synthetic melanin and their hypotensive effects on pigmented and nonpigmented rabbit eyes. Jpn J Ophthalmol 37:32-8, 1993.
Abstract: The binding of ocular hypotensive drugs to synthetic melanin was studied spectrophotometrically in vitro. The ocular hypotensive effects of the drugs, namely, timolol, befunolol, carteolol, pilocarpine, epinephrine, prostaglandin A2, F2 alpha and E2, also were compared in vivo on eyes of pigmented and albino rabbits. At an initial concentration of 10(-4) M, each of the three beta-blockers exhibited a binding rate of 80-85% as compared to only 40% for pilocarpine and 50% for epinephrine. Almost none of the prostaglandins were found to bind to synthetic melanin. Topically applied, 0.5% timolol and 3% pilocarpine significantly lowered the intraocular pressure in albino but not in pigmented rabbits. Epinephrine (1%) caused a significant reduction in the intraocular pressure both in albino and pigmented rabbits; however, the maximum reduction was greater in albino than in pigmented rabbits. Intraocular pressure was reduced to the same extent and with a similar time-course in both albino and pigmented rabbits by 0.02% prostaglandin A2, F2 alpha and E2. These findings show that several ocular hypotensive drugs bind to melanin and suggest that this process can modify the extent of their pharmacological effects when tested in a single dose, or the time-course of their effects when used to treat chronic conditions.

- Napolitano A, Crescenzi O, Prota G.
Copolymerization of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2- carboxylic acid in melanogenesis: isolation of a cross-coupling product. Tetrahedron Lett. 34: 885-888, 1993.
Abstract: Under biol. relevant conditions, co-oxidn. of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid affords, in addn. to a complex mixt. of homopolymers of the two indoles, a small but significant amt. of cross-coupling product I which was isolated as the tetra-O-acetyl deriv.

- Napolitano A, Palumbo A, Misuraca G, Prota G.
Inhibitory effect of melanin precursors on arachidonic acid peroxidation. Biochim Biophys Acta 1168:175-80, 1993.

- Reszka Krzysztof J, Chignell Colin F.
EPR and spin-trapping investigation of free radicals from the reaction of 4-methoxybenzenediazonium tetrafluoroborate with melanin and melanin precursors. J. Am. Chem. Soc. 115:7752-7760, 1993.

- Sarna T, Swartz HM.
Interactions of melanin with oxygen (and related species). Atmos Oxid Antioxid V 3:129-169, 1993.
Abstract: A review with 145 refs. The structure, formation, and reactivity of melanin are discussed emphasizing those aspects needed to provide a suitable basis for understanding its interactions with oxygen. The role of oxygen in the generation of melanin, the chem. reactions of melanin that involve oxygen, the photochem. of melanin, and melanin's phys. interactions with oxygen are also discussed.

- Sato, Masako.
Mechanism in binding of thiamin to melanin. Kenkyu Kiyo - Kagoshima Daigaku Kyoikugakubu, Shizen Kagakuhen 43:83-90, 1991.
Abstract: The binding of thiamin was investigated in eye melanin of carp and pigeon. The release of thiamin from eye melanin was not affected by protease such as pronase, trypsin and chymotrypsin. Thiamin was strongly released from eye melanin by divalent metal ion comparable with monovalent metal ion, though there were differences among these metal ions. The release of thiamin from eye melanin was smallest at pH 4.5. These results suggest that the binding of thiamin is electrostatic in nature.

- Shibata T, Prota G, Mishima Y.
Nonmelanosomal regulatory factors in melanogenesis. J. Invest. Dermatol. 100:274, 1993.

- Shosuke Ito.
Biochemistry and physiology of melanin. Pigm Pigm Disord 33-59, 1993.
Commentary: Useful review.
Abstract: A review, with 145 refs., on: the characterization of melanins; the biochem. of melanins; biochem. of pheomelanins; melanins and melanogenesis; and physiol. of melanins.

- Takamatsu S, Rho M, Hayashi M, Komiyama K, Tanaka H, Omura S, Imokawa G.
New inhibitors of melanogenesis, OH-3984 K1 and K2. II. Physico-chemical properties and structural elucidation. J Antibiot (Tokyo),46(10):1526-9, 1993.
Commentary: Isolation and structure of novel melanogenesis inhibitors. Unexpected, interesting structures.
Abstract: New melanin synthesis inhibitors, OH-3984 K1 and K2, were isolated from the fermentation broth of Streptomyces sp. OH-3984, and their structures were elucidated by spectroscopic methods and by chemical transformations. OH-3984 K1 (M.W.: 306; C18H26O4) and K2 (M.W.: 308; C18H28O4) have unique gamma-lactone rings, both of which correspond to oxidative products derived from C1-O14 cleavage of the 14-membered lactone group.

- Vas G, Vekey K, Czira G, Tamas J, Favretto D, Taldi P, Bertazzo A, Costa C, Allegri G.
Characterization of melanins by pyrolysis/gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom V 7:870-873, 1993.
Commentary: Comparative work and as such of interest. The method is questionable with respect to melanin structure because of possible artifacts at high temperature pyrolysis.

2. Biology of pigment cells and pigmentary disorders

(Comments by Dr M. Picardo)

- Alvarez J, Peteiro C, Toribio J.
Linear and whorled nevoid hypermelanosis. Pediatr Dermatol 10:156-8,1993.

- Asahina A, Chi HI, Otsuka F.
Subungual pigmented nevus: evaluation of DNA ploidy in six cases. J Dermatol. 20(8):466-72, 1993.
Abstract: The discrimination between subungual pigmented nevus and subungual melanoma in situ is still a clinical problem. We measured DNA ploidy in six cases of subungual melanotic lesions which exhibited the features of subungual pigmented nevus or lentigo simplex histologically. Five cases presented a diploid pattern with or without a slight increase of hyperdiploid cells. One case presented a polyploid pattern, it also exhibited histologically abnormal melanocytes with large nuclei and pigment-filled elongated dendrites. The DNA ploidy pattern and histologic features suggest that the lesion of this latter case contains abnormal melanocytes which probably have the potential to undergo a malignant transformation into a subungual melanoma. DNA ploidy analysis, therefore, is likely to provide information for evaluating the biologic behavior of subungual melanotic lesions.

- Bergamaschi O, Kon S, Doine AI, Ruben MP.
Melanin repigmentation after gingivectomy: a 5-year clinical and transmission electron microscopic study in humans. Int J Periodontics Restorative Dent 13:85-92, 1993.

- Bhatnagar V, Anjaiah S, Puri N, Darshanam BN, Ramaiah A.
pH of melanosomes of B 16 murine melanoma is acidic: its physiological importance in the regulation of melanin

biosynthesis. Arch Biochem Biophys 307(1):183-92, 1993.

- Boyera N, Cavey D, Delamadeleine F, Bouclier M, Hensby C, Shroot B.
A novel in vitro model for the study of human keratinocyte/leucocyte interactions under autologous conditions. Br J Dermatol. 129(5):521-9, 1993.

- Broekhuyse RM, Kuhlmann ED, Winkens HJ.
Experimental autoimmune anterior uveitis (EAAU): induction by melanin antigen and suppression by various treatments. Pigment Cell Res Feb 6:1-6, 1993.
Commentary: This paper presents different points of interest. The studies on the extracutaneous pigmentary system are important to better understand the possible functions of the melanin. The induction of an autoimmune syndrome by purified ocular and skin melanins can open a new view on the pathogenesis of some diseases involving the pigmentary system. Moreover, the therapeutic activity of vitamin E, suggest a free radical-dependent mechanism in the onset of these manifestations.

- Brooks G, Goss MW, East JA, Hart IR.
Growth of melanocytic cells is associated with down-regulation of protein kinase C alpha, delta, and epsilon isoforms. Possible role of diacylglycerol. J-Biol-Chem. :268(32):23868-75, 1993.
Commentary: The correlation between melanocyte duplication, melanin synthesis and Protein kinase C modifications have not still completely defined. This study add some new observations to the topic. The down regulation of specific isoform of PKC seems tobe correlated with the proliferation of normal melanocytes, and the constitutive down regulation in transformed melanocytes may be the consequence of elevated diacylglicerol levels in these cells; in fact, stimulation of quiescent cells with synthetic diacylglicerol induce a significant increase of DNA synthesis. Stimulation of alteration of the membrane lipid component with the subsequent release of phosphatidynilnositol could be a trigger factor in these cellular responses.

- Cesarini JP.
Repartition of mankind on the earth and role of cutaneous melanization. Ann Dermatol Venereol 120(5):359-62, 1993.

- Chakraborty AK, Chakraborty A, Chakraborty DP.
Hydroquinone simultaneously induces indoleamine 2,3-dioxygenase (IOD) and inhibits tyrosinase in Bufo melanostictus. Life Sci. 52:1695-1698, 1993.

- Farooqui JZ, Auclair BW, Robb E, Sarkisian E, Cooper C, Alexander JW, Warden G, Boissy RE, Norlund J.
Histological, biochemical, and ultrastructural studies on hyperpigmented human skin xenografts. Pigment Cell Res, 6(4 Pt 1):226-33, 1993.

- Fakhfakh AC, Humbert P, Aubin F, Kantelip B, Agache P.
Cutaneous pigmentation induced by minocycline: ultrastructural analysis and X-ray microanalysis. Ann Dermatol Venereol 119:975-9, 1992.

- Fechner GA, Jacobs JJ, Parsons PG.
Inhibition of melanogenesis in human melanoma cells by novel analogues of the partial histamine (H2) agonist nordimaprit. Biochem Pharmacol 46:47-54, 1993.
Commentary: The pharmacological inhibition of melanogenesis is one of the main projects in pigment cell studies. Chakraborty et al and Fechner et al, by different models, have produced new contributes on in vivo and in vitro mechanism of action of some possible depigmenting drugs. The Parson group has focused his attention on the relationship between the chemical structure, i.e. polarity of the molecules, and the in vitro activity on pigmented melanoma cells, which is probably one of the clues of the problem. Probably different mechanisms may account for in vitro results: the chemical reactivity of the tested substances and the antienzimatic properties. Interesting are some suggestions offered by Chakraborty et al, on the elevated levels of urinary indole metabolites found in vitiligo subjects and the in vivo metabolism of hydroquinone.

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Vitiligo autoantibodies are effective against melanoma. Cancer 72(8):2365-9, 1993.

- Fukai K, Ishii M, Kadoya A, Hamada T, Wakamatsu K, Ito S.
Nevus depigmentosus systematicus with partial yellow scalp hair due to selective suppression of eumelanogenesis.

Pediatr Dermatol 10(3):205-8, 1993.
Abstract: We report a Japanese patient with congenital hypomelanosis with a segmental pattern on the left abdomen, whorl-like pattern on the back; mosaic pattern on the chest, right abdomen, and proximal extremities; and with yellow hair on a portion of the scalp. Chemical analysis of the yellow hair revealed decreased eumelanin content, whereas the pheomelanin content was normal.

- Gulyaev ABA, Dontsov EI, Il'yasova BM, Ostrovskii A.
Determination of melanin content in retinal pigment epithelium melanosomes as a function of human age. Dokl Akad Nauk V 333:257-259, 1993.
Abstract: The concn. of paramagnetic centers, as measured by EPR, in melanosomes of human retinal pigment epithelium did not change with age between the ages of 20 to .apprx.65 yr. Apparently, on aging, the concn. of melanin in individual melanosomes does not change but the no. of melanosomes diminish.

- Hashimoto O, Miyamoto K, Moritomo T, Saito H, Watanabe T, Mochizuki K.
Characterization of cultured cells derived from Mongolian gerbil's. J Vet Med Sci 55 (2) 227-32, 1993.
Abstract: A cell line was established from a Mongolian gerbil's (Meriones unguiculatus) homotransplantable malignant melanoma. Ascitic tumor cells detected in a gerbil when transplanted intraperitoneally were adapted to culture. In primary culture, cells were divided into 2 types, multipolar and polygonal cells. Cell masses which adhered to polygonal cells were observed after the 6th passage. The adhering cells were removed and transferred into another flasks. The cells showed multipolar and possessed projections. Then after, the cells increased in number vigorously and formed acinous structures. At early passages, abundant melanin granules in some of the cells were demonstrated by light and electron microscopical observations. Most of the cells were positive by DOPA reaction. Melanin pigments were gradually decreased through the 20th to 30th passage and most of cells became amelanotic. The doubling time of the cell line was 32 hr. Chromosome number of the cell line ranged from 68 to 82. Whitish tumors were produced in the abdominal cavity within 30 days when intraperitoneally inoculated the cells to gerbils. This cell line, designated as MGM-A, has been subcultured for more than 100 passages during 2 years.

- Hearing VJ, King RA.
Determinants of skin color: melanocytes and melanization. Pigm Pigm Disord:3-32, 1993.
Abstract: A review, with 282 refs., on: tissue distribution; melanocyte structure; biochem. determinants; mol. biol. of melanocytes; and interactions and levels of regulation.

- Hedin CA, Pindborg JJ, Axell T.
Disappearance of smoker's melanosis after reducing smoking. J Oral Pathol Med 22:228-30, 1993.

- Kidson SH, Richards PD, Rawoot F, Kromberg JG.
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3. MSH, MCH, other hormones, differentiation

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administration of DMPEA elevated plasma titers of alpha-MSH in a dose- and time-related manner. Pretreatment with the opiate antagonist naltrexone had no effect on basal plasma levels of alpha-MSH but blocked DMPEA-induced alpha-MSH release. Treatment with a dose of naltrexone sufficient to block DMPEA-induced secretion of alpha-MSH had no effect on stress-induced secretion of alpha-MSH. Although pretreatment with the dopamine receptor agonist apomorphine prevented DMPEA-induced alpha-MSH secretion, DMPEA had no effect on the synthetic activity of tuberohypophysial dopamine neurons as gauged by measuring the accumulation of 3,4-dihydroxyphenylalanine in the neurointermediate lobe (NIL) following administration of NSD-1015. In vitro treatment of isolated NILs with DMPEA resulted in a significant increase in alpha-MSH release. Naltrexone completely blocked the stimulatory effects of DMPEA on alpha-MSH release in vitro. Our results indicate that DMPEA stimulates alpha-MSH secretion by acting directly through opiate receptors at the level of the NIL.

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4. Photobiology and photochemistry

(Comments by Dr M. Picardo)

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Abstract: The kinetic properties of ascorbic acid oxidation by light-activated melanin granules demonstrate the presence of a specific reactive site on the melanin granule saturable by ascorbic acid. Increased light intensity increased the Vmax and reduced the Km of this reaction, indicating increased affinity of the active site for ascorbic acid. The kinetics of this reaction are not markedly changed in a reduced-oxygen environment. Ascorbic acid oxidation is competitively inhibited by isoascorbic acid, an epimer of ascorbic acid, while other tested reducing agents are inactive. The Ki for isoascorbic acid is 1 mM, about the same as the Km of ascorbic acid.

- Hruza GJ, Dover JS, Flotte TJ, Goetschkes M, Watanabe S, Anderson RR.
Q-switched ruby laser irradiation of normal human skin. Histologic and ultrastructural findings. Arch Dermatol 127 :1799-805, 1991.

- Kobayashi N, Muramatsu T, Yamashina Y, Shirai T, Ohnishi T, Mori T.
Melanin reduces ultraviolet-induced DNA damage formation and killing rate in cultured human melanoma cells. J Invest Dermatol 101(5):685-9, 1993.
Commentary: These studies, by different methods, illustrate the photoprotective role of melanin. Associated with

the physical properties, a chemical scavenging activity of melanin pigment is documented. The definition of these functions of melanin, not strictly correlated with UV absorption, underlines a more general mechanism by wich melanocytes and their product contribute to the normal homeostasis of the skin.

- Lam Kwok Wai, Glickman RD.
Prevention of light-induced free radical production from melanin granules by ascorbic acid. Int. Congr. Ser. - Excerpta Med. 998:633-636, 1992.
Abstract: Rapid ascorbic acid oxidn. has been demonstrated during exposure of the retinal pigment epithelium cells to light. Because this rapid reaction is a suitable candidate for a detoxification mechanism, and because it appears to be dependent on the presence of melanin, the present study was designed to characterize the light-dependent ascorbic acid oxidn. reaction in the presence of melanin granules in a cell free incubation mixt.

- Lee Ki Hong, Kim Jae Hong, Myung Ki Bum, Kook Hong Il.
Effect of ultraviolet-A irradiation on the number of melanocytes and the amount of melanin in cultured melanocytes. Hanyang Uidae Haksulchi V13:501-508, 1993.
Abstract: The effect of UVA irradn. was studied on the no. of melanocytes and the amt. of melanin in cultured human melanocytes. Subcultures of melanocytes were examd. on the 2nd and 10th day after UVA irradn. at 1-4 J/cm2. There was no change in melanocytes proliferation after UVA irradn. On the 2nd day of cultivation after UVA irradn., the amt. of melanin was increased in proportion to the increment of dose of UVA irradn. After irradn. at 4 J/cm2, the reading was 3.79 .times. 10-4 .mu.g/cell, which was 3.11-fold larger than that of the nonirradiated group. On the 10th day of cultivation after UVA irradn., the amt. of melanin was slightly influenced according to the increment of dose of UVA irradn. After irradn. at 4 J/cm2, it was 1.60 .times. 10-6 .mu.g/cell, which was 1.33-fold larger than that of the nonirradiated group. After UVA irradn., the amt. of melanin of the 2nd day of subcultivation was larger than that of the 10th day. From this data, it is thought that UVA irradn. is capable of directly stimulating melanogenesis; however, melanocyte proliferation is not influenced at all by UVA irradn.

- Nacht S.
Melanin, the natural biopolymer for ultraviolet protection. Biotechnol. Polym. 104-22. Edited by: Gebelein, Charles G. Technomic: Lancaster, Pa. 1993.

- Liu YT, Sui MJ, Ji DD, Wu IH, Chou CC, Chen CC.
Protection from ultraviolet irradiation by melanin of mosquitocidal activity of Bacillus thuringiensis var. israelensis. J Invertebr Pathol 62(2):131-6, 1993.
Abstract: A process for production, isolation, and purification of melanin produced by the fermentation of Streptomyces lividans 66 harboring a recombinant plasmid pIJ702-bearing tyrosinase gene has been developed. The efficacy of melanin in the protection of mosquito larvacidal activity of Bacillus thuringiensis var. israelensis against uv light has been studied. Results obtained by the live cell counts and the bioassay of residual mosquitocidal activity of B. thuringiensis var. israelensis after exposure to uv radiation showed that melanin is an excellent photoprotective agent.

- Setlow RB, Grist E, Thompson K, Woodhead AD.
Wavelengths effective in induction of malignant melanoma. Proc Natl Acad Sci U S A 90:6666-70, 1993.

- Tokura Y, Yagi H, Satoh T, Takigawa M.
Inhibitory effect of melanin pigment on sensitization and elicitation of murine contact photosensitivity: mechanism of low responsiveness in C57BL/10 background mice. J Invest Dermatol 101(5):673-8, 1993.

5. Neuromelanins

Comments by Dr M. d'Ischia:

Owing to the exponential growth in the litterature on brain neuromelanin, the selection of material is necessarily more subjective than comprehensive. In the present survey, an attempt is made to discern and delineate important areas of research activity and major developments, rather than to present a disconnected set of isolated topics.

Structure and properties of neuromelanin.
Interest in the structure and physicochemical properties of neuromelanin from human substantia nigra has continued unabated in 1993. Despite considerable difficulties with the purification and spectral analysis of intact

pigment, matching with those of the cutaneous counterparts, important breakthroughs appear to be just behind the door. Two papers by H. Swartz and his associates (J. Neurochem. 61:68-79, 1993; J. Neural.Transm. Park. Dis. Dement. Sect.5:203-213, 1993) deal with the use of electron paramagnetic resonance (EPR) as an effective tool for directly studying the nature of the free radical centres in both natural and synthetic neuromelanins, and the levels of metal cations bound to the pigment polymer. The results are consistent with a concept of neuromelanin as a highly heterogeneous, sulfur-containing pigment, which serves the function of a metal reservoir in melanised dopaminergic neurons. This contributes to the current controversy on the role of cysteinyldopamine(s) as biosynthetic precursor(s) of substantia nigra neuromelanin (cfr. Pigment Cell Res.:333-335; 1993, not abstracted in this issue), and would reinforce contentions for iron-neuromelanin interaction as a causative factor in Parkinson's disease.

Neuromelanin function and role in Parkinson's disease.

Current emphasis in studies of the biochemical pathology of Parkinson's disease tends to be on oxidative stress. This trend is apty documented by Oertel and Kupsch's review (Curr. Opin. Neurol. Neurosurg 6:323-332, 1993) and surfaces in many outstanding papers of the last few months. A study by Chiueh et al. (Ann NY Acad Sci 679:370-375, 1993) deals with in vivo markers for oxidant injury of neuromelanin-containing neurons, whereas a contribution from Agid's group (Neuroscience, 55:167-175, 1993) provides evidence that the expression of the copper-zinc-dependent superoxide dismutase messenger RNA is higher in pigmented mesencephalic dopaminergic neurons as compared to non-pigmented neurons. Wether this reflects a protective response to elevated metabolic fluxes of superoxide in pigmented neurons, or represents rather a vulnerability factor, causing the accumulation of toxic levels of hydrogen peroxide, remains a major focus for future research.

- Aime S, Fasano M, Bergamasco B, Lopiano L, Valente G.
Evidence for a glycidic-lipidic matrix in human neuromelanin, potentially responsible for the enhanced iron sequestering ability of substantia nigra. J Neurochem 62(1):369-71, 1994.
Abstract: The high-resolution solid-state 13C-NMR spectrum of a neuromelanin specimen (from patients dying from nonneurological diseases) is compared with that obtained from enzymatically prepared dopamine- melanin . The main differences between the two spectra suggest the occurrence in neuromelanin of a glycidic/lipidic matrix tightly associated with the melanin macromolecule. Atomic emission spectroscopy revealed high iron content (1.5%) in the neuromelanin specimen, in full agreement with previous reports. These observations support the view that neuromelanin acts as a strong chelating (and insolubilizing) system for iron ions and further suggest that the attack to this compact composite substrate may be an important step to allow the release of iron ions responsible for the increased lipid peroxidation reported in the pathogenesis of Parkinson's disease.

- Chiueh CC, Murphy DL, Miyake H, Lang K, Tulsi PK, Huang SJ.
Hydroxyl free radical (OH) formation reflected by salicylate hydroxylation and neuromelanin. In vivo markers for oxidant injury of nigral neurons. Ann N Y Acad Sci 679:370-5, 1993.

- Du XP.
Kanamycin ototoxicity and melanin in the inner ear. Chung Hua Erh Pi Yen Hou Ko Tsa Chih 28:14-6,58, 1993.
Abstract: In order to clarify the relationship between ototoxicity of aminoglucoside and inner ear melanin, we have undergone observations on the cochleas of albino and pigmented guinea pigs with surface preparation, paraffin section. SEM and ECochG following chronic administration of kanamycin. The damage in the pigmented animal was more serious than that of the albino ones. It seems that the cochlea of the pigmented animal is more susceptible to kanamycin than that of the albino ones. It suggests that melanin may be implicated in the ototoxicity of aminoglucoside antibiotics that might be due to its capacity to take and accumulate the drug.

- Enochs WS, Nilges MJ, Swartz HM.
Purified human neuromelanin, synthetic dopamine melanin as a potential model pigment, and the normal human substantia nigra: characterization by electron paramagnetic resonance spectroscopy. J Neurochem 61:68-79, 1993.

- Herrero MT, EC, Hirsch Kastner A, Ruberg M, Luquin MR, Laguna J, Javoy-Agid F, Obeso JA, Agid Y.
Does neuromelanin contribute to the vulnerability of catecholaminergic neurons in monkeys intoxicated with MPTP? Neuroscience, 56(2):499-511, 1993.
Commentary: The present literature update features a paper by Herrero et al. addressing a critical issue in research on Parkinson's disease: Is neuromelanin a contributory factor in MPTP-induced parkinsonism. The study, carried out on monkeys chronically exposed to the neurotoxin, contributes to a vast literature on the subject, and provides evidence for a greater vulnerability of pigmented dopaminergic neurons to MPTP-toxicity with respect to non-pigmented neurons. From this, it is inferred that neuromelanin plays a role in MPTP-induced parkinsonism. A word

of caution is however in order. It is now established that MPTP is oxidatively activated by MAO-B in extranigrostriatal compartments, e.g. glial cells, to give MPP^+. This latter then enters the dopaminergic neurons via the dopamine uptake system and interacts with mitochondrial Complex I, inducing various noxious effects, such as an increased susceptibility of the Complex to oxidative damage and augmented free radical generation. Several lines of evidence suggest, on the other end, that neuromelanin formation is but an epiphenomenon of a defect in the antioxidant defenses of certain populations of dopaminergic neurons, whereby a small but significant portion of the intraneuronal dopamine pool does not survive oxidative stress and is slowly consumed to produce the pigment. In the light of the foregoing, it is conceivable that in neuromelanin-containing neurons, a defect in the antioxidant armamentarium reduces the cellular ability to counteract MPP^+ toxicity, thereby accounting for enhanced susceptibility to MPTP. if so, this can be taken to mean that the preferential loss of pigmented vs. non-pigmented neurons after MPTP intoxication is per se not a sufficient basis for postulating a role of neuromelanin: most likely, the pigment is only a visible indicator of a metabolic deficiency which may be the actual factor underlying selective vulnerability.

- Herrero MT, Hirsch EC, Kastner A, Luquin MR, Javoy-Agid F, Gonzalo LM, Obeso JA, Agid Y.
Neuromelanin accumulation with age in catecholaminergic neurons from Macaca fascicularis brainstem. Dev Neurosci, 15(1):37-48, 1993.
Abstract: Neuromelanin (NM) is an auto-oxidation by-product of catecholamine synthesis which is observed almost exclusively in primates. We have estimated the distribution and the number of NM-positive neurons of the upper brainstem and the degree of their melanization from birth to the onset of senescence in 5 monkeys (Macaca fascicularis) aged 0, 1.5, 3.5, 8 and 13 years. Series of sections taken at 640-microns intervals were examined either unstained to detect unstained NM, stained for NM with Masson silver impregnation or processed by tyrosine hydroxylase (TH) immunohistochemistry to analyze catecholaminergic neurons. The proportion of NM-containing cells among TH-positive neurons varied from one catecholaminergic region to another: low in the hypothalamus and central gray substance (cgs); moderate in the cell group A8, and high in the ventral tegmental area (VTA), locus coeruleus (LC) and substantia nigra (SN). TH-positive neurons were detected in the SN, VTA, catecholaminergic cell group A8, LC, cgs and hypothalamus. At birth, although no unstained NM-positive neurons were detected, Masson-stained cells were observed, though only in the LC. At 1.5 and 3.5 years, Masson-positive neurons were observed despite the absence of visible pigment. At 8 and 13 years, unstained NM was present in Masson-positive neurons. The number of unstained NM-positive neurons and Masson-positive neurons and the amount of NM per neuron increased with age in each subregion studied. Nevertheless, some TH-positive neurons were found to be without NM. The data indicate a differential increased NM content with age in the neurons of midbrain catecholaminergic cell groups. However, its functional significance remains to be determined.

- Howard MJ, Gershon MD.
Role of growth factors in catecholaminergic expression by neural crest cells: in vitro effects of transforming growth factor beta 1. Dev Dyn 196:1-10, 1993.
Abstract: The differentiation of neural crest cells into catecholaminergic neurons is dependent upon both intrinsic properties and signals from the embryonic microenvironment. In tissue culture, the development of catecholaminergic traits is dependent upon factors present in chick embryo extract (CEE). This dependency suggests that soluble growth factors affect catecholaminergic differentiation in vivo. We have studied the role of CEE-derived factors and the potentially related influence of characterized growth factors on catecholaminergic phenotypic expression in avian neural crest cells. In this report, we show that CEE-derived factors and transforming growth factor beta1 (TGF-beta 1) differentially influence catecholaminergic phenotypic expression as well as melanogenesis. TGF-beta 1 substituted for CEE-derived factors and supported the in vitro differentiation of tyrosine hydroxylase (TH) and dopamine-beta-hydroxylase (DBH) immunoreactivities, as well as catecholamine biosynthesis and storage. Differentiation of catecholaminergic cells was dependent on factors present in 10% CEE during the first 1-4 days in culture suggesting an initial critical period for exposure. One day of initial exposure to either CEE-derived factors or TGF-beta 1 was sufficient to support the subsequent expression of catecholaminergic phenotypic characteristics. The time course of responsiveness to TGF-beta 1 was different than for CEE-derived factors. Neural crest cells remain responsive to TGF-beta 1 for at least 5 days, which is past the critical period for CEE-derived factors. Bioassay of CEE shows that endogenous levels of TGF-beta are less than or equal to 0.5 ng/ml. Immunoprecipitation of TGF-beta from CEE or blockade by neutralizing antibodies did not result in a loss of catecholaminergic differentiation by neural crest cells. Although CEE supports melanogenesis under all of the growth conditions tested, TGF-beta 1 was found to be inhibitory.

- Nikiforidis GC, Tsambaos DG, Karamitsos DS, Koutsojannis CC, Georgiou SV.
Abnormalities of the auditory brainstem response in vitiligo. Scand Audiol 22:97-100, 1993.
Abstract: Accumulating evidence suggests that vitiligo is a systemic disease affecting the entire pigmentary system

and possibly the melanin-containing cellular elements of the nervous system. In the present paper we comparatively study the auditory brainstem response (ABR) of 30 patients with active vitiligo and 50 healthy human subjects in order to detect possible subclinical abnormalities of the auditory system in this disorder. Our findings reveal a statistically significant ($p < 0.01$) decrease of the I peak latency and a statistically significant ($p < 0.01$) increase of the I-III interpeak latency in the patients as compared to the controls. The decrease of the first peak latency may be due to a numerical decrease of active melanocytes in the inner ear which results in an impairment of the ion exchange between the endolymph and perilymph. The increase of the I-III interpeak latency may be explained in terms of an abnormal synaptic activity and transmission of the action potential from the auditory nerve to the superior olive.

- Oertel WH, Kupsch A.
Pathogenesis and animal studies of Parkinson's disease. Curr Opin Neurol Neurosurg 6:323-32, 1993.

- Touzani K, Tramu G, Nahon JL, Velley L.
Hypothalamic melanin-concentrating hormone and alpha-neoendorphin-immunoreactive neurons project to the medial part of the rat parabrachial area. Neuroscience 53:865-76, 1993.

- Zecca L, Swartz HM.
Total and paramagnetic metals in human substantia nigra and its neuromelanin. J Neural Transm Park Dis Dement Sect 5: 203-13 1993.

- Zhang P, Damier P, Hirsch EC, Agid Y, Ceballos-Picot I, Sinet PM, Nicole A, Laurent M, Javoy-Agid F.
Preferential expression of superoxide dismutase messenger RNA in melanized neurons in human mesencephalon. Neuroscience 55:167-75, 1993.

6. Genetics, molecular biology

(Comments by Dr B. Bouchard)

- Adema GJ, de Boer AJ, van 't Hullenaar R, Denijn M, Ruiter DJ, Vogel AM, Figdor CG.
Melanocyte lineage-specific antigens recognized by monoclonal antibodies NKI-beteb, HMB-50, and HMB-45 are encoded by a single cDNA. Am J Pathol 143(6):1579-85, 1993.
Abstract: The glycoproteins recognized by monoclonal antibody (MAb) NKI-beteb are among the best diagnostic markers for human melanoma. MAb NKI-beteb reacts with melanoma cells throughout tumor development and does not cross-react with other tumor or normal cells, except for cells of the melanocytic lineage. Two other melanocyte lineage-specific MAbs, HMB-50 and HMB-45, show a specificity and staining pattern strikingly similar to the ones observed for NKI-beteb. Herein, we demonstrate that all three MAbs recognize protein products encoded by a single cDNA. Expression of this cDNA in BLM cells results in immunoreactivity with all three MAbs. In addition, we demonstrate co-distribution of the RNA species detected by the cDNA with the proteins recognized by the MAbs in tissue sections.

- Ando O, Mishima Y, Hanada S, Suemoto Y, Atobe J, Kurimoto M.
Analyses of mixed melanogenesis in tyrosinase cDNA-transfected human amelanotic melanoma cells. J Invest Dermatol,101(6):864-70, 1993.

- Breton C, Schorpp M, Nahon JL.
Isolation and characterization of the human melanin-concentrating hormone gene and a variant gene. Brain Res Mol. 18:297-310, 1993.

- Brossart P, Keilholz U, Willhauck M, Scheibenbogen C, Mohler T, Hunstein W.
Hematogenous spread of malignant melanoma cells in different stages of disease. J Invest Dermatol. 101(6):887-9, 1993.

- Dakour J, Vinayagamoorthy T, Jimbow K, Chen H, Luo D, Dixon W, Munoz V.
Identification of a cDNA coding for a Ca(2+)-binding phosphoprotein (p90), calnexin, on melanosomes in normal and malignant human melanocytes. Exp-Cell-Res. 209(2):288-300, 1993.
Abstract: In order to have a proper biosynthesis and secretion of the melanin-pigment granules (melanosomes) the melanocyte may require a melanosome-associated molecule that provides a signal for assembly and organization of melanogenic enzymes and proteins within the compartment of melanosomes. This study reports the presence

of a Ca(2+)-binding phosphoprotein, p90, which can be engaged in such melanogenic function, located on the melanosomal membrane of human melanocytes. A human melanoma cDNA expression library in lambda Zap II was screened with a rabbit polyclonal antibody raised against human melanosomes isolated from cultured human melanoma cells, SK MEL 23. A cDNA encoding a melanosomal protein, M(r) 90 kDa, was identified through this immunoscreening. A partial sequencing of nucleotides (822 bp from the N-terminal domain) of this clone (3.8 kb) and predicted amino acids showed more than 90% homology with dog calnexin, a previously reported endoplasmic reticulum (ER) transmembrane protein. A fusion protein of this p90 with beta-galactosidase expressed in Escherichia coli revealed both the immuno-cross-reactivity with anti-dog calnexin and anti-human melanosome antibodies and the Ca(2+)-binding property. Upon immunohistochemistry, the anti-dog calnexin antibody revealed the positive immunoreactivities with both normal and malignant human melanocytes, showing a much higher expression of antigenic epitope than nonmelanocytic human cells. The laser scanning confocal immunofluorescence, using an antibody against a human melanosome-specific antigen (HMSA-5), and immunoelectron microscopy, using immunogold, confirmed the major localization of anti-dog calnexin antibody epitope on the melanosomes and ER.

- Duttlinger R, Manova K, Chu TY, Gyssler C, Zelenetz AD, Bachvarova RF, Besmer P.
W-sash affects positive and negative elements controlling c-kit expression: Ectopic c-kit expression at sites of kit-ligand expression affects melanogenesis. Development (Cambridge, UK) 118(3):705-17, 1993.
Abstract: The receptor tyrosine kinase c-kit and its cognate ligand KL are encoded at the white spotting (W) and steel (Sl) loci of the mouse, resp. Mutations at both the W and the Sl locus cause deficiencies in gametogenesis, melanogenesis and hematopoiesis (erythrocytes and mast cells). The W-sash mutation differs from most W mutations in that it affects primarily mast cells and melanogenesis but not other cellular targets of W and Sl mutations. Thus, Wsh/Wsh mice are fertile and not anemic, but they lack mast cells in their skin and intestine and are devoid of coat pigment. Heterozygotes are black with a broad white sash/belt in the lumbar region. To det. the basis for the phenotypes of W-sash mice, the authors investigated c-kit RNA and protein expression patterns in adult Wsh/Wsh mice and during embryonic development. The authors show that c-kit expression is absent in bone-marrow-derived Wsh/Wsh mast cells, the fetal and the adult lung, and the digestive tract at embryonic day 13 1/2 (E13 1/2), tissues that normally express c-kit. Unexpectedly, in E10 1/2 and 11 1/2d Wsh/Wsh embryos, the authors found c-kit expression in the dermatome of the somites, the mesenchyme around the otic vesicle and the floorplate of the neural tube, structures known to express the c-kit ligand in wild-type embryos. The ectopic c-kit expression in Wsh homozygous embryos does not affect c-kit ligand expression. The presumed Wsh/Wsh melanoblasts appeared to be normal and, at E10 1/2, similar nos. were found in normal and homozygous mutant embryos. At E13 1/2 +/+ embryos had a graded distribution of melanoblasts from cranial to caudal with a min. in the lumbar region. Whereas E13 1/2 homozygous Wsh/Wsh embryos essentially lacked c-kit-pos. cells in the skin, E13 1/2 heterozygous Wsh/+ embryos had reduced nos. of melanoblasts compared to +/+ with few or none in the lumbar region (future sash). It is proposed that ectopic c-kit expression in the somitic dermatome affects early melanogenesis in a dominant fashion. Mol. anal. of Wsh chromosomal DNA revealed a deletion or rearrangement in the vicinity of the c-kit gene. These results provide an explanation for the Wsh phenotype and have implications for the control of c-kit expression.

- Easty DJ, Ganz SE, Farr CJ, Lai C, Herlyn M, Bennett-DC.
Novel and known protein tyrosine kinases and their abnormal expression in human melanoma. J Invest Dermatol. 101(5):679-84, 1993.

- Fuqua WC, Weiner RM.
The melA gene is essential for melanin biosynthesis in the marine bacterium Shewanella colwelliana. J Gen Microbiol 1395:1105-14, 1993.
Commentary: The presence of a single gene, melA, previously characterized in the marine bacterium Shewanella colwelliana, has been shown to be an essential requirement for melanin synthesis. MelA can completent null mutants unable to synthesize the pigment, and antibodies confirm the presence of the melA gene product in the wild type S. colwenallia. However, the expression of this gene does not appear to control the amount of melanin produced.

- Kimura N, Tsuge T.
Gene cluster involved in melanin biosynthesis of the filamentous fungus Alternaria alternata. J Bacteriol 175: 4427-35, 1993.
Commentary: Three genes shown to be essential for melanin synthesis in the fungus Alternaria alternata have been recently isolated. The expression of theses genes correlate with the presence of mycelial melanization. Furthermore, expression of BRM1, BRM2 and ALM restore the wild type phenotype in Brm1-(light brown), Brm2- (brown) and Alm-(albino) mutants, respectively. Future studies will be of interest to determine the similarities between these

genes and the corresponding mammalian genes.

- Lee ST, Strunk KM, Spritz RA.
A survey of protein tyrosine kinase mRNAs expressed in normal human melanocytes. Oncogene. 8(12):3403-10, 1993.
Abstract: We have used the reverse transcription-polymerase chain reaction to survey the repertoire of protein tyrosine kinases expressed in cultured normal human melanocytes, a differentiated cell type derived from the neural crest. We identified 25 different tyrosine kinase cDNAs among a total of 608 protein tyrosinase kinase-related cDNAs analyzed. Six encode receptor tyrosine kinases for known ligands, several of which have been implicated in controlling melanocyte proliferation in vitro. Two others encode apparent receptor tyrosine kinases for unknown ligands. Four encode known non-receptor tyrosine kinases and five encode previously identified anonymous protein tyrosine kinases. Of the eight other melanocyte-associated protein tyrosine kinases, most or all appear to be novel. These 25 protein tyrosine kinase genes exhibit distinct patterns of expression in cultured human melanocytes, human erythroleukemia cells, and a variety of normal human tissues. We mapped 16 of the corresponding protein tyrosine kinase genes to specific human chromosomes, identifying a total of 19 human genetic loci, some of which may constitute candidate genes for genetic disorders of mammalian development.

- Melani C, Silvani A, Parmiani G, Colombo MP.
Lymphotoxin gene expression by melanocytes and melanoma cell lines and persistence of unspliced mRNA. FEBS Lett. 335(1):114-8,1993.

- Oetting WS, Stine OC, Townsend D, King RA.
Evolution of the tyrosinase related gene (TYRL) in primates. Pigment Cell Res 6(3):171-7, 1993.

- Presse F, Nahon JL.
Differential regulation of melanin-concentrating hormone gene expression in distinct hypothalamic areas under osmotic stimulation in rat. Neuroscience, 55(3):709-20, 1993.

- Shattuck RL, Wood LD, Jaffe GJ, Richmond A.
MGSA/GRO transcription is differentially regulated in normal retinal pigment epithelial and melanoma cells. Mol Cell Biol. 14(1):791-802, 1994.

- Spritz RA.
Molecular genetics of oculocutaneous albinism. Semin Dermatol, 12(3):167-72, 1993.
Abstract: Oculocutaneous albinism (OCA) is a group of autosomal recessive disorders characterized by deficient synthesis of melanin pigment. Type I (tyrosinase-deficient) OCA results from deficient enzymatic activity of tyrosinase, which catalyzes at least three steps in the melanin biosynthetic pathway. Type II (tyrosinase-positive) OCA results from abnormalities of the "P" polypeptide. Recent application of molecular genetic techniques to the study of these disorders has led to extraordinary advances in knowledge of their molecular pathogenesis, paving the way to improved diagnosis, carrier detection, and even treatment.

- Tripathi RK, S, Bundey Musarella MA, Droetto S, Strunk KM, SA, Holmes Spritz RA.
Mutations of the tyrosinase gene in Indo-Pakistani patients with type I (tyrosinase-deficient) oculocutaneous albinism (OCA). Am J Hum Genet 53(6):1173-9, 1993.
Abstract: Oculocutaneous albinism (OCA) is a group of autosomal recessive disorders characterized by deficient synthesis of melanin pigment. Type I (tyrosinase-deficient) OCA results from mutations of the tyrosinase gene (TYR gene) encoding tyrosinase, the enzyme that catalyzes the first two steps of melanin biosynthesis. Mutations of the TYR gene have been identified in a large number of patients, most of Caucasian ethnic origin, with various forms of type I OCA. Here, we present an analysis of the TYR gene in eight Indo-Pakistani patients with type I OCA. We describe four novel TYR gene mutations and a fifth mutation previously observed in a Caucasian patient.

- Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ.
Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell 75(2):229-40, 1993.
Abstract: bcl-2-/-mice complete embryonic development, but display growth retardation and early mortality postnatally. Hematopoiesis including lymphocyte differentiation is initially normal, but thymus and spleen undergo massive apoptotic involution. Thymocytes require an apoptotic signal to manifest accelerated cell death. Renal failure results from severe polycystic kidney disease characterized by dilated proximal and distal tubular segments and hyperproliferation of epithelium and interstitium. bcl-2-/-mice turn gray with the second hair follicle cycle,

implicating a defect in redox-regulated melanin synthesis. The abnormalities in these loss of function mice argue that Bcl-2 is a death repressor molecule functioning in an antioxidant pathway.

- Wang LR, Crossland JP, Dawson WD.
Coat color genetics of Peromyscus: II. Tan streak--a new recessive mutation in the deer mouse, P. maniculatus. J Hered 84:304-6, 1993.
Commentary: Wang et al. report an interesting new mutation in the deer mouse. The locus affected by this new recessive mutation (tns) has not been identified, but cross studies rule out loci presenting with previously characterized mutations. Further studies defining the nature of this mutation and its implications for the genetic control of melanogenesis should be of interest to the field of pigmentation.

- Winder AJ, Wittbjer A, Rosengren E, Rorsman H.
Fibroblasts expressing mouse c locus tyrosinase produce an authentic enzyme and synthesize phaeomelanin. J Cell Sci 104:467-75, 1993.
Commentary: A paper by AJ.Winder et al. reports the study of the enzyme tyrosinase, the product of the c-albino locus, in transfected cells. This study confirms previous work demonstrating that tyrosinase is stably expressed in fibroblasts, is catalycally active and can induce eumelanin synthesis. The authors further demonstrate that translational or post-translational modifications can occur in transfected fibroblasts through mecanisms similar to those existing in melanocytic cells.

- Winder AJ, Wittbjer A, Rosengren E, Rorsman H.
The mouse brown (b) locus protein has dopachrome tautomerase activity and is located in lysosomes in transfected fibroblasts. J Cell Sci 106(Pt 1):153-66, 1993.
Abstract: Many genes mapping to pigmentation loci are involved in the regulation of melanin synthesis in the mouse. The brown (b) locus controls black/brown coat coloration, and its product has significant homology to the key melanogenic enzyme tyrosinase. This has led to suggestions that the b-protein is itself a melanogenic enzyme. In order to investigate its function, we have established lines of mouse fibroblasts stably expressing the b-protein by co-transfection of a b-protein expression vector and a plasmid conferring resistance to the antibiotic G418. The b-protein synthesised by these cells has the expected molecular mass of 75 kDa and reacts with three different anti-b-protein antibodies. We were unable to confirm previous reports that the b-protein has tyrosinase or catalase activity, but detected stereospecific dopachrome tautomerase activity in b-protein-expressing fibroblasts. This dopachrome tautomerase binds to Concanavalin A-Sepharose, and the major product of its action on L-dopachrome is 5,6-dihydroxyindole-2- carboxylic acid. Since this activity is not present in untransfected cells we conclude that the b-protein has dopachrome tautomerase activity. Fibroblasts do not contain melanosomes, the specialised organelles in which the b-protein is located in melanocytes. Nevertheless, indirect immunofluorescence localisation of the b-protein in transfected fibroblasts produces a distinctive pattern of intense juxtanuclear staining combined with punctate cytoplasmic staining. Double-labelling shows co-localisation of the b-protein with the late endosomal/lysosomal markers beta-glucuronidase and LAMP-1, both in transfected fibroblasts and in mouse melanoma cells. These findings are consistent with the hypothesis that melanosomes are closely related to lysosomes.

- Yoshii T, Tamura K, Taniguchi T, Akiyama K, Ishiyama I.
Water-soluble eumelanin as a PCR-inhibitor and a simple method for its removal. Nippon Hoigaku Zasshi 47(4):323-9, 1993.
Abstract: It has been confirmed that water-soluble eumelanins often extracted together with DNAs from natural black hairs act as an inhibitor of Taq DNA polymerase in the polymerase chain reaction (PCR). In the present investigation, an attempt to amplify the non-coding 333-bp region of mitochondrial DNA (mt333DNA) produced the following results: 1) Water-soluble preparations made from chemically synthesized melanin (Sigma products), as well as natural black eumelanins , inhibited the PCR amplification of mt333DNA at concentrations of more than 2 micrograms/ml. 2) Quantitative measurement of Taq DNA polymerase-catalyzed DNA synthesis in terms of the amount of [alpha-32P] dCMP incorporated into activated calf thymus DNA showed that both of the water-soluble melanins had the same inhibition activity as represented by the sigmoidal curve derived from a quadratic equation of melanin concentration. This observation suggested that Taq DNA polymerase combined with two molecules of melanin to form an inactivated complex. 3) Melanins did not appear to affect either the thermostability of Taq DNA polymerase at 94 degrees C, or the step of primer-annealing to template DNAs. On the other hand, we established a simple and useful method for removal of water-soluble eumelanins contaminating DNA preparations from hairs. The method was based on the adsorption of melanins to Bio-Gel. When a Bio-Gel P-60 minicolumn was equilibrated with 10 mM sodium acetate buffer, pH 4.2, water-soluble melanins were completely adsorpted to it whereas DNAs passed through, although the melanins showed incomplete adsorption to the gel when it was

equilibrated with TE (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA).

7. Tyrosinase, TRP1, TRP2 and other enzymes

Comments by Prof. J.C. Garcia-Borron:

The fact that three of the papers summarized below address the role of tyrosinase and TRP1 in the formation of eumelanins and phaeomelanins reflects an increasing interest not only for the role of these proteins but also for the control of the switch between eu and phaeomelanogenesis. The paper by del Marmol et al (FEBS Lett 327:307-10) is particularly relevant, since the behaviour of a variety of human pigment cell lines is compared. The authors present clear cut evidence pointing to an important role of TRP1 in eumelanogenesis. This "eumelanogenic" role of TRP1 has also been demonstrated in the B16 mouse melanoma cell model by Kumuzaki et al. (Exp Cell Res 207:33-40). Conversely, Winder et al. (J. Cell Sci. 104:467-475) demonstrate that fibroblasts expressing the c locus tyrosinase produce phaeomelanin. This is an interesting work since transfection is used in combination with techniques that allow for the characterization of key melanogenic intermediates. Taken together, these papers could lead to the hypothesis that the c locus tyrosinase is a phaeomelanogenic enzyme, whereas the b protein, TRP1, may direct melanin biosynthesis towards eumelanogenesis. Interestingly, it has been shown by Bennett and coworkers that b mutant melanocytes expressing a functional c protein synthesize a brown pigment, that switches to black upon transfection with a vector able to direct the expression of the b protein (Bennett et al, 1990, Development 110:471-5). However, the increasing evidence pointing to an important role of TRP1 in eumelanogenesis will be difficult to assess until the actual catalytic potentials of the b protein are definitively established.
Two other relevant papers deserve a brief comment. Park et al (J. Biol. Chem. 268:11742-9) demonstrate that in normal human melanocytes protein kinase C (PK C) activates tyrosinase and melanogenesis. This is not the first report pointing to an activatory role of PK C and/or phorbol esters in human melanogenesis, which, therefore, appears to be proved. However, an inhibitory effect or phorbol esters and/or PKC in mouse melanoma cells has been demonstrated by several authors. Are these differences due to the use of mouse versus human melanocytes or do they indicate that melanin synthesis regulation is different in malignant melanocytes as opposed to normal melanocytes? In any case, the observation of an activatory role for PK C in normal human cells emphasizes the need of caution for the extrapolation of results obtained either in mouse melanocytes or in malignant melanocytes to the situation in normal human cells.
Finally, the paper by Chen et al (J. Biol. Chem. 268:18710-6) is the first demonstration of the involvement of a chaperone in the correct folding of tyrosinase. Since the experimental model used in this work is Streptomyces antibioticus, the situation in mammalian cells is still completely unknown. However, the observation that the correct folding and binding of copper is complex and dependent on a specific chaperone may help to explain why transfection experiments often lead to ambiguous results. It will be important to extend these studies to mammalian cells.

- Chen LY, Chen MY, Leu WM, Tsai TY, Lee YH.
Mutational study of Streptomyces tyrosinase trans-activator MelC1. MelC1 is likely a chaperone for apotyrosinase.
J Biol Chem 268:18710-6, 1993.

- Chiu E, Lamoreux ML, Orlow SJ.
Postnatal ocular expression of tyrosinase and related proteins: disruption by the pink-eyed unstable (p(un)) mutation. Exp Eye Res. 57(3):301-5, 1993.
Abstract: Ocular pigmentation in the mouse occurs primarily postnatally as a result of the melanization of neural crest-derived melanocytes. Using immunologic and biochemical techniques, we demonstrate that in normal mice the expression of tyrosinase and the related proteins TRP-1 and TRP-2, rises during the first week of life, remains elevated for a week, and then steadily declines to low levels by adulthood. Sucrose gradient density centrifugation demonstrates that tyrosinase, TRP-1 and TRP-2 are present in high molecular weight forms in the eyes of wild-type mice. The normal time course is disrupted in mice carrying the pink-eyed unstable (p(un)) mutation at the P-locus, a model for tyrosinase-positive albinism in man. Tyrosinase and TRP-2 are present at wild-type levels in the eyes of p(un)/p(un) mice at birth, but, rather than rising, their levels rapidly decline over the first week of life. TRP-1 is almost undetectable, even at birth. High molecular weight complexes could not be detected in eyes of p(un)/p(un) mice. Our results suggest that postnatal ocular melanogenesis in the mouse presents an attractive model for the study of the orderly expression and action of the proteins involved in eumelanin synthesis, and that the p(un) mutation disrupts this temporally controlled process.

- De bruyn A, Mendelbaum K, Sandkuijl LA, Delvenne V, Hirsch D, Staner L, Mendlewicz J, Van Broeckhoven C.

Nonlinkage of bipolar illness to tyrosine hydroxylase, tyrosinase, and D2 and D4 dopamine receptor genes on chromosome 11. Am J Psychiatry. 151(1):102-6, 1994.
Abstract: OBJECTIVE: Previous linkage and allelic association studies using DNA polymorphisms, cosegregation of cytogenetic abnormalities with psychiatric illness, and assignment of genes involved in neutotransmitter metabolism suggested that chromosome 11 may harbor a gene predisposing to bipolar illness. The authors examined linkage in the families of 14 probands with bipolar illness, with the candidate genes tyrosine hydroxylase (TH), D4 dopamine receptor (DRD4) at 11p15, tyrosinase (TYR) at 11q14-q21, and D2 dopamine receptor (DRD2) at 11q22-q23, as well as with the c-Harvey-ras oncogene (HRAS) and insulin gene (INS), both located at 11p15, a region that previously showed linkage to bipolar illness. METHOD: The genetic data were analyzed with both lod score analysis (parametric) and affected-sib-pair analysis (nonparametric); both narrow and broad definitions of the clinical phenotype were used. Further influences of diagnostic uncertainties were accounted for by using diagnostic probability classes weighing the stability of each phenotype. RESULTS: Two-point linkage results excluded close linkage of bipolar illness to each candidate gene; negative results were also obtained when the narrow definition of the clinical phenotype was used. Moreover, multipoint linkage analysis of HRAS and INS excluded the 11p15 region encompassing both DRD4 and TH. In agreement with the negative linkage results, affected-sib-pair analysis did not show preferential sharing of marker alleles at any of the candidate genes. CONCLUSIONS: The negative results obtained under different genetic models exclude a frequent role for DRD4, TH, TYR, and DRD2 in the pathogenesis of bipolar illness.

- del Marmol V , Solano F, Sels A, Huez G, Libert A, Lejeune, Ghanem G.
Glutathione depletion increases tyrosinase activity in human melanoma cells. J Invest Dermatol 101(6):871-4, 1993.

- del Marmol V, Ito S, Jackson I, Vachtenheim J, Berr P, Ghanem G, Morandini R, Wakamatsu K, Huez G.
TRP-1 expression correlates with eumelanogenesis in human pigment cells in culture. FEBS Lett 327:307-10, 1993.

- Jacobsohn GM, Iskandar R, Jacobsohn MK.
Activity of mushroom tyrosinase on catechol and on a catechol estrogen in an organic solvent. Biochim Biophys Acta 1202(2):317-24, 1993.

- Kuzumaki T, Matsuda A, Wakamatsu K, Ito S, Ishikawa K.
Eumelanin biosynthesis is regulated by coordinate expression of tyrosinase and tyrosinase-related protein-1 genes. Exp Cell Res 207:33-40, 1993.

- Labudova O, Kollarova M.
Thioredoxin-reductase: structure, properties, and function. Biokhimiia 58(8):1240-6, 1993.
Abstract: Thioredoxin reductase (TR-RED) pertains to the family of pyridine nucleotide disulfide oxidoreductases distinguished by their remarkable structural homology. The enzyme is a constituent component of the thioredoxin complex which is present in all types of organisms and is universal in respect of the numerous physiological functions it performs. The ability of TR-RED to protect the skin from UV-generated free oxygen species has been found. Owing to its ability to control melanin biosynthesis, the enzyme "doses" the suntan.

- Odh G, Hindemith A, Rosengren AM, Rosengren E, Rorsman H.
Isolation of a new tautomerase monitored by the conversion of D-dopachrome to 5,6-dihydroxyindole. Biochem Biophys Res Commun 197(2):619-24, 1993.
Commentary: The paper by Odh et al deserves a word of caution concerning the name assigned to the new enzyme acting on D-Dopachrome. Since the product of the reaction, identified by HPLC, is dihydroxyindole (DHI), the reaction catalysed is not an isomerization , but rather a decarboxylative rearrangement. The enzyme cannot, therefore, be described as a tautomerase, i.e. as a special isomerase catalyzing the interconversion between two tautomers. The name dopachrome tautomerase should be reserved for the enzyme transforming dopachrome into its tautomeric form, DHICA, and in the case of the new enzyme, it should be substituted by a name describing more closely the reaction catalysed.

- Park HY, Russakovsky V, Ohno S, Gilchrest BA.
The beta isoform of protein kinase C stimulates human melanogenesis by activating tyrosinase in pigment cells. J Biol Chem 268:11742-9, 1993.

- Rauth S, Davidson RL.
Suppression of tyrosinase gene expression by bromodeoxyuridine in Syrian hamster melanoma cells is not due to

its incorporation into upstream or coding sequences of the tyrosinase gene. Somat Cell Mol Genet 19:285-93, 1993.
Abstract: 5-Bromodeoxyuridine (BrdU), a thymidine analog, suppresses melanogenesis in Syrian hamster melanoma cells. Tyrosinase, which is the key enzyme for the synthesis of melanin , is suppressed by exposure to BrdU, and the drop in enzyme activity is correlated with a drop in tyrosinase mRNA level. In order to investigate whether suppression of tyrosinase mRNA by BrdU is due to BrdU substitution into coding sequences or upstream sequences of the tyrosinase gene, we carried out stable and transient transfection assays with constructs containing either the human tyrosinase cDNA sequence under the control of a nontyrosinase promoter or a chloramphenicol acetyltransferase (CAT) reporter gene under the control of 5' flanking sequences of the mouse tyrosinase gene. When the plasmid containing the tyrosinase cDNA was stably transfected into mouse fibroblasts, tyrosinase activity in the transfectants was not suppressed by BrdU. Since BrdU would be incorporated into the tyrosinase cDNA integrated in these transfectants, the results suggest that BrdU suppression of tyrosinase gene expression is not due to its incorporation into coding sequences of the tyrosinase gene. When plasmids with tyrosinase regulatory sequences were transfected into melanoma cells for transient expression assays, CAT gene expression was suppressed by BrdU. Because the CAT plasmids do not contain a mammalian origin of replication and should not replicate under the conditions of transient transfection, BrdU would not be incorporated into the DNA of those plasmids. Therefore, these results suggest that the suppression of tyrosinase gene expression by BrdU also is not due to the incorporation of BrdU into upstream sequences of the tyrosinase gene.

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Specific tyrosinases associated with melanoma replicative senescence and melanogenesis. Cancer Res. 53:2469-2471, 1993.

- Valverde P, Jimenez Cervantes C, Salinas C, Garcia-Borron JC, Solano F, Lozano JA.
Preparation of purified tyrosinase devoid of dopachrome tautomerase from mammalian malignant melanocytes. Pigment Cell Res. 6(3):158-64, 1993.

- Vass E, Nappi AJ, Carton Y.
Alterations in the activities of tyrosinase, N-acetyltransferase, and tyrosine aminotransferase in immune reactive larvae of Drosophila melanogaster. Dev Comp Immunol 17:109-18, 1993.

- Winder AJ, Wittbjer A, Rosengren E, Rorsman H.
Fibroblasts expressing mouse c locus tyrosinase produce an authentic enzyme and synthesize phaeomelanin. J. Cell Sci. 104:467-475, 1993.

8. Melanoma and other pigmented tumours

(Comments by Dr R. Peter)

- Abdel Wahab ZA, Darrow TL, Vervaert CE, Giannopoulou AA, Li W, Seigler HF.
Inhibition of the growth of human melanoma metastases in nude mice by melanoma-specific murine monoclonal antibody. Surg-Oncol. 1(2):115-25, 1992.
Abstract: The administration of anti-melanoma murine monoclonal antibody (MAB) 16.C8 (IgG2a) to nude mice bearing established human melanoma lung or liver metastases resulted in a significant inhibition of tumour growth. A total dose of 2 mg of affinity purified 16.C8 caused complete inhibition of tumour growth in 89 and 100% of animals in the liver and lung model, respectively. In contrast, a significant tumour growth was found in most control animals which received an irrelevant IgG2a MAB or 2% human serum albumin in Hanks Balanced Salt Solution (HBSS). The MAB was most effective when treatment was started on day 1 or 4 following tumour inoculation. When the 16.C8 MAB treatment was delayed 7 or 14 days, 33 and 67% of 16.C8 treated animals, respectively, developed tumours. The MAB-mediated anti-tumour activity appeared to be dose dependent, and the effect of a suboptimal dose was potentiated by the concomitant administration of recombinant interleukin 2 (rIL-2). Recombinant IL-2 alone in a similar dose did not elicit comparable anti-tumour activity. Moreover, the MAB 16.C8 inhibited tumour growth in irradiated animals which may suggest the involvement of host-radioresistant cellular elements in the 16.C8 antibody-mediated anti-tumour activities in nude mice. These results suggest that MAB 16.C8 alone or combined with rIL-2 may prove useful in the immunotherapy of metastatic melanoma.

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Overexpression of c-erbB-2 and c-myc but not c-ras, in canine melanoma cell lines, is associated with metastatic potential in nude mice. Anticancer Res. 13(5A):1365-71, 1993.

- Akasaka T, Imamura Y, Kon S.
Multiple agminated juvenile melanoma arising on a hyperpigmented macule. J Dermatol 20(10):638-42, 1993.

- Anstey A, McKee P, Jones EW.
Desmoplastic malignant melanoma: a clinicopathological study of 25 cases. Br J Dermatol 129(4):359-71, 1993.

- Bennett DC.
Genetics, development, and malignancy of melanocytes. Int Rev Cytol 146:191-260, 1993.

- Bjornhagen V, Mansson Brahme E, Lindholm J, Mattsson A, Auer G.
Morphometric, DNA and PCNA in thin malignant melanomas. Med Oncol Tumor Pharmacother 10(3):87-94, 1993.
Abstract: Morphometric assessment of nuclear area, shape and density, nucleolar area, analysis of DNA content and expression of proliferating cell nuclear antigen (PCNA) was performed in a case control study of 72 malignant melanomas, thickness < or = 0.8 mm and Clark level II-III. Twenty-four thin metastasizing melanomas (TMM) were individually compared to two thin non-metastasizing melanomas (TNM) after individual matching for site of primary tumor, tumor thickness, level of invasion, tumor regression and duration of follow-up. Conditional logistic regression analysis with maximum likelihood estimates showed significant differences between TMM and TNM with regard to the nuclear correlation coefficient ($p = 0.005$), standard deviation of nuclear shape NCI ($p = 0.017$), and nuclear density ($p = 0.030$), indicating that thin melanomas with pleomorphic and possibly densely packed nuclei are associated with recurrence. No significant differences were found regarding nuclear or nucleolar area, mean nuclear shape NCI, nuclear DNA content or expression of PCNA.

- Casazza S, Gambini C, Tunesi G, Rovida S, Caruso F, Pastorino A, Canepa M.
Expression of P53 protein in cutaneous melanoma. Pathologica 85(1097):335-42, 1993.
Abstract: P53 protein in cutaneous melanoma. We report the results of an immunohistochemical analysis about the nuclear phosphoprotein P53 expression performed on 48 primary and 10 metastatic cutaneous melanoma in order to assess the prevalence of the expression of mutant P53 protein (m-P53) in this skin tumour. In our study m-P53 was found in about 46% of primary tumours without any significant relationship with the corresponding metastatic lesions. Therefore the P53 count in cutaneous melanoma is not a prognostic marker of tumour spread and aggressiveness.

- Chu L, Abdul A, Takahashi T, Kojima A, Himiya T, Kusama K, Komiyama K, Hori M, Matsumoto M, Tanaka H et al.
Amelanotic melanoma of the oral cavity. J Nihon Univ Sch Dent 35(2):124-9, 1993.
Abstract: A case of amelanotic melanoma arising in the upper molar region, which was difficult to diagnose histologically, is reported. The patient was a 79-year-old woman, who complained of a painful swelling in the gingiva of the left upper molar region. Routine histological examination showed that the lesion was composed of diffusely scattered atypical cells with round, spindle-shaped and irregular nuclei and scanty fibrous connective tissue. A fascicular arrangement was often found in the lesion, and no cancer nests were observed. Immunohistochemical study demonstrated positive staining for S-100 protein in both the nuclei and cytoplasm of the tumor cells. Electron microscopic examination revealed that cell organelles were abundant, and an interrupted basal lamina was often found along the cell membrane. The preliminary diagnosis was a non-epithelial malignant tumor. After surgery, histological examination of metastases in lymph nodes from the submandibular region revealed that the tumor cells contained melanin pigment in the cytoplasm, as confirmed by Masson's melanin stain. The final pathological diagnosis was therefore amelanotic melanoma. Immunohistochemical staining for S-100 protein may be useful for differential diagnosis of amelanotic melanoma in conjunction with electron microscopic examination.

- Damle BD, Sridhar R, Desai PB.
Dipyridamole modulates multidrug resistance and intracellular as well as nuclear levels of doxorubicin in B16 melanoma cells. Int J Cancer. 56(1):113-8, 1994.

- Eisenthal A, Skornick Y, Ron I, Zakuth V, Chaitchik S.
Phenotypic and functional profile of peripheral blood mononuclear cells isolated from melanoma patients undergoing combined immunotherapy and chemotherapy. Cancer Immunol Immunother 37(6):367-72, 1993.
Abstract: In the present study we tested the phenotypic profile as well as several immunological responses of peripheral blood mononuclear cells (PBMC) isolated from melanoma patients. These patients underwent chemotherapy with dacarbazine and carboplatin from day 1 to day 22, followed by immunotherapy of low-dose recombinant interleukin-2 and recombinant interferon alpha administered subcutaneously from day 36 to day 75.

The PBMC from 14 patients were isolated on day 0 before chemotherapy, on day 36 after chemotherapy and on day 76 after immunotherapy. After chemotherapy, a decrease in CD16+ cells and increase in CD3+ and CD4+ cells correlated with a significant decrease in the generation of lymphokine-activated killer (LAK) activity. After immunotherapy, an increase in CD16+ cells correlated with an increase in the induction of LAK activity. A comparison between responding and non-responding patients revealed statistically significant differences in LAK activity of PBMC and response to concanavalin A following chemotherapy, and in the percentage of CD8+ cells following immunotherapy. Our results point toward the value of continuing such a study on a larger population of cancer patients in order to select the appropriate bioassays for monitoring and predicting the clinical responsiveness to combined therapies.

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Melatonin binding sites are functionally coupled to phosphoinositide hydrolysis in Syrian hamster RPMI 1846 melanoma cells. Life-Sci 53(24):PL393-8, 1993.

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Quantification of melanin and iron content in uveal malignant melanomas and correlation with magnetic resonance image. Br J Ophthalmol 77:297-301, 1993.

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A pulsed laser and pulse radiolysis study of amphiphilic chlorophyll derivatives with PDT activity toward malignant melanoma. Photochem Photobiol 58(4):506-11, 1993.
Abstract: Two amphiphilic derivatives of chlorophyll, which have high potential as photodynamic therapy sensitizers for malignant melanoma have been investigated by a combination of laser flash photolysis and pulse radiolysis. It is shown that direct excitation of monomeric forms of these molecules in both hydrophilic and hydrophobic environments produces significant yields of the corresponding triplet states, which have been characterized in terms of spectral and kinetic parameters. In both environments, scavenging of the triplets by oxygen produces singlet oxygen, O2(1 delta g), with essentially unit efficiency as evidenced by time-resolved IR luminescence measurements.

- Fogue Calvo L, Villanueva C, Madrigal L.
Melanocytic schwannoma: a rare tumor originating in the neural crest. Apropos a case associated with Horner's syndrome. Rev Clin Esp 192:386-8, 1993.
Abstract: Schwann cells tumors, producing melanin , are extremely rare and pose difficult problems for its differential diagnoses from melanomas, which are tumors with different prognostic and treatment. In this paper a new case is described, which debuts as Claude-Bernard-Horner syndrome being diagnosed with the help of Immunohistochemistry and Electronic Microscopy.

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Pigmented pulmonary carcinoid tumor. An immunohistochemical and ultrastructural study. Arch Pathol Lab Med 117:832-6, 1993.

- Garbe-C.
Chemotherapy and chemoimmunotherapy in disseminated malignant melanoma. Melanoma Res 3(4):291-9, 1993.

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Modulation by MHC class I antigens of the biology of melanoma cells Non-immunological mechanisms. Melanoma Res 3(4):285-9, 1993.

- Geiger JD, Wagner PD, Shu S, Chang AE.
A novel role for autologous tumour cell vaccination in the immunotherapy of the poorly immunogenic B16-BL6 melanoma. Surg-Oncol 1(3):199-208, 1992.
Abstract: The growth of immunogenic tumours stimulates the generation of tumour-sensitized, but not functional, pre-effector T cells in the draining lymph nodes. These pre-effector cells can mature into effector cells upon in-vitro stimulation with anti-CD3 and IL-2. In the current study, using a defined, poorly immunogenic tumour, B16-BL6 melanoma, the pre-effector cell response was not evident during progressive tumour growth but was elicited by vaccination with irradiated tumour cells admixed with Corynebacterium parvum. After anti-CD3/IL-2 activation, these cells were capable of mediating the regression of established pulmonary metastases. The efficacy of the vaccine depended on the doses of both tumour cells and the adjuvant. While higher numbers of tumour cells were more effective, an optimal dose (12.5 micrograms) of C. parvum was required. The dose of irradiation was not a

critical factor. After vaccination, kinetic studies revealed that the pre-effector cell response was evident 4 days later and declined after 14 days. These observations illustrate the potential role of active immunization in the cellular therapy of cancer.

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Control of metastatic properties of BL6 melanoma cells by H-2Kb gene: immunological and nonimmunological mechanisms. Clin-Exp-Metastasis. 11(6):439-52, 1993.
Abstract: The effect of class I H-2 antigen expression on the metastatic properties of BL6 melanoma cells was investigated. The BL6-8 clone isolated from the highly metastatic BL6 melanoma did not express H-2Kb gene. Following transfection with the H-2Kb gene, BL6-8 cells displayed a low metastatic potential in the immunocompetent as well as immunosuppressed (X-irradiated) or triple-immunodeficient mice with impaired T, B and natural killer (NK) cells function. The expression of H-2Kb gene and the low metastatic ability of transfected BL6 melanoma cells were associated with appearance of cell membrane soybean agglutinin (SBA) and Griffonia simplicifolia 1B4 (GS1B4) lectin-binding carbohydrates. These alterations in cell surface carbohydrates were found to be a result of reduction in sialylation of SBA binding sites and upregulation of the alpha 1.3 galactosyltransferase (alpha 1.3GT) gene. To assess the importance of H-2Kb-induced alterations in cell surface carbohydrates for metastasis formation, BL6-8 melanoma cells were transfected with H-2Kb gene without neor gene cotransfection and selected for adherence to SBA-lectin-conjugated agarose beads. The transfected clones that expressed SBA and GS1B4 lectin-binding carbohydrates were low metastatic. Further analysis of these clones showed that presence of SBA and GS1B4 lectin-binding carbohydrates rather than expression of H-2Kb molecules per se might be responsible for low metastatic potentials of H-2Kb-transfected cells in the immunocompromised mice. Studies of the possible mechanisms responsible for low metastatic ability of H-2Kb-transfected melanoma cells revealed that these cells displayed a reduced ability to adhere to murine pulmonary endothelial cells as well as to laminin and collagen IV. We hypothesized that the observed nonimmunological effects of H-2Kb gene in BL6 melanoma cells is a result of an interaction between the H-2Kb gene and B16 melanoma-specific ecotropic retrovirus. It results in inhibition of this retrovirus production with consecutive alteration in the expression of cellular genes controlling cell surface glycosylation and adhesion properties essential for the metastatic phenotype of BL6 melanoma.

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GD3 vaccines for melanoma: superior immunogenicity of keyhole limpet hemocyanin conjugate vaccines. Cancer-Res. 54(1):197-203, 1994.

- Hoon DS, Hayashi Y, Morisaki T, Foshag LJ, Morton DL.
Interleukin-4 plus tumor necrosis factor alpha augments the antigenicity of melanoma cells. Cancer Immunol Immunother. 37(6):378-84, 1993.
Abstract: Immune cytokines are important regulators of the immune response to neoplastic cells. We previously reported that interleukin 4 (IL-4) and either tumor necrosis factor alpha (TNF) or interferon gamma (IFN) synergistically inhibit melanoma cell growth and induce cell differentiation. In the present study we used various combinations of IL-4, IFN and TNF to enhance the antigenicity of melanoma cells. IL-4 plus TNF significantly increased the ability of melanoma cells to stimulate cytotoxic T cells (CTL) and act as targets of these CTL; IL-4 plus IFN was somewhat less effective, while TNF plus IFN was not as effective. IL-4 plus TNF also increased the expression of HLA class I and HLA-DR antigens on melanoma cells. The CTL lines examined in this study were CD3+CD4+ and oligoclonal. These preclinical results suggest that the immune response to melanoma whole-cell vaccines might be enhanced by pretreating vaccine cells with IL-4 plus TNF.

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A malignant melanoma imaging agent: synthesis, characterization, in vitro binding and biodistribution of iodine-125-(2-piperidinylaminoethyl)4-iodobenzamide. J Nucl Med. 34(12):2169-75, 1993.

- Kan Mitchell J, Liggett PE, Taylor CR, Rao N, Granada ES, Danenberg KD, White WL, Van Eldik LJ, Horikoshi T, Danenberg PV.
Differential S100 beta expression in choroidal and skin melanomas: quantitation by the polymerase chain reaction. Invest Ophthalmol Vis Sci. 34(12): 3366-75, 1993.
Abstract: PURPOSE. S100 beta, a member of a calcium-binding protein family (S100s), is an important clinical marker for skin melanoma. In contrast, uveal melanomas appeared to express S100 beta protein less frequently and to a lesser degree. This study was performed to verify and extend this finding to the mRNA level. METHODS. A quantitative polymerase chain reaction (PCR)-based method was used. A ratio, comparing the S100 beta PCR

fragment to that of beta-actin (an internal reference gene), was generated to compare S100 beta mRNA expression among samples. RESULTS. The ratios for skin melanomas (1.2 to 3.9; three tissues and two cell lines) were significantly higher than that for choroidal melanomas (0.1 to 0.63; seven of eight primary tumors and four of four cell lines). Only one choroidal melanoma biopsy had a ratio greater than 1. The PCR products from choroidal melanoma were identical in size and sequence to the S100 beta, as determined by gel electrophoresis and RNA conformational polymorphism. Because the ratios were also low in choroidal melanoma cell lines, the S100 beta phenotype appears to be genetically stable. CONCLUSION. S100 beta is differentially expressed at the RNA and protein levels by skin and choroidal melanomas, which are derived from distinct populations of melanocytes. However, choroidal melanomas expressing little or no S100 beta were significantly stained by antiserum specific for the S100 protein family. Taken together, these data suggest that choroidal melanocytes express another, perhaps even novel, S100 protein(s).

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Characterization of ganglioside expression in human melanoma cells: immunological and biochemical analysis. J Biochem Tokyo. 114(2):186-93, 1993.
Abstract: The expression of N-glycolylneuraminic acid (NeuGc)-containing gangliosides in human melanoma cells grown both in culture and as xenografts in athymic (nu/nu) mice was analyzed extensively with specific mouse monoclonal antibodies (MAbs). Three MAbs (GMR8, GMR14, and GMR3) specific for GM3(NeuGc), GM2(NeuGc), and GD3(NeuGc-NeuGc-), respectively, were used. Significant differences were observed in the ganglioside compositions between the cultured cells in vitro and the tumors grown in vivo. The major difference was that the cells cultured in serum-free medium did not express any NeuGc-containing gangliosides, whereas those grown in nude mice expressed a number of NeuGc-containing gangliosides, namely GM3(NeuGc), GM2(NeuGc), GD3(NeuAc-NeuGc-), GD3(NeuGc-NeuAc-), and GD3(NeuGc-NeuGc-). The structures of these gangliosides were also determined chemically. No activity of CMP-NeuAc hydroxylase was demonstrated either in the melanoma cells cultured in vitro or in those grown in nude mice, suggesting that these cells incorporated NeuGc-containing glycoconjugates from the mouse sera and converted them to other NeuGc-containing gangliosides. The mouse sera contained only GM2(NeuGc), but not the other NeuGc-containing gangliosides or any NeuAc-containing gangliosides.

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Chemoimmunotherapy of metastatic malignant melanoma. The Salpetriere Hospital (SOMPS) experience. Eur J Cancer. 29A Suppl 5:S2-5, 1993.

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In vivo antimelanoma effects of 4-S-cysteaminylphenol, a newly synthesized therapeutic agent specific to melanoma. J Cancer Res Clin Oncol 119:470-4, 1993.

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According to the authors these effects might be caused by cytotoxic oxidation products. This study is an interesting morphologic access to investigate cell changes after irradiation. The study is well designed. However, the long duration of cell growth between exposure to X-rays and harvesting requiring numerous changes of the medium resulting in different concentrations of L-Dopa at different stages of the cell cycle migh lead to less reproducible results as observed in varying degrees of cell damage after 8 Gy and L-Dopa in identically treated cell cultures. It might be of interest to study the morphological changes during defined steps of the cell cycle and in cell lines derived from different kinds of melanomas and different tumor stages as well as especially lower doses of radiation. Correlation of morphological effects with molecular changes in apoptosis are desirable. Further studies on L-Dopa as kind of a radiosensitizer for melanoma treatment - side effects not even taken into account - will certainly require not only electronmicroscopical results.

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(5 ng) or saline as control, twice a day for three weeks beginning eight days after tumor inoculation. LH, using hot water circulator at 43 +/- 0.2 degrees C for 30 min, was induced to the limb with tumor twice a week for two weeks. Size of the primary tumor was measured every other day for five weeks. Mice were sacrificed five weeks after tumor inoculation. The size of the primary tumor and the number of lung metastases were reduced in mice treated either with IL-7 or LH alone. As a control for IL-7, granulocyte colony stimulating factor (G-CSF) alone had no effect on primary tumor size or number of lung metastases. The greatest antitumor effect was observed in mice treated with IL-7 in combination with LH. Survival was prolonged significantly only in mice treated with IL-7 plus LH compared with that of mice treated with saline. Decreased natural killer (NK) cell activity, number of Thy1.2 cells, and ratio of L3T4+/Lyt2+ cells were associated with tumor growth. These parameters were restored in mice treated with IL-7 plus LH. Increases in levels of IL-1 alpha, lL-6, tumor necrosis factor (TNF alpha) and interferon (IFN gamma) were associated with an increase in the survival of tumor-bearing mice treated with IL-7 and/or LH. These results suggest that changes in T-cell, NK cell and cytokines such as IL-1 alpha, IL-6, TNF-alpha and IFN-gamma in response to IL7 and/or LH might account for prolonged survival of B16a melanoma-bearing mice and that IL-7 might be useful as a potential antitumor agent combined with other therapy in certain malignant solid tumors with metastases.

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Abstract: Ocular pigmentation in the mouse occurs primarily postnatally as a result of the melanization of neural crest-derived melanocytes. Using immunologic and biochemical techniques, we demonstrate that in normal mice the expression of tyrosinase and the related proteins TRP-1 and TRP-2, rises during the first week of life, remains elevated for a week, and then steadily declines to low levels by adulthood. Sucrose gradient density centrifugation demonstrates that tyrosinase, TRP-1 and TRP-2 are present in high molecular weight forms in the eyes of wild-type mice. The normal time course is disrupted in mice carrying the pink-eyed unstable (p(un)) mutation at the P-locus, a model for tyrosinase-positive albinism in man. Tyrosinase and TRP-2 are present at wild-type levels in the eyes of p(un)/p(un) mice at birth, but, rather than rising, their levels rapidly decline over the first week of life. TRP-1

is almost undetectable, even at birth. High molecular weight complexes could not be detected in eyes of p(un)/p(un) mice. Our results suggest that postnatal ocular melanogenesis in the mouse presents an attractive model for the study of the orderly expression and action of the proteins involved in eumelanin synthesis, and that the p(un) mutation disrupts this temporally controlled process.

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Melanins and Melanogenesis

Giuseppe Prota

Universita Degli Studi di Napoli Federico II
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This book focuses on all aspects of melanin pigmentation, providing a concise, comprehensive picture of new knowledge gained at the frontiers of research. It draws heavily on the authors's 30-year activity in the field and his continuing work with specialists of widely diverse disciplines. The core of the book deals with the structure, physicochemical properties, and biosynthesis of the major classes of melanin pigments, including neuromelanins. Other discussions include the biology of the various types of pigment producing cells, the structure and mode of action of tyrosinase, and the chemistry of urinary melanogens and their biomedical applications as metabolic markers of melanocyte activity, especially for the follow-up of malignant melanoma. Finally, the book considers progress in the photobiology and photochemistry of melanins, with special emphasis on the controversial role of these pigments in skin photoprotection. This book is ideally suited as a basic guide for newcomers, and a handy source of specific information for practioners in academic, medical and industrial settings.

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