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Periclico quadrimestrale della European Society for Pigment Cell Research (Associazione Europea per lo Studio della Pigmentazione), realizzato con il contributo della Fondazione Pro Ricerca Dermatologica e della Pifer Italiana.

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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 from his group has been presented at the meeting), which is known to be a growth and chemokinetic factor for neonatal melanocytes, is also chemotactic for the melanocytes. In response to PUVA therapy, the increased intrapidermal 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 colonized 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 HLA 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 become 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, underlining 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 melanocytic 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 situations 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 constitutive leghorn 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. Pusi and 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 was irrespective to the clinical appearance of the disease, i.e. segmental, acrofacial or generalised. This group proposed that the increased release of catecholamines at the nerve endings can induce high level of catecholamines on the skin which can produce a cytotoxic effect through two mechanisms: a direct one via the spontaneous oxidation of the catecholamines and the generation of free radicals; the second one via the vasoconstruction 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 bioppterin 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 phenylethanalamine-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 bioppterin 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.
1. Melanins and other pigments chemistry

(Comments by Prof. M. Peter)


**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.


**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 of 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.


**Abstract:** Melanins isolated from human hair (black, brown, red, dark and light blond, and gray) were purified and extended by ESR. Differences were found in the ESR signal parameters showing a dependence on the concm. 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 purif. procedure.


**Commentary:** Important information on regulation of melanogenesis in melanosoma.

**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.


**Commentary:** Interesting technology and application to polymeric materials.

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• Cuevas AA, Garcia-Cattineras S. Melanin 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 pigments. 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 pernitrogenate under strong acidic conditions. This procedure has been utilized for the characterization of melanins and results in the well defined products, thiainole-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 2-hydroxy-5-2-carboxylic acid. TDCA derives from phaeomelanins 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 (DES2 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 DES2 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.


• Komiyama K, Takamatsu S, Takahashi Y, Shinose M, Hayashi M, Tanaka H, Iwai Y, Oumura S, Intowaka G. New inhibitors of melanogenesis. OH-3984 K1 and K2. J. 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 R16 melanoma cells at concentrations of 7.5 and 3.8 micrograms/ml, respectively, whereas inhibition of tyrosiase activity has not been observed. The microbial metabolites showed no antimicrobial biological 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. Kensu 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.


Abstract: Optical techniques and pulsed-laser, time-resolved photoacoustics (PA) were employed to obtain information on the mechanism of interaction between cationic zinc tetraethylpypyridylporphin(ZnTBPYP) and synthetic L-Dopa melanins. Synthetic eumelanin and pheomelanin strongly quench the fluorescence of ZnTBPYP, 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 observed, whereas for the complexed form of ZnTBPYP 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.


Commentary: Valuable data on drug binding to melanin in ocular tissues.

Abstract: Norfloxacin (NFLX) content was determined in pigmented ocular tissues by HPLC. [14C]-NFLX was administered to rabbits and melanin-contg. ocular tissues were isolated and analysed 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 extm. procedure. Greater than 90% of NFLX was extd. into the supernatant as extd. by HPLC as well as by radioassay. The calibration curve was linear in the range 0.05-10.0µg/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.


Abstract: The binding of ocular hypotensive drugs to synthetic melanin was studied spectrophotometically in vitro. The ocular hypotensive effects of the drugs, namely, timolol, benedol, cafoline, 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 intracocular pressure in albino but not in pigmented rabbits. Epinephrine (1%) caused a significant reduction in the intracocular pressure both in albino and pigmented rabbits; however, the maximum reduction was greater in albino than in pigmented rabbits. Intracocular 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.


Abstract: Under biol. relevant conditions, co-oxidn. of 5,5'-dihydroxyindole and 5,5'-dihydroxyindole-2-carboxylic acid afforded, 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.


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 phy. interactions with oxygen are also discussed.
2. Biology of pigment cells and pigmentary disorders
(Comments by Dr. M. Picardo)


- Bhattacharjee V, Anjaria S, Puri N, Darshanam BN, Ramia A. pH of melaninomes of B 16 murine melanoma is acidic: its physiological importance in the regulation of melanin


Commentary: This paper presents different points of interest. The studies on the extracutaneous pigmentary mechanisms 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.


Commentary: The correlation between melanocyte duplication, melanin synthesis and Protein kinase C modifications have not still completely defined. This study adds some new observations to the topic. The down regulation of specific isoform of PKC seems to be correlated with the proliferation of normal melanocytes, and the constitutive down regulation in transformed melanocytes may be the consequence of elevated diacylglycerol levels in these cells; in fact, stimulation of quiescent cells with synthetic diacylglycerol induce a significant increase of DNA synthesis. Stimulation of alteration of the membrane lipid component with the subsequent release of phosphatidylglycerol could be a trigger factor in these cellular responses.


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 antiflammatory 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.


Fukui K, Ishii M, Kudoy A, Hamada T, Wakamatsu K, Ito S. Nevus depigmentosus systematics with partial yellow scalp hair due to selective suppression of eumelanogenesis. 502

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 phaeomelanin content was normal.


Abstract: The concn. of paramagnetic centers, as measured by EPR, in melanomas of human retinal pigment epithelium did not change with age between the ages of 20 to approx.65 yr. Apparently, on aging, the concn. of melanin in individual melanomas does not change but the no. of melanosomes diminish.


Abstract: A cell line was established from a Mongolian gerbil's (Meriones unguiculatus) homotransplantable malignant melanoma. Asotic 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 observation. 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.


Abstract: A review, with 282 refs., on: tissue distribution; melanocyte structure; biochem. determinants; mol. biol. of melanocytes; and interactions and levels of regulation.


Abstract: Research designed to establish procedures for DNA anal. of hair samples revealed that some kinds of hair samples, such as dyed hairs and hairs longer than 20 cm, contain certain potent inhibitors of DNA anal., while no such effect is found in grey hairs. Hairs treated with hydrogen peroxide always contain a potent inhibitor of DNA polymerase and DNases. This inhibitor is a kind of melanin deriv., which is denatured during the oxid. procedure; i.e. water sol. melanin. This deriv. is also contained in hairs longer than 30 cm suggesting that the melanin could be slowly converted into water sol. substances in air. This substance inhibits the DNA polymerase by a kind of 2 mol. inhibition, while it reacts in the DTNase by a kind of one mol. inhibition. Water sol. melanin could be derived from natural melanin and synthesized melanin by hydrogen peroxide treatment, but inhibition by the former is much more potent than by the latter. Inhibition by water sol. melanin is much stronger than that of several kinds of antibiotics. Water sol. melanin can be eliminated from the DNA ext. of hair by applying to Bio-Gel and a spin column. The results distinctly suggest that pretreatment of DNA ext. of the hair by these processes for the elimination of the inhibitory substances is indispensable for the success of DNA anal. in forensic practice.

Abstract: A review with 293 rfs. of inherited hypopigmentation syndromes such as albinism Waardenburg syndrome, piebaldism, Zivkovic-Margolis syndrome, nevus depigmentous, and nevus anemicus, with primary focus on oculocutaneous albinism.


Commentary: It is now presented a new possible role of melanocytes: an immunological function. Preliminary results have been presented at the last IPIC in London and have stimulated a great interest. Up to now, we have not completely understood the role of the pigmentary activity of melanocytes and of the melanin produced in response to UV light and to inflammatory process, and the results of this study open a novel view on some of the biological properties of the melanocytes suggesting a possible clue for the pathogenesis of pigmentary disorders. This study indicates that all the epidermal cells participate both in the physiology and in the pathology of the skin.


Abstract: Photolysis of aerated solns. of acid sol. type I calf skin collagen with UV-11 (mainly 254 nm) radiation results in photolysis, which may involve crosslinking and/or chain cleavage. Addn. of either synthetic melanin or sepia melanin obtained from cuttlefish appears to provide protection against photolysis, induced loss of fluorescence. However, the mechanism by which each of these chemicals, distinct entities protect may differ from each other. The protection provided by the sepi melanin is less than what might be expected from the ability of the melanin to act as a simple optical inner filter whereas the opposite is true for the synthetic melanin. Synthetic melanin appears to be photooxidized whereas sepi melanin may be photoreduced in the course of the reaction. These observations are consistent with the involvement of, as well as phys. mechanisms in photoprotection by melanin. Electron transfer reactions and/or active O intermediates may participate.


Abstract: Growth/cytokinetic factors, encountered by dispersing neural crest cells, affect the development of different crest-derived subpopulations. Here, we address the dependence by cells of the crest-derived melanogenic lineage on Steel factor (SLF). Specifically, we have asked when SLF-responsive cells first appear in cultured neural crest cell populations and whether there is a defined period during which this melanogenic subpopulation requires the presence of the SLF. We demonstrate that survival of a melanogenic subpopulation of cultured murine crest cells requires SLF for a critical period, which begins only after the second day of dispersal in vitro. We also conclude that the critical period of SLF dependence lasts about 4 days, ending about the time that melanocytes differentiate, as indicated by the presence of functional melanosome, before their overt melanization. Finally, we demonstrate that although the final stages of melanogenesis (i.e., melanization) do not appear to require SLF, this process may be enhanced by extended exposure to high levels of SLF in vitro.


Abstract: It is presented a six-year-old girl with silvery hair syndrome, of Griscelli-Prunieras variety, hereditary sickness with regressive autosomic and distinguished by partial albinism and leukocytic alterations. She presented the acute phase of the sickness distinguished by: hepatosplenomegaly, thrombocytopenia, lymphenadenopathy generalized, and systemic infection; it is corroborated how a hemophagocytic syndrome; during her evolution developed pancerebellar syndrome. By laboratory were corroborated: decrease phagocytes, degranulation 0%, decrease of globulins gamma, neuroepitma, skin test of PPD and Candida negatifs, there were not find the giant inclusions in bone marrow leukocyte and peripheral blood that are feature of Cheik-Diag-Higashi syndrome. Another alteration that was the distribution of mote of melanin on the hair that in the Griscelli-Prunieras syndrome are six times bigger in the Cheik-Diag-Higashi syndrome.

- Shaikh P, Aqiel KL.

Abstract: In isolated scale melanophores of L. rohita the melanosome aggregating effect of K+ was inhibited in Ca2+-deprived medium. Moreover, the Ca2+-antagonists verapamil and lanthanum inhibited the action of K+ in a connc.-dependent manner. The elevation of extracellular Ca2+ could compromise the verapamil-induced inhibition in a concn.-dependent manner. The cation Ca2+ appeared to be required only for K+-induced aggregation and not melanosome aggregation per se, as in this fish adrenaline and melanin-conc. hormone effectively caused aggregation of melanosomes in Ca2+-free medium.

- Si SP, Tsou HC, Lee X, Pesacce M.

- Slominski A, Paus R.

- Slominski A, Paus R, Schade-Stor D.

Abstract: Epidermal melanocytes (MC) are pigment-producing and secreteactively cells of neural crest origin that communicate directly with multiple targets. Here, we propose that normal epidermal MC also are "sensory" and regulatory cells operating in the conAbstract: of a regulatory network for the maintenance of epidermal homeostasis. Altered regulatory MC functions may play a role in selected skin diseases, and racial pigmentation may affect cutaneous functions.

- Slominski A, Pruski D.

- Sukhanov VA, Voronkova IM, Shevtsov SV, Morozova LF.

Abstract: The melanocyte-stimulating hormone (alpha-MSH) used at 10(-6)-5 x 10(-8) M concentrations inhibited the growth of amelanotic cells of human malignant melanoma B16 and influenced cell morphology without any effect on melanization or tyrosinase activity. Inhibition of tumour cell growth was accompanied by marked elevation of intracellular cAMP levels but not that of cGMP. Dibutyryl-CAMP and the CAMP-dependent protein kinase A inhibitor also inhibited the cell growth. Alpha-MSH increased mono-, di- and 1,4,5-monoinositol triphosphate concentrations and influenced the activities of phosphatidylinositol kinase and phosphatidylinositol-4-phosphate kinase and phosphatidylinositol-4,5-diphosphate levels. Myoinositol phosphate concentrations changed on a second scale and levelled off by the 3rd-5th min, whereas that of cAMP increased drastically by the 30th min.

- Tobin D, Mandie N, Dover R.

- Todd C, Hewitt SD, Kempannaj J, Noz K, Thody AJ, Ponec M.
Complementary: A new method for the co-culture of melanocytes and keratinocytes has been defined by the authors, utilizing a de-epidermized dermis as substrate. The most interesting finding is the functional interaction between melanocytes and keratinocytes in this model, and possibly studies performed more in depth may clarify some of the intercellular control mechanisms of melanogenesis.


- Yimin L. Mechanism and regulation of coloring in soy sauce. Zhongguo Nianzao 2:1-8, 1993. Abstract: A review with 8 refs. on the biosynthesis of pigment (e.g., melanin) during soy sauce fermentation, effects of Fe2+, oxygen, sugars, carbohydrates, and amino acids on the pigment formation, and methods for control of the color of soy sauce.

3. MSH, MCH, other hormones, differentiation


- Breton C, Press F, Hervé G, Nahon JL. Structure and regulation of the mouse melanin-concentrating hormone mRNA and gene. Mol Cell Neurosci 4:271-284, 1993. Abstract: Melanin-conc. hormone (MCH) and associated peptides, designated NEI and NGE, are predominantly expressed in hypothalamic neurons which project widely throughout the mammalian brain. These peptides might be involved as neuromodulators in the control of goal-directed behaviors, the integration of stress response, and/or the regulation of arousal (in general). In vivo studies of this peptide system using a transgenic mouse model call for information on the structure and regulation of the mouse MCH (mMCH) gene. One cDNA for mouse prepro-MCH was isolated from a brain library by using a rat MCH cDNA as probe. This cDNA contains an open reading frame coding for a 165-amino acid precursor that displays about 90% sequence identity with rat and human prepro-MCH. Most of the structural portion of the mMCH gene was cloned and characterized using the polymerase chain reaction (PCR). Strong conservation in exon-intron organization and primary sequences was found among the mouse, rat, and human genes, suggesting that coding and noncoding regions have homologous biological functions. Developmentally regulated expression of mMCH gene in mouse hypothalamus was examined by Northern blot hybridization. There were 30-40-fold changes in the relative mMCH mRNA contents observed during postnatal development, characterized by a peak at the weaning period. Moreover, striking variations in mMCH mRNA length, due to a poly(A) tail, were revealed during postnatal life. Tissue distribution of mMCH gene transcripts was investigated by the PCR and Northern blot procedures. Expression of the mMCH gene was revealed in heart, intestine, spleen, and testis and was regulated in a developmentally programmed manner. Strikingly, short as well as long mMCH RNA species were identified at the periphery. Taken together, the authors' data indicate that both transcriptional and post-transcriptional mechanisms regulate the expression of the MCH gene in mouse brain and at the periphery.

- Carr JA, Saldan LC, Samora A, Tejeda D. Effects of the enkephalin analog (D-Met2,Pro5)-enkephalinamide on alpha-melanocyte-stimulating hormone secretion. Regul Pept 47(2):141-50, 1993. Abstract: We used the met-enkephalin analog (D-Met2,Pro5)-enkephalinamide (DMP2) to investigate enkephalinergic control of alpha-melanocyte-stimulating hormone (alpha-MSH) secretion. Systemic (i.c.)
administration of DMPEA elevated plasma titers of alpha-MSH in a dose- and time-related manner. Pretreatment with the opiate antagonist naloxone had no effect on basal plasma levels of alpha-MSH but blocked DMPEA-induced alpha-MSH release. Treatment with a dose of naloxone 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, DMAPA had no effect on the synthetic activity of tuberohypophyseal 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. Naloxone 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.

• Carania A, Lipton JM. 

• Colao A, Merola B, Carali M, La Tessa G, Boudouresque F, Oliver C, Di Riento G, Annunzio L, Lombardi G. 
alpha-Melanocyte-stimulating hormone is present in the inferior petrosal sinuses in patients with Cushing's disease. 

• Chakraborty A, Pawelczyk J. 

Abstract: Receptors for melanotropin (MSH) were found to be expressed by immortalized primary human epidermal keratinocytes (HaKEK-1). Using 125I-beta MSH as a probe, the MSH receptors from mouse melanoma cells and human keratinocytes were found to be remarkably similar. In each cell line, there were high and low affinity receptors, with the high affinity classes showing positive cooperativity. Competition of 125I-beta MSH for binding with non-radioactive MSH revealed similar profiles. Cross-linking studies, followed by gel electrophoresis and autoradiography, showed almost identical gel migration patterns. Both cell types expressed internal as well as plasma membrane binding sites. MSH receptors on both cell types were up-regulated by ultraviolet light and by MSH itself. Although the function of MSH receptors expressed by the immortalized keratinocytes is unknown, the results are consistent with recent reports that proliferation of epidermal keratinocytes is stimulated by MSH and that prolactin and melanocortin genes are expressed in the epidermis. These results support a model in which keratinocytes and melanocytes, interacting in an "epidermal- melanin unit," each respond to UV light signals with increased MSH receptor activity.

• De Luca M, Siegrist W, Bondanza S, Matyor M, Cancrada R, Eberle AN. 

Abstract: The combined action of cholecalciferol (CT)-dependent activation of the p34-rat cyclase signaling pathway, stimulation of protein kinase C, and activation of the tyrosine kinase activity of cell surface receptors and proto-oncogene products, have been shown to stimulate melanocyte proliferation. However, natural factors responsible for the optimal stimulation of normal human melanocyte growth, either isolated or co-cultured with keratinocytes, remain largely unknown. alpha MSH (alpha melanocyte stimulating hormone) has previously been shown to bind to murine and human melanoma cells and to stimulate their adenylate cyclase and tyrosine activity. In contrast, very little is known about the presence and function of alpha MSH receptors in normal human melanocytes. We now report that alpha MSH: (i) binds to normal human melanocytes through a single class of high-affinity receptors, (ii) does not induce per se melanocytes to enter the G0-phase of the cell cycle, (iii) does indeed stimulate melanocyte proliferation in a dose-dependent fashion, but its stimulatory effect requires BFGF and/or the activation of protein kinase C.

• Drozdz R, Baker BI, Zeller A, Eberle AN. 

• Dyer JR, Ahmed AR, Oliver GW, Poulton CW, Haynes LW. 

• Eberle AN, Siegrist W, Drozdz R, Verin VJ, Baggett C, Solca F, Girard J, Zeller A. 
Radiolabelled melanotropic peptides (alpha-MSH and MG2) for receptor identification. Synth. Appl. Inot. Labelling

Abstract: A possible inter-relationship between oestradiol, alpha-melanocortin (alpha MSH) and NA in the ventromedial nucleus (VMN) has been studied in ovariectomised-adrenalectomised rats primed with a low dose of oestradiol benzoate (2 or 5 micrograms OB), which induces receptivity in approximately half the rats. Priming with OB increased NA turnover in the VMN (as assessed by the decline in NA concentrations 1 hour after 250 mg/kg alpha-methyl tyrosine alpha MT) and also enhanced the release of NA from basolateral hypothalamic fragments in vitro. This occurred whether the rats were receptive or non-receptive. Injection of 20 micrograms/rat alpha MSH, 4 hours before autopsy in OB-primed rats, reduced NA turnover in the VMN but only in the receptive animals. Alpha MSH had no effect on NA content in the VMN, but prevented the decline normally induced by the alpha MT, indicating an inhibition of release. Application of 1 microgram/ml alpha MSH to incubated hypothalamic fragments enhanced release of NA in the tissue obtained from untreated controls and the OB-non-receptive group, but had no effect on the tissue of the OB-receptive animals. Perhaps NA release had already occurred in vivo in the latter group. Alpha MSH (100 mg/rat) and NA (20, 200 and 2,000 mg/rat) were injected into the VMN of ovariectomised-adrenalectomised rats primed with 1 microgram OB. Both agents stimulated lordosis in non-receptive animals with a peak activity at 60 min.


Abstract: A review, with 102 refs., on the role of MSH in human skin color; on models of UV light-induced skin pigmentation; on the control of skin pigmentation by sex steroid, cytokines and growth factors, eicosanoids, and thyroid hormones; on hormonal control of follicular melanogenesis; and therapeutic uses of melanotropic peptides.


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4. Photobiology and photochemistry
(Comments by Dr M. Piccardo)

Dawson BV.

Dissanayake NS, Greenoak GE, Mason RS.
Abstract: The effects of UVA, mixed UVA + B, and solar-simulated irradiation were examined in human keratinocytes and melanocytes cultured in vitro. Irradiation with UVA, UVA + B, or the solar simulator caused a dose-dependent decrease in keratinocyte cell numbers and thymidine incorporation after 24 hours, with recovery after 48 and 72 hours. Divided dose regimens reduced the inhibitory effect of ultraviolet (UV) irradiation on cell numbers measured 24 hours after the last irradiation. Exposure to both UVA and UVA + B increased formation of cornified envelopes. Similar irradiance doses of UVA 80 minutes (1.12 J/cm2) and UVA + B 40 minutes (1.04 J/cm2) caused 2.5- and 3.3-fold increases in cornified envelope formation, respectively. With solar-simulated irradiation, the cornified envelope formation was increased by 3.5-fold after exposure of 8 minutes (2.6 J/cm2). Irradiation of melanocytes with UVA, UVA + B, or solar-simulated irradiation resulted in a dose-dependent decrease in melanocyte numbers after 24 hours compared with sham-irradiated controls. As a result of UV irradiation, tyrosinase activity of melanocytes measured at 24 hours was stimulated. UVA + B irradiation (1.04 J/cm2) increased tyrosinase activity approximately twofold, while UVA alone (1.1 J/cm2) increased tyrosinase four to sixfold and solar-simulated irradiation (1.3 J/cm2) increased tyrosinase approximately twofold compared to the control cells. Melanin content increased in cells after both UVA and mixed UVA + B irradiation. These results indicate that both UVA and mixed UVA + B irradiation had qualitatively similar effects on the proliferative and functional activity of skin-derived cells but that the type of irradiation and the dosage regimen affect the dose-response relationship.

Gia O, Mobillo S, Rahumbo M, Pathak MA.

Glechrest BA, Zhai S, Eller MS, Yarosh DB, Yaar M.

Glickman RJ, Sowell R, Lam KW.
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 isosorbidic acid, an epimer of ascorbic acid, while other tested reducing agents are inactive. The Km for isosorbidic acid is 1.25 mM, about the same as the Km of ascorbic acid.

Hruz A, Dewar JS, Fritche JT, Gechtschkes M, Watanabe S, Anderson RR.

Kobayashi N, Moramatsu T, Yamashita Y, Shiomi T, Ohishi T, Mori T.
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 which melanocytes and their product contribute to the normal homeostasis of the skin.

- Lam Kwok Wai, Glickman RD.
Abstract: Rapid ascorbic acid oxidizes, has been demonstrated during exposure of the retinal pigment epithelium cells to light. Because its rapid reaction is a possible 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 oxidation reaction in the presence of melanin granules in a cell free interaction mix.

- Lee Ki Hong, Kim Jae Yong, Myung Ki Bum, Kook Hong Il.
Abstract: The effect of UVA irradiation 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 irradi. at 1.4 J/cm2. There was no change in melanocytes proliferation after UVA irradi. On the 2nd day of cultivation after UVA irradi., the amt. of melanin was increased in proportion to the increment of dose of UVA irradi. After irradi. at 4.4 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 irradi., the amt. of melanin was slightly influenced according to the increment of dose of UVA irradi. After irradi. 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 irradi., 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 irradi. is capable of directly stimulating melanogenesis; however, melanocyte proliferation is not influenced at all by UV A irradi.

- Nacht S.

- Liu YT, Sui MJ, Ji DD, Wu HH, Chou CC, Chen CC.
Abstract: A process for production, isolation, and purification of melanin produced by the fermentation of Streptomyces lividus 66 harboring a recombinant plasmid pJJ/202-bearing tyrosinase gene has been developed. The efficacy of melanin in the protection of mosquito larval activity of Bacillus thuringiensis var. israelensis against UV light has been studied. Results obtained by the live cell counts and the bioassay of residual mosquito cuticular activity of B. thuringiensis var. israelensis after exposure to UV radiation showed that melanin is an excellent photoprotective agent.

- Setlow RB, Grist S, Thompson K, Woodhead AD.

- Tokura Y, Yagi H, Sato H, Takigawa M.

5. Neuromelanins

Comments by Dr. M. Ishida:
Owing to the exponential growth in the literature 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. 61:66-79, 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 neuropeptides, and the levels of metal cations bound to the pigment polymer. The results are consistent with a concept of neuropeptide as a highly heterogeneous, sulfur-containing pigment, which serves the function of a metal reservoir in mechanised dopaminergic neurons. This contributes to the current controversy on the role of cysteylin dopaminergic neurons as biosynthetic precursors of substantia nigra neuropeptide (cfr. Pigment Cell Res. 3:33-35; 1993, not abstracted in this issue), and would reinforce contentsions for iron-neuropeptide interaction as a causative factor in Parkinson's disease.

Neuropeptide 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 aptly documented by Cernot and Kupchik's review (Curr. Opin. Neurol. Neurosurg. 6:323-332, 1993) and surfaces in many outstanding papers of the last few months. A study by Chiuheh et al. (Ann NY Acad Sci 679:370-375, 1993) deals with in vivo markers for oxidant injury of neuropeptide-containing neurons, whereas a contribution from Agid's group (Neuroscience, 55:167-172, 1995) 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. Whether 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.


Abstract: The high-resolution solid-state 13C-NMR spectrum of a neuropeptide specimen (from patients dying from neurologcal diseases) is compared with that obtained from enzymatically prepared dopamine- melanin. The main differences between the two spectra suggest the occurrence in neuropeptide of a glycidic/lipidic matrix tightly associated with the melanin macromolecule. Atomic emission spectroscopic revealed high iron content (1.5%) in the neuropeptide specimen, in full agreement with previous results. These observations support the view that neuropeptide 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.


Du XP. Kanamycin toxicity and melanin in the inner ear. Chung Hua Erh Pi Yen Hou Ts Tsa Chih 28:14-6,58, 1993.

Abstract: In order to clarify the relationship between toxicity of aminoglycoside and inner ear melanin, we have undergone observations on the cochlea 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 toxicity of aminoglycoside antibiotics that might be due to its capacity to react and detoxicate the drug.


Commentary: The present literature update feature a paper by Herrero et al. addressing a critical issue in research on Parkinson's disease: is neuropeptide 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 neuropeptide 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 extrastraiostriatal 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 hand, that neureiomelanin formation is not 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 neureiomelanin-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 neureiomelanin: most likely, the pigment is only a visible indicator of a metabolic deficiency which may be the actual factor underlying selective vulnerability.


Abstract: Neureiomelanin (NM) is an auto-oxidation 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 unstrained 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 unstrained 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.


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.


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 ionic 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.


6. Genetics, molecular biology
(Comments by Dr B. Bouchard)


Abstract: The glycoproteins recognized by monoclonal antibody (MAB) NKG-betab are among the best diagnostic markers for human melanoma. MAB NKG-betab 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 NKG-betab. 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.


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, Mr(4) 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 calf, 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 calf and anti-human melanosome antibodies and the Ca(2+)

- Duttlinger R, Manova K, Chu TY, Gyssler C, Zelenetz AD, Bachvarova RF, Bemser P.


Abstract: The receptor tyrosine kinase c-kit and its cognate ligand RQ 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 1/2 Wsh/Wsh embryos, the authors found c-kit expression in the dermome of the somites, the mesenchyme around the oric 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 E12 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 dermome affects early melanogenesis in a dominant fashion. Mel. 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, Parr CJ, Lai C, Herlyn M, Bennett DC.


- Puqua WC, Weiner RM.


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 competent null mutants unable to synthesize the pigment, and antibodies confirm the presence of the melA gene product in the wild type S. colwelliana. However, the expression of this gene does not appear to control the amount of melanin produced.

- Kimura N, Troupe T.


Commentary: Three genes shown to be essential for melanin synthesis in the fungus Alternaria alternata have been recently isolated. The expression of these 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.

  **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 60B protein tyrosine 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 other 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.


  **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.

  **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.

  **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.


Commentary: Wang et al. report an interesting new mutation in the deer mouse. The locus affected by this new recessive mutation (tm) 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.


Commentary: A paper by A.J. Winder et al. reports the study of the enzyme tyrosinase, the product of the c-albinism locus, in transfected cells. This study confirms previous work demonstrating that tyrosinase is stably expressed in fibroblasts, is catalytically active and can induce melanin production. The authors further demonstrate that translational or post-translational modifications can occur in transfected fibroblasts through mechanisms similar to those existing in melanocytes.


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 Concanaavalin 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 results are consistent with the hypothesis that melanosomes are closely related to lysosomes.


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 synthesised melanin (Sigma products), as well as natural black melanin, inhibited the PCR amplification of mt333DNA at concentrations of more than 2 mg/μg/ml. 2) Quantitative measurement of Taq DNA polymerase-catalysed DNA synthesis in terms of the amount of [α-glycerophosphate] dATP incorporated into activated calf thymus DNA showed that both of the water-soluble melanins had the same inhibiting 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-50 minipill was equilibrated with 10 mL sodium acetate buffer, pH 4.2, water-soluble melanins were completely adsorbed to it whereas DNAs passed through, although the melanins showed incomplete adsorption to the gel when it was
7. Tyrosinase, TRP1, TRP2 and other enzymes

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

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 phaenomelanogenesis. The paper by del Marmol et al. (PSSB Lett 327:307-310) is particularly relevant, since the behavior 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 Kumazaki et al. (Exp Cell Res 207:38-40). Conversely, Windler et al. (J Cell Sci 104:467-475) demonstrate that fibroblasts expressing the c locus tyrosinase produce phaeomelan. 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, TRP3, may direct melanin biosynthesis towards eumelanogenesis. Interestingly, it has been shown by Bennett and coworkers that b mutants 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. 268:13742-9) demonstrate that in normal human melanocytes protein kinase C (PK C) activates tyrosinase and melanogenesis. This is 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 of phorbol esters and/or PK C 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.


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(um)) 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(um)/p(um) 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(um)/p(um) 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(um) mutation disrupts this temporally controlled process.


Abstract: OBJECTIVE: Previous linkage and allelic association studies using DNA polymorphisms, cosegregation of cyrogemonic abnormalities with psychiatric illness, and assignment of genes involved in neurotransmitter 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 glyceraldehyde-3-phosphate dehydrogenase (GRH) 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.


- O'Neill G, Hindemith A, Rosengren AM, Rosengren E, Rorsman H. Comment: Odh et al deserve 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 dihydroxydopinal (DHL), the reaction catalyzed 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, DHLCA, and in the case of the new enzyme, it should be substituted by a name describing more closely the reaction catalyzed.


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

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Abstract: S-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 mouse tyrosinase 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 transfecants was not suppressed by BrdU. Since BrdU would be incorporated into the tyrosinase cDNA integrated in these transfecants, 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.

- Rieber M, Strandberg, Rieber M.


- Vass E, Nappi AJ, Carton Y.


- Winder AJ, Wirttjer A, Rosengren E, Rosenman H.


8. Melanoma and other pigmented tumours

(Comments by Dr R. Peters)

- Abdel Wahab ZA, Darrow TL, Vervaat CE, Giannopoulos AA, Li W, Seigier HF.


Abstract: The administration of anti-melanoma murine monoclonal antibody (MAB) 16.68 (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 16.68 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 Hank's Balanced Salt Solution (HBSS). The MAB was most effective when treatment was started on day 1 or 4 following tumour inoculation. When the 16.68 MAB treatment was delayed 7 or 14 days, 33 and 67% of 16.68 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 16.68 inhibited tumour growth in irradiated animals which may suggest the involvement of host-radioresistant cellular elements in the 16.68 antibody-mediated anti-tumour activities in nude mice. These results suggest that MAB 16.68 alone or combined with rIL-2 may prove useful in the immunotherapy of metastatic melanoma.

- Ahern TE, Bird RC, Bird AE, Wolfe LG.

\textbf{Akasaka T, Imamura Y, Kon S.} 

\textbf{Arksey A, McKee P, Jones EW.} 

\textbf{Bennett DC.} 

\textbf{Bjornhagen V, Mansson Brahe E, Lindholm J, Mattsson A, Auer G.} 

\textbf{Abstract:} Morphometric assessment of nuclear area, shape and density, nuclear 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 < 4 mm and Clark level II-III. Twenty-four thin metastasizing melanomas (TMM) were individually compared to two thin non-metastasizing melanomas (TNN) 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 TNN 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 nuclear area, mean nuclear shape NCI, nuclear DNA content or expression of PCNA.

\textbf{Casazza S, Gambini C, Tunesi G, Rovida S, Caruso F, Pastoreno A, Canepa M.} 

\textbf{Abstract:} PS3 protein in cutaneous melanoma. We report the results of an immunohistochemical analysis about the nuclear phosphoprotein PS3 expression performed on 48 primary and 10 metastatic cutaneous melanomas in order to assess the prevalence of the expression of mutant PS3 protein (m-PS3) in this skin tumour. In our study m-PS3 was found in about 46% of primary tumours without any significant relationship with the corresponding metastatic lesions. Therefore the PS3 count in cutaneous melanoma is not a prognostic marker of tumour spread and aggressiveness.


\textbf{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 Mason'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.

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

\textbf{Essenbelt A, Skornick Y, Ron I, Zakut V, Chaitchik S.} 

\textbf{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.


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 nonionic 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.


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.


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-LD3 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-LD3/IL-2 activation, these cells were capable of m-daring 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

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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 0.62; 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 melanomas 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).

Kapadia SB, Priesman DM, Hitchcock CL, Ellis GL, Popek EJ.
Malignant uveal and dermal tumor of infancy. Clinicopathological, immunohistochemical, and flow cytometric study.

Kawashima I, Ozawa H, Kotani M, Suzuki M, Kawano T, Gomibuchi M, Tai T.
Characterization of ganglioside expression in human melanoma cells: immunological and biochemical analysis.

Abstract: The expression of N-glycolyneuraminic 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(NeuAc-NeuAc), and GD3(NeuAc-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 glycosconjugates 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.

Chemoinmunotherapy of metastatic malignant melanoma. The Salpetriere Hospital (SOMPS) experience.

Kitaoka M, Nemoto T, Seki S, Ito S, Kasuga T.
In vivo antimelanoma effects of 4-5-cysemamphenol, a newly synthesized therapeutic agent specific to melanoma.

Cellular accumulation of fluorine-18-labelled boronophenylalanine depending on DNA synthesis and melanin incorporation: a double-tracer microautoradiographic study of B16 melanomas in vivo.

Lane H, O’Loughlin S, Powell F, Magee H, Dervan PA.
A quantitative immunohistochemical evaluation of lentigo maligna and pigmented solar keratosis.

Lyng H, Jagen DR, Southon TE, Rofstad EK.
31P-nuclear magnetic resonance spectroscopy in vivo of six human melanoma xenograft lines: tumor bioenergetic status and blood supply.

Transduction of human melanoma cell lines with the human interleukin-7 gene using retroviral-mediated gene transfer: comparison of immunologic properties with interleukin-2.
Blood. 82(12):3686-94, 1993. 524
Abstract: Two human melanoma cell lines were transduced with the human interleukin (IL)-7 and IL-2 genes using retroviral-mediated gene transfer. Stable, high-level cytokine expression was achieved. The in vitro growth of transduced tumors was unaltered. Neither of the IL-2-transduced melanoma cell lines grew in athymic mice, whereas one IL-7-transduced melanoma line showed retarded in vivo growth. This is consistent with animal studies suggesting a predominantly T-cell response to IL-7-transduced tumors and a more nonspecific response to IL-2-transduced tumors. Both IL-7- and IL-2-transduced melanoma cell lines could induce cytotoxic lymphocytes in mixed lymphocyte-tumor cultures. The expression of putative melanoma antigens (MAGE-1 and MAGE-3) was unaltered.


Abstract: Uveal melanomas are unique among the malignant tumors of the eye investigated by MRI in that both T1 and T2 are relatively shortened due to the paramagnetic effect of melanin. Bearing in mind this property, we conducted a comparative study between MRI and CT in 11 patients with histologically proven choroidal malignant melanoma. The results of this study confirm that MRI is superior to CT in both differential diagnosis and in determining the extent of the tumor which is crucial if conservative treatment is to be undertaken.


Abstract: Two cases of presumed Spitz naevus, whose diagnosis on clinical and histological grounds was uncertain, were examined for cellular DNA content using the technique of DAPI-DNA microfluorometry. They were compared with 20 cases, respectively, of clinically and histologically confirmed, Spitz naevus, malignant melanoma and acquired pigmented naevus. The two Spitz naevi showed a diploid pattern in a distribution histogram of cellular DNA content. The pattern was similar to that of confirmed Spitz naevi and of acquired pigmented naevi but different from the aneuploid pattern of malignant melanomas. DNA index values of the two cases were within the range of confirmed Spitz naevi and different from those of malignant melanomas. The DAPI-DNA microfluorometric method thus provided confirmatory evidence for the diagnosis of Spitz naevus. The method appears to reflect sensitively the biological behaviour of tumour cells, and is a useful aid to the diagnosis of uncertain Spitz naevi.


Commentary: Monolayer cultures of murine Harding-Passey melanoma cells were exposed to 8 and 16 Gy of X-ray X-irradiation. 8 Gy treated cells revealed little ultrastructural changes, those cells exposed to 16 Gy showed ultrastructural signs of damage like segregates, swollen mitochondria and vacuoles. Treatment with L-3,4-dihydroxyphenylalanine (2x10 m6 L-Dopa) showed no major effects. 8 Gy and L-Dopa after 6 days of incubation revealed final cell desintegration with more vacuoles and segregates, a decreased endoplasmic reticulum, swollen mitochondria, less pinocytic vesicles, less microsill on the surface and more melanin containing organelles.

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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 might 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 is 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 electromicroscopical results.


Abstract: We previously reported a hamster animal model of melanoma in which the tumor tissue expresses gangliosides GM3, GD3, and O-acetyl GD3. This ganglioside pattern is similar to that in human melanomas (Ren, S., A. Slominski, and R. K. Yu. 1989 Cancer Res. 49: 7051). In this study, we isolated and purified these gangliosides using chloroform-methanol extraction, Folch partition, chromatographies on DEAE-Sephadex A25, and fastprotein columns. The yields of gangliosides GM3, GD3, and O-acetyl GD3 were 44.1 mg, 19.6 mg, and 9 mg per 100 g of Ma melanotic melanoma tissues, respectively. The structures of these gangliosides were characterized by periodate oxidation, gas chromatographic (GC) analysis, fast-atom bombardment-mass spectrometry (FAB-MS), and nuclear magnetic resonance (NMR) studies. The structure of hamster melanoma O-acetyl GD3 is different from the 9-O-acetyl GD3 previously reported in human melanoma. The major fatty acids of this ganglioside are C16:0, C18:0, C20:0, C22:0, and C24:0 and the long-chain base is C18-sphingozine.


Abstract: Bacterioloroitin a (BCA), a new photosensitizer for photodynamic therapy, was labelled with 99mTc-pertechnetate following a reversible coupling of 99mTc-pertechnetate to protein. Biodistribution studies were conducted in male Syrian Golden hamsters with hamster Greene melanoma implanted s.c. on both sides of the abdomen. After i.v. administration of 99mTc-pertechnetate-labelled BCA 17 tissue and fluid samples were analysed at time intervals ranging from 1 to 24 h. Technetium-labelled BCA showed a pronounced affinity for tissues belonging to the reticuloendothelial system. Peak activities, 1 h post-injection, were distributed as follows: lung, liver, spleen, urine > small intestine, kidney, blood, heart, stomach, large intestine > thyroid, tumour, skin, muscle, eye > > brain. It is concluded that the technetium-labelled photosensitizer BCA does not accumulate selectively in neoplastic tissue.


Abstract: Ascorbate has been shown to be involved in essential fatty acid (EFA) metabolism, resulting in the suggestion that the effect of ascorbate on cell growth may be mediated through an influence on the metabolism of these FAs. This study examined the effect of ascorbate, supplemented over the nutritional concentration range of 0-100 micrograms/mL, on the in vitro growth of non-malignant LLCMK (monkey kidney) cells and malignant B16 murine melanoma cells. The effect of ascorbate on EFA composition was also investigated, and involved the determination of the levels of linoleic acid (LA), gamma-linoleic acid (GLA), dihomogamolinoleic acid (DGLA) and arachidonic acid (AA) present in the stroma and membrane of the two cell types. Ascorbate had no significant inhibitory or stimulatory effect on the growth of either the LLCMK or B16 cells. EFA levels detected in the LLCMK cells were generally higher than those detected in the B16 cells. The % composition of the various FAs in the stroma fractions of the two cell types were higher than the level of the corresponding FAs in the membrane

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fractions. 0LA levels were not detectable in the membrane fractions of the B16 cells. AA 4% composition determined in both cell types, was greater than that of any other EFA 4% composition.


Abstract: PURPOSE. During the process of metastasis, changes in cell-cell and cell-matrix contacts occur; therefore, expression of integrins, a superfamily of adhesion molecules, may be important. Expression of integrins has been studied extensively in cutaneous melanoma. Because it is known that uveal melanoma has a metastatic behavior different from cutaneous melanoma, the authors investigated integrin expression in uveal melanoma. METHODS. The authors used monoclonal antibodies recognizing integrin subunits alpha 1, beta 1, and beta 2-4 and integrins alpha v beta 3 and alpha v beta 5 on frozen sections of 32 human primary uveal melanomas and 4 metastases, followed by an avidin-biotin-peroxidase complex-immunoperoxidase technique. RESULTS. As in cutaneous melanoma, alpha 4 expression was rare, but most lesions expressed alpha 3 and alpha v beta 3. In contrast to cutaneous melanoma, in which alpha 2 is well expressed in lesions and alpha 5 is expressed in only a small percentage of lesions, alpha 2 expression was rare in uveal melanoma and alpha 5 expression was found in all lesions. A major difference was observed with regard to the alpha v beta 3 vtronecin receptor: in contrast to cutaneous melanoma, in which alpha v beta 3 is expressed in advanced primary melanomas and metastases, alpha v beta 3 was not detected in any of the primary uveal melanomas, but all lesions strongly expressed alpha v beta 5. CONCLUSIONS. Integrin expression in uveal melanoma cannot be correlated with cell type or invasiveness. In contrast to cutaneous melanoma, it seems that determination of the integrin expression profile is not suitable for categorizing uveal melanomas as less malign and significantly malignant.


Abstract: Chromone, flavone and xanthone analogues of geriparvain (1) are described. Compounds 2a and 2b are more active in inhibiting the proliferation of F10 metastatic murine melanoma cells. In particular, 2a is 13 times more active at micromolar concentration after 48 h exposure.


Abstract: Interleukin (IL)-7 has been evaluated for its influence, alone or in combination with local hyperthermia (LH), in B16a melanoma-bearing mice. Six- to eight-week-old C57BL/6J male mice were inoculated s.c. with 5 x 10(5) tumor cells into the left hind limb. Mice were randomly divided into four groups, and treated s.c. with IL-7
(5 ng) or saline as control, twice a day for three weeks beginning eight days after tumor inoculation. IL-4, 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+ /Ly2+ cells were associated with tumor growth. These parameters were restored in mice treated with IL-7 plus LH. Increases in levels of IL-1 beta, IL-6, tumor necrosis factor (TNF alpha) and interferon (IFN gamma) were associated with an increase in tumor-bearing mice treated with IL-7 and/or LH. These results suggest that changes in T-lymphocyte and cytokines such as IL-1 alpha, IL-6, TNF alpha and IFN-gamma in response to IL-7 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 therapies in certain malignant solid tumor with metastases.


Abstract: Dermoscopy (epiluminescence microscopy) is an in vivo technique that enables the clinician to visualize a variety of structures in pigmented cutaneous lesions that are not discernible by naked-eye examination. To identify the histologic correlates of these structures, a series of 71 pigmented neoplasms was documented photographically with and without dermoscopy. These lesions then underwent total excision and careful step-sectioning so that the resulting histologic slides could be correlated with the dermoscopic photographs. The histologic correlates of the pigment network, brown globules, black dots, blotches, hypopigmented areas, white areas, grey-blue areas, and whitemeal are identified. The structures seen under dermoscopy have specific histologic correlates. Understanding these histopathologic correlates will allow clinicians to better evaluate the dermoscopic features of pigmented lesions.


Abstract: Melanotic tumor from huge amts. of melanin. The relationship between melanotic neoplasm and the intensity of lipid peroxidation (measured as the level of TBARS, namely malondialdehyde) was examined. The level of TBARS in the blood serum and brain and lung homogenates of mice with melanomas was higher than that seen in healthy controls. This suggests that melanotic neoplasm may influence the oxidative potential and lipid peroxidation in the blood and inner organs of mice.

9. Eye


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 demonstrated 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 weeks, and then steadily declines to low levels by adulthood. Surplus glucose 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 (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.


- Kochan ska-Dziurovicz A, Bilinska B, Ko czynska U, Stadlik J. Interaction of iron ions with melanin isolated from choroid and iris of cattle eyes. Klin. Orzna 94:232-235, 1992. Abstract: Anal. of Moessbauer and IR spectra of melamins from cattle eyes complexed to Fe3+ at pH = 3 indicates that melanin at pH = 3 is able to fix more Fe3+ ions than at pH = 1. The value of isomer shift and quadrupole splitting indicates that the iron bound to melanin is the high-spin Fe3+ ion. Changes in IR spectra of melanin complexed with Fe3+ ions indicates, that the iron ions interact with carbonyl groups of melanin macromols.


10. Other


- Hanio ka T, Tanaka M, Tamagawa H, Shinzuki S. Epidemiologic study of melanin pigmentation in the attached gingiva in relation to cigaret smoking. Koku Eisei Gakkai Zasshi 43:40-47, 1993. Abstract: In the studies by oral exam. and questionnaire about smoking habit for 317 males, the presence of pigmentation was eobd. in 82% of current smokers, 51% of former smokers and 29% of non-smokers. The melanin index showed that ginvival pigmentation is intimately related to ages, and duration of smoking. The results indicate that ginvival melanin pigmentation is strongly associd. with cigaret smoking habits.

- Horak V, Weeks G. Poly(3,5-dihydroxyindole) melanin film electrode. Bioorg. Chem. 21:24-33, 1993. Abstract: Melanoid pigment deposited electrochem. at solid anodes from a 5,6-dihydroxyindole (DHI) soln. showed properties of a polymeric quinole-quinone. The structure of this material as a DHI polymer was confirmed with secondary-ionization mass spectra (SIMS) and IR, its redox properties were characterized with cyclic voltammetry, and its chem. properties were ascertained in a reaction with H2O2. The poly(DHI) electrode that exchanges electrons with hydroquinone may serve as a probe in the studies of the reaction of melamin with bio. systems carrying a redox system.
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