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Editorial Office: G. Ghanem (Editor), C. Meunier, R. Morandini (Production Team), Laboratory of Oncology and Experimental Surgery (L.O.C.E.), Université Libre de Bruxelles, Institut J. Bordet, Rue Héger-Bordet 1, B - 1000 Brussels, Belgium.
Phone: 32-2-535.35.46 Fax: 32-2-534.95.50 E-Mail: gghanem@resulb.ulb.ac.be

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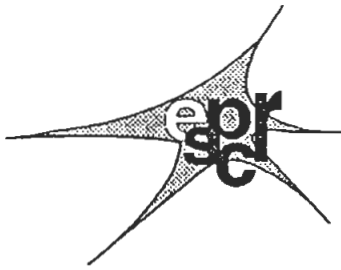

LETTER TO THE EDITOR
DISCUSSION, REVIEW, SHORT COMMUNICATION, ...

Meeting Report

VII[th] Annual Meeting of the PASPCR
June 15th - 18th, 1997 The Westin Hotel Providence, RI, USA

Overview / Summary of Meeting - Walter Quevedo

On behalf of Hal Swartz and myself, I would like to congratulate the 129 scientists that participated in the 7th Meeting of the PanAmerican Society for Pigment Cell Research and the fine Providence weather for making it a good one. The clambake organized by Tom Holstein and the Reception and Banquet arranged by Fae Best Carletti were standout features of the social program. The scientific sessions were well attended throughout and discussions following presentations were of high quality. One had the feeling that pigment cell research is at the cutting edge of science not only in pursuing leads provided by workers in other fields but also in compensating such workers with new investigative directions that they might follow. Hal's concept of the sunrise sessions was vindicated by the consistently high attendance and unabating enthusiastic exchanges between "teachers" and "students" during classes that ended all too soon. There were moments of pride and sentimentality such as those accompanying Vince Hearing's Gelb Award, Joe Bagnara's Career Investigator Award, and Aaron Lerner's and Tom Fitzpatrick's acceptance of the Meeting Chairmen's Award for Outstanding Contributions to Pigment Cell Research and its Pursuit in New England. It was an honor for Providence to host the 7th Meeting.

Symposium I - Molecular Aspects of Malignant Melanoma - Frank Meyskens

This was an exciting symposium that elicited many questions and comments. Two major types of papers were presented: strategies related to the underlying basis for immunotherapy in human melanoma and characterization of molecular changes in certain genes related to pigmentation.
The symposium opened with a useful historical overview of the field of biologic and immunologic approaches to the management of melanoma (H Wanebo). To date, almost all approaches to melanoma management have produced no or modest benefit in the clinical situation, although the studies presented by D Shrayer and colleagues suggested that in an animal model of human disease that both primary melanoma tumor growth and lung metastases could be prevented. The emphasis of subsequent speakers was on the necessity of characterizing the specific features of immunologic alterations. Precise identification of human melanoma antigens by tumor infiltrating T lymphocytes (Y Kawakami), the immune response to melanosomal differentiation antigen induced by altered antigen (S Bartido). The new human melanocyte specific gene factor MSG1 was presented in two papers; T Shioda showed that the factor was a nuclear protein, and was induced by phorbol ester and was transcriptionally regulated. M Fenner provided further information that MSG1 promoter activity was correlated with pigmentation.
In two studies from Yale, it was shown that the regulation of FGF-2, a requirement for melanocyte proliferation, was regulated by c-Myb proto-oncogene (M Miglarese). In a second paper, R Halaban and her colleagues showed that when tyrosinase was inadvertently retained in the endoplasmic reticulum, its degradation was accelerated, contributing to the amelanotic phenotype, and suggesting perhaps that this property is phenotypical rather than from direct genetic alteration. Finally, a paper fitting neither of the two major areas, but dealing with an important, relatively neglected, aspect was presented (F Meyskens). NKFB and AP-1 were shown to be regulated differently in melanocytes and

melanoma cells and the response in terms of redox suppression between the two cell types was markedly different. Overall, this symposium offered considerable anticipation that new approaches to the management of melanoma may evolved from these and other basic advances in understanding described throughout the conference.

Symposium II - Role of Melanocyte Death during Development and Adaptive *Responses of Skin to Damaging Agents* - Raymond Boissy

This symposium began with the keynote address presented by James H Wyche entitled "A model system for studying cell death regulation". Dr Wyche presented a review of molecular events occurring in the initial stages of apoptosis prior to DNA damage by describing the molecules modulating susceptibility to apoptosis (i.e., bax, bcl2, etc.) and the various caspases involved in proteolysis during initiation of apoptosis. He highlighted this review by describing his research on staurosporine treated promyelocytic leukemia cells which exhibited activation of caspase-3 causing the proteolysis of the DNA-dependent protein kinase which then correlated with the initiation of apoptotic chromosomal DNA degradation. Zalfa Abdel-Malek then presented "Elucidation o f the signaling pathway which mediates the responses of human melanocytes to UVB light" in which she demonstrated that cultured human melanocytes exposed to UVB undergo proliferation arrest and can progress to apoptosis after overexpression of p53. In addition, cAMP inducers like MSH can accentuate the increased melanization response of cultured melanocytes to UVB and promote movement of cells to the S phase of proliferation. Fan Yang next presented "The effects of tyrosinase activity and commonly used mitogens on the cytotoxicity of 4-tertiary butyl phenol (TBP) to human melanocytes" in which she demonstrated that the development of apoptosis in cultured human melanocytes after exposure to 4TBP was not influenced by the level of tyrosinase activity or melanin content. In contrast, the presence of either MSH or bFGF could accentuate cytotoxicity of 4TBP exposed melanocytes. William Pavan then presented "Met-HGF signaling is critical for melanocyte development: Implications for Waardenburg Syndrome type II" in which he demonstrated, using genetically engineered mice as either knockouts or overexpressers for the Met tyrosine kinase receptor or its ligand HGF/SF respectively, that this signaling pathway is crucial for continued development of ckit/steel positive neural crest cells towards melanocytes. E. Michelle Southard-Smith next presented "Physical mapping and embryological analysis of Dominant Megacolon, a mouse model of Hirschsprung's disease" in which she described the mapping of the Dom locus to a 0.01cM region proximal to D15Mit2 by linkage disequilibrium and physical mapping. In addition, expression *** tructure". S Orlow (NY) began the session with a discussion of the *pink-eyed dilution* gene product and its role in melanogenesis and melanosome formation. Melanocytes cultured from p-null mice possess smaller, more numerous melanosomes, and this is corrected in part by culture in high concentrations of exogenous tyrosine, as are alterations in levels of tyrosinase and some other melanosomal proteins. V Setaluri (Winston-Salem) discussed the sequences responsible for exit from the ER and trafficking to melanosomes of gp75 (aka TRP-1). By the use of site-directed mutagenesis, the information necessary has been shown to be present in the short cytosolic tail of gp75, and the presence of a dileucine motif is an important criterion for proper trafficking, though it may not be the only one. VJ Hearing (Bethesda) gave the Gelb Keynote Address in the context of this symposium. He summarized a large body of work from his own lab and others, focusing especially on the differences between pheomelanosomes and eumelanosomes. Levels of tyrosinase are decreased and levels of TRP-1, TRP-2, p and silver proteins are almost undetected in pheomelanic hair follicle extracts. Cultured melanocytes can now be treated with recombinant agouti signal protein, enabling the process of pheomelanosome formation in response to ASP to begin to be dissected *in vitro*. J Hammer (Bethesda) presented work from his laboratory on myosin V^a, the *dilute* locus gene product. This unconventional myosin can be shown to be present at the melanosomal surface by immunofluorescence, immuno-EM and by biochemical techniques. In cells from mice with dilute mutations, melanocytes extend dendrites, but melanosomes fail to traffic down these dendrites and remain in a perinuclear distribution, thus implicating myosin V^a in the transport of melanosomes as

a prelude to their transfer. J Bhawan (Boston) discussed various theories regarding the transfer of melanosomes from melanocyte to keratinocyte. Careful EM studies provide evidence in support of not one, but rather two or three modes of transfer that may be operative in sun-exposed skin.
P Samaraweera (Orlow lab) described a 65 kDa protein which localizes to the cytosolic face of the melanosome, and unlike previously described melanosomal proteins is peripheral rather than integral to the membrane. It shows altered detergent solubility in melanocytes cultured from buff mice, which may have a melanosomal transport defect. K Sato (Orlow lab) used site directed mutagenesis and the construction of chimeric molecules to show that both TRP-1 and TRP-2 are able to stabilize tyrosinase protein in cotransfection experiments. The amino terminal portion of TRP-1 is critical to its ability to effect this stabilization. B Potterf (Hearing/Gahl labs) examined the transport of sulfhydryl compounds by melanosomes. Although glutathione transport seems not to be an issue, melanosomes show robust transport of cysteine. The role of such transport in the control of melanogenesis was discussed. Finally M K Cullen (St. Louis) examined tyrosine uptake by isolated melanosomes. Cytosolic factors appear to regulate this accumulation, and interestingly, extracts from melanocytes cultured from mice with "melanolysosomal" defects such as ruby exhibit an altered ability to enhance this tyrosine transport.

Symposium IV - Ocular Melanocytes and RPE

Role of Transcription Factors in Pigment Cell Biology - **Seth J Orlow**

A symposium on Ocular Melanocytes and RPE/ Transcription Factors in Pigment Cell Biology was chaired by Seth Orlow. Richard Sidman (Southborough) delivered the keynote address. He and his colleagues have examined the effects of two white-spotting mutations, namely mi^{vit} and W^{sh}, on RPE and choroidal melanocytes. Careful analysis of the RPE of mi^{vit} mutant mice reveals that this gene controls not only pigmentation but proliferation as well, with more numerous taller RPE cells, many of them hypopigmented, noted early on in the mutants. An unusual tigroid pattern can be discerned in ocular tissue from W^{sh} mice, with the additional unexpected observation that giant clumped melanosomes are present in pigment cells from this mutant, reminiscent of those in beige mice. Since W^{sh} mi^{vit} double mutant mice are indistinguishable from the mi^{vit} single mutants, Dr Sidman deduced that Kit may act downstream of MITF. Ongoing studies are geared towards determining how nuclear localization may play a role in controlling MITF function.
Sylvia Smith (Augusta) presented work on the expression of the TRP family in RPE/choroid of mi^{vit} mice. Levels of TRP-1 are dramatically reduced even at the earliest time points examined, and levels of tyrosinase diminished to a lesser extent, whereas levels of TRP-2 were unaffected, suggesting that MITF in vivo plays its most critical role in TRP-1 gene expression regulation. A Zervos (Charlestown) and his group have identified an MITF-interacting protein using the yeast 2-hybrid system. This protein is the human homolog of RKR2, which contains both multiple zinc finger repeats and basic regions. The protein interacts with and inhibits the transcriptional regulatory function of MITF.
Rivka Rachel (NY) discussed her efforts to determine how the ability of RPE cells to make melanin affects the retinofugal projections during embryonic development. She has looked at both albino mice which lack all tyrosinase activity as well as those from mice homozygous and heterozygous for dark-eyed albinism (c^{44H}) which have low but demonstrable levels of tyrosinase activity to ask whether the pigment-producing effects are graded or there is a "gate" level needed below which normal development will not occur. Her results suggest that the reality may be a mixed-situation. Dan-Ning Hu (NY) and Ray Boissy (Cincinnati) each discussed their laboratories efforts to study melanogenesis and growth of cultured human ocular melanocytes. These cells can now be cultured from the uveal tract and will synthesize melanin and melanosomes in culture. Agents which upregulate cyclic AMP stimulate growth and melanogenesis by these cells, including via the bet adrenergic receptor. By contrast muscarinic agonists inhibit their growth. MSH receptors appear not to be expressed by ocular melanocytes in vitro. Finally Brian Potterf (Hearing/Gahl labs) expanded on his previous work to show that melanosomal tyrosine transport is upregulated by pretreatment of cells with MSH or dbcAMP.

Whether this regulation is at the translational or transcriptional levels is under investigation. Of a wide range of small molecules, those that compete with tyrosine best are L-phenylalanine, L-DOPA and L-leucine. The potential role of tyrosine transport in the regulation of melanogenesis and in the switch from eumelanin to pheomelanin was discussed.

MiniSymposium I - Pigment Cell Genetics and Molecular Biology - Gregory Barsh

Synopsis pending

MiniSymposium II - Signaling Pathways in Pigment Regulation - Zalfa Abdel-Malek

Synopsis pending

MiniSymposium III - Comparative Aspects of Vertebrate Pigmentation - Joseph Bagnara

In his introduction to the session, Joe Bagnara pointed out that in most life science disciplines, the word "comparative" is inferred immediately to mean cold-blooded vertebrates and, as such, are looked at as model systems by those who investigate mammals. He emphasized that this is a misconception and that lower and higher vertebrate pigmentation systems are related primarily by homology rather than by analogy. He enumerated some of these homologies and several of these facets were discussed in more detail by subsequent speakers. Thus, Ken Mason next discussed molecular, genetic, and evolutionary aspects of the pigmentation of the Mexican axolotl, a salamander species for which many mutants are available. Comparative analysis of these mutants was discussed in relation to relevant mammalian mutants. Mason indicated that "the cladistic analysis of the TRP-1 cDNA from the axolotl along with members of the tyrosinase gene family from a number of species, clarified the evolutionary relationships of these molecules." The molecular biology of other axolotl pigmentary enzymes was discussed. Several shorter presentations related to the development of pigmentation were presented later. Thus, Mark Reedy disclosed that, in the chick embryo, later chromatoblasts emigrating from the neural crest follow a dorsolateral route and differentiate into melanocytes. He suggested that specification of these neural crest cells as melanoblasts occurred *in situ* and dictated their later migration and their route. Mark Moody then discussed his work on developmentally regulated expression of TRP-1 in the axolotl. The axolotl was also used by Susan Holder who discussed developmental regulation of xanthophore differentiation by region-specific influences. Perhaps, one such region-specific factor is melanization inhibiting factor (MIF) which appears to function much as does the product of the *agouti* gene implicated in several aspects of mammalian pigmentation. Thus, J Newton presented his work on the cloning and molecular characterization of two genes, the *agouti* and *extension*, that are involved in the regulation of pheomelanogenesis in breeds of domestic dogs. The Smyth line chicken (SL), long considered a model for autoimmune human vitiligo, was discussed by G.P. Sreekumur who presented a penetrating analysis of the genetic and molecular linkage of the Smyth line chicken system. For his presentation, he was later honored with the award for the most outstanding presentation by a graduate student. The functional aspects of chromatophores were reviewed by Mac Hadley who emphasized the role of MSH and its receptor MC1R. The physiology of chromatophores was further discussed by Ana Maria Castrucci who considered the roles of a variety of neural and hormonal agents in the normal regulation of chromatophore function in fishes. A remarkable means of chromatophore control was considered by Mark Rollag who discussed photo-transduction in *Xenopus* melanophores. He reported that such light-sensitive melanophores contain a unique opsin that represents a class of opsins that became distinct at about the time that vertebrate and invertebrate opsins diverged during evolution.

MiniSymposium IV - Photobiology and Biophysics of Melanin and Melanocytes - Helene Hill

This symposium was held on Tuesday, June 17th and was cochaired by Drs. Harold M Swartz and Helene Z Hill. Three of the papers dealt with effects of iron in one form or another on melanin. Jeffrey Tosk noted that the brain stem has one of the highest concentrations of ferritin in the body and this iron-rich substance may interact with catecholaminergic precursors to form melanin. This could

account for the presence of melanin in this area of the brain. Dr. Eisner is also interested in neuromelanins and the structure around the Fe-site. Natural and synthetic neuromelanins were compared and showed small but significant differences. Dr. Jacobson's talk focused on the role of extracellular melanin of pathogenic yeast as a redox buffer the activity of which is enhanced by Fe++. Iron may provide the reducing equivalents needed in order for melanin to neutralize pro-oxidants generated by the immune system, thereby enhancing the pathogenesis of the yeast.

Determining the routes taken by various precursors to synthesize the various melanins is key to our understanding of the balance of pigments in the final products and ultimately to the understanding of pigment function. Dr. Ito showed in a most elegant fashion that phaeomelanin synthesis is favored in the presence of high cysteine and low melanogenic activity. Eumelanin will dominate when the opposite conditions exist. In the presence of TRP1, DHICA is oxidized to its corresponding quinone and this in turn will oxidize DHI resulting in a mixed polymer.

Understanding the nature of the radical spectra generated by melanins of various types and sources is key to understanding the myriad effects attributed to melanins. Mark Nilges, in his studies of the free radicals generated by melanins, cast doubt on the hypothesis that dehydrohydroxybenzothiozine is the source of the radical spectrum of phaeomelanin.

'Is melanin photoprotective, photosensitizing or neutral?' remains a question that has yet to be answered. The literature is rife with conflicting results which have only served to muddy the waters. Endpoints studied are survival, pyrimidine dimers, growth arrest, to mention a few. Yet it is to skin cancer and especially melanoma that we should direct our attention. In tissue culture studies, this boils down to studying cellular transformation and mutation. These endpoints are technically difficult to investigate. Ms. Kaur, a graduate student in Dr. Hill's lab, has developed an artificial melanocyte so-to-speak by transfecting a plasmid containing the tyrosinase gene into Chinese hamster ovary cells which are exquisitely sensitive to mutation. Her preliminary studies show that cells which express pigment are less sensitive to killing by ionizing radiation and UVA and more sensitive to UVC than plasmid controls lacking the gene. The responses of the pigmented cells were no different from the controls after exposure to UVB and FS20 radiation. The pigment in the cells was eumelanin by ESR analysis. These cells will be useful in future mutational analyses.

2. Biology of pigment cells and pigmentary disorders

(Comments by Dr M. Picardo)

Some papers have been published on vitiligo in the last few months. Nordlund and Majumder presented a clear review on this topic reporting many of the most recent studies on this disease. Yu et al reported an alteration of the mononuclear cell function in vitiligo subjects particularly in the cytokine production. These authors suggest that the observed increase in the secretion of pro-inflammatory cytokines may have an important role in the melanocyte toxicity through an enhancement of effector cell migration and attachment to target cells. In the same issue of J.I.D. the group of Thomas Luger present evidence showing additional activities of α-MSH. UV irradiation or application of tumour promoters may stimulate the release of α-MSH which may have an important role in the modulation of epidermal stress response as well as of inflammatory reaction. Carroll and co-workers using an interesting model of transgenic mice which over express γ-IFN in the epidermis, together with eczema, present hair loss and hypopigmentation suggesting that the production of this cytokine may be one of the mechanisms involved in post-inflammatory hypopigmentation. On the possible mechanisms of UV-induced pigmentation, Romero-Graillet et al. showed that normal human keratinocytes are able to produce nitric oxide in response to UVB and UVA irradiation. Conditioned media from UV exposed keratinocytes stimulate dendriticity and melanin synthesis in cultured melanocytes and this effect is reversed by NO scavengers. The observations of these authors, partly previously reported, indicate that NO is one of the paracrine mediators of UV-induced melanogenesis and they provided further support to the theory indicating a free-radical mediated process as an important step in UV-induced tanning. With a similar view, Filipe et al. have showed that topical application of superoxide dismutase prevent inflammation and histological alterations due to PUVA treatment on human skin. Among the papers published on the biological characteristics of melanoma cells, Wei and co-workers have presented data showing a direct correlation between DNA repair capacity and metastatic potential in a murine melanoma cell line. Following exposure to UV light of different metastatic or non metastatic clones, these authors have evaluated , by the host-cell reactivation assay, the DNA repair capacity of each clone and have found, as expected, a direct correlation between DNA repair capacity and cell survival, and with metastatic potential. The authors suggest that their data may partly explain the resistance to chemotherapy of melanoma. Moral et. al. , evaluating immunoistochemically the expression of the different classes of glutathione S transferase in melanocytes and melanoma cells, have showed that in melanoma cells alfa GSTs were only occasionally present whereas mu form was found. These results present new questions on the role and the correct distribution of all the forms of the isoenzymes in the skin. Ledda et al. report that the expression of the secreted protein acidic and rich in cysteine (SPARC) is associated with the neoplastic progression in human melanoma. SPARC is a secreted glicoprotein associated with cell-matrix interactions during morphogenesis, migration and proliferation. Immunoistochemical analysis showed that the protein is strongly expressed by primary and metastatic melanoma cells and is not expressed in normal melanocytes. The authors also describe a specific metabolism by melanoma cells with a post-transcriptional glycosylation and extracellular cleavage. Jiang and co-workers demonstrated that melanotropic membrane receptors are characteristic of human keratinocytes, melanocytes and melanoma cells and suggest that the expression of these receptors could be targets for melanotropic peptides for the identification, localisation and possibly chemotherapy of melanoma. In order to detect primary and metastatic melanoma cells, Sarantou et. coll. developed a multimarker reverse transcription PCR plus Southern blot assay for tyrosinase, TRP-!, TRP-2, Pmel 17 and MART-1/Melan-A. Evaluating melanoma cell lines, primary and metastatic melanoma, tumour involved lymph nodes and patients'blood, the authors found at least one mRNA in 86% of the specimens assayed and concluded that this multimarker melanoma associated antigen analysis is more reliable than a single molecular marker to detect melanoma cells.

Finally, Hedley et al. have evaluated the influence of the extracellular matrix proteins on cutaneous and uveal melanocytes. The data reported in the paper, based on previous work and on some new experiments, indicate that, at least in vitro, ECM strongly influence morphology, tyrosinase activity and proliferation of both cutaneous and uveal melanocytes and suggest that also in vivo ECM may have a regulator in controlling proliferation and melanin synthesis. In the same issue of Pigment Cell Res. Bowers and coll. presented data on the effect of alpha MSH in vitro avian melanocytes. These authors, using the model of feather melanocytes from Barred Plymounth Rock, showed that these cells have alpha-MSH receptors and are able to respond to the hormone through the activation of c-AMPC as a second messenger.

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Melanocyte cultures

(Comments by Dr N. Smit)

Recently Bessou et al already described the use of human skin reconstructs of different phototypes and they used this system to study the responses to UVB irradiations (JID 107, 684, 1996). Now they have shown that the system can also be used to investigate reagents that can influence melanogenesis, such as isobutylmethylxanthine, 1-oleoyl-2-acetyl sn-glycerol, kojic acid, and mequinol, either by addition to the culture medium or used topically at the air-liquid interface. In the study by Benathan melanocytes from different skin phototypes were used showing that the cells basically maintain their differences in pigment production. The authors show that the phaeomelanin precursor 5-S-cysteinyl-dopa is present in both cell types and is probably formed by the direct addition of cysteine to dopaquinone. Two papers in Current Eye Research describe melanocyte cultures originating from the eye (Diebold et al and Smiththomas et al). Especially the latter study compares the melanogenic properties of choroidal melanocytes and retinal pigment epithelial (RPE) cells. They show that the RPE cells were unpigmented in culture whereas the choroidal melanocytes showed different properties of pigment

production (TRP1 and 2 expression and tyrosinase activity). Tobin and Bystryn described in 1995 (JID 104; 86) a method to isolate and culture hair follicle melanocytes (HFM). In their present study they distinguish two types of HFM and epidermal melanocytes(EM). In primary cultures very dark pigmented HFM were still present but did not proliferate in culture. The second major population of HFM was much less pigmented and had smaller cell size than the EM but these cells showed a strong proliferation.
Sviderskaya et al describe very interesting mouse melanocyte and melanobla-st cultures with mutation in the P gene. The p-null melanocytes lacking the P gene product, a melanosomal transmembrane protein, were strongly hypopig-mented. The deficient melanin biosynthesis can be corrected by transfection of the cells with the human P cDNA. Mutant P cDNA as found in OCA2 patients could only partially correct the defective melanogenesis. The work clearly demonstrates the important function of the P gene product in melanin synthesis. Other interesting mutant melanocytes are described by Provance et al who cultured melanocytes from dilute mutant and wild type mice. They argue that the dilute myosin is necessary for the localization of melanoso-mes in the cytoplasm and may be required for transport of melanosomes throughout the cell. This concept is confirmed by the work of Wu et al who show that myosin V and F-actin are both concentrated in dendrites and dendritic tips, a localization where indeed the concentration of melanosom-es is cultured melanocytes is high. Le Poole et al have produced a melanocyte cell line (PIG1) which, after they had undergone more than twice the population doublings of the parental cells, showed proliferation exceeding that of early passage normal melanocytes by 100%. The PIG1 cells which contained more pigment than parental cells were produced by introducing E6 and E7 open reading frames of HPV 16 into the cultured melanocytes, by using a retroviral vector. Use of this cell line in different laboratories or production of similar lines may be very useful for future pigment cell research.

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Beneficial effects of the melanocortin alpha-melanocyte-stimulating hormone on clinical and neurophysiological recovery after experimental spinal cord injury. Neurosurgery. 40(1):122-30;discussion 130-1, 1997.

4. Photobiology and photochemistry

(Comments by Dr M. d'Ischia)

The present literature search collects together papers dealing with disparate aspects of the photochemistry and photobiology of cutaneous melanocytes, ranging from the molecular mechanisms of UV-induced hyperpigmentation through apoptosis and the genetics of melanoma formation to photodynamic therapy of the tumour.
In two related papers by Romero-Graillet et al. evidence is reported showing that nitric oxide, constitutively by keratinocytes on exposure to UV radiation, plays a critical role as paracrine of UV-induced melanogenesis via activation of a cGMP-dependent kinase and the cGMP signal transduction pathway. The rise in melanogenesis induced by NO in cultured melanocytes is associated with an increase in the levels of tyrosinase and TRP-1.
The crucial function of keratinocytes in activating and protecting melanocytes from
UV-induced injury emerges also from the study by Zhai et al. showing that NGF, induced by UV-stimulated keratinocytes, protects epidermal pigment cells from apoptosis after physiologie UV exposure.
Additional reports on UV-induced pigmentation and skin defense mechanisms include the first demonstration of the expression of the corticotropin releasing factor (CRF) gene and production of CRF peptide by UVB-stimulated human melanocytes (Slominski et al); the regulation of production and release of proopiomelanocortin derived peptides by cultured human melanocytes and keratinocytes by UVB radiation (Chakraborty et al.) and the characterization of the alterations in melanogenic enzyme activities induced by PUVA (Mengeaud and Ortonne JP).
The relationship between UV exposure and melanoma is investigated from different viewpoints in a series of papers dealing with the expression of E and P-cadherin in melanomas with tumour progression and following UV exposure (Seline et al.); the role of immune effector mechanisms in UV-induced growth of melanoma (Donawho et al.); the genetics of UV-induced melanoma formation in Xiphophorus (Nairn et al.); the enhanced expression of protein kinase C subspecies in melanogenic compartments of B16 melanoma cells induced by UV or MSH (Oka et al.) and the changes in ferritin and glutathione levels in human melanoma cells induced by oxidative stress and UVA radiation (Applegate et al.). Other papers deal with the effects of photosensitizers on human melanoma cells and murine melanoma (Hadjur et al.) and a review of the evidence relating cutaneous melanoma to sun exposure, highlighting the importance of intermittent sun exposure and a history of sunburn (Elwood JM).

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Susceptibility of human melanoma cells to oxidative stress including UVA radiation. Int J Cancer. 67(3):430-4, 1996.

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Effect of photosensitizer system and irradiation parameters on the efficiency of photodynamic therapy of B16

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Enhanced growth of murine melanoma in ultraviolet irradiated skin is associated with local inhibition of immune effector mechanisms. J Immunol. 157(2):781-6, 1996.

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Melanoma and sun exposure. Semin Oncol. 23(6):650-66, 1996.

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6. Genetics, molecular biology

(Comments by Dr F. Beerman)

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Molecular basis of congenital hypopigmentary disorders in humans - a review. Pigm Cell Res. 10(1-2):12-24, 1997.

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An improved and combined reverse transcription polymerase chain reaction assay for reliable detection of metastatic melanoma cells in peripheral blood. Melanoma Res. 7(2): 111-116, 1997.

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Correction of retinal abnormalities found in albinism by introduction of a functional tyrosinase gene in transgenic

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Selective increase in specific alternative splice variants of tyrosinase in murine melanomas - a projected basis for immunotherapy. Proceedings of the National Academy of Sciences of the United States of America. 94(10):5332-5337, 1997.
Summary: The existence of alternative splice variants of mouse tyrosinase has been published by Ruppert et al. (EMBO J. 7, 2715-2722, 1988) and Porter & Mintz (Gene 97, 277-282, 1991). In this paper, the presence of such alternative transcripts is analysed in murine melanomas, as they are available in the Tyr-SV40E transgenic mice. Several splice variants were equally represented in skin and in tumor samples, for example deletions of exon 3. However, the Delta 1b and Delta 1d alternatively spliced transcripts, due to deletions within the first exon, were specifically augmented in most of the tumors over their very low levels in skin. The level of Delta 1b rose as high as 11.3 % of total tyrosinase mRNAs as compared with 0.6 % in skin. Such alternative transcripts might lead to unusual peptides, which could have antigenic potential in melanotic tumors.

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Sequence analysis of the human tyrosinase promoter from a patient with tyrosinase-negative oculocutaneous albinism. Pigm Cell Res. 10(1-2):64-67, 1997.

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Apparent digenic inheritance of Waardenburg-syndrome type 2 (WS2) and autosomal recessive ocular albinism (AROA). Human Mol Genet. 6(5):659-664, 1997.
Shortened abstract: Two previously described families seemed to delineate a new subtype characterized by WS2 in conjunction with ocular albinism (OA). We screened for mutations in one of the WS2-OA families and discovered a 1 bp deletion in exon 8 of MITF.. OA previously has been associated with compound heterozygosity for a mutant TYR allele and the TYRR402Q allele, a functionally significant polymorphism that is associated with moderately reduced tyrosinase catalytic activity, In this family, all of the individuals with the OA phenotype are either homozygous or heterozygous for TYRR402Q, and heterozyous for the 1 bp deletion in MITF. This suggests that the WS2-OA phenotype may result from digenic interaction between a gene for a transcription factor (MITF) and a gene that it regulates (TYR).

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Prevalent and novel mutations of the tyrosinase gene in Korean patients with tyrosinase-deficient oculocutaneous albinism. Molecules & Cells. 7(2):187-191, 1997.

- Sato S, Masuya H, Numakunai T, Satoh N, Ikeo K, Gojobori T, Tamura K, Ide H, Takeuchi T, Yamamoto H.
Ascidian tyrosinase gene - its unique structure and expression in the developing brain. Develop Dynamics. 208(3):363-374, 1997.
Summary: This paper reports on the isolation of the tyrosinase gene from an anchestral chordate, the ascidian *Halocynthia roretzi*. Larvae of this animal have two sensory pigment cells in the brain, which express tyrosinase RNA. Moreover, DOPA oxidase activity is detected on protein extracts of tailbud stages. The isolated tyrosinase cDNA has an open reading frame of 596 amino acids, which is 36-39 % identical in amino acid sequence to vertebrate tyrosinases.

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Technical advances in prenatal diagnosis of tyrosinase-negative oculocutaneous albinism. Acta Dermato Venereologica. 77(1):10-13, 1997.

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Complementation of hypopigmentation in p-mutant (pink-eyed dilution) mouse melanocytes by normal human P cDNA, and defective complementation by OCA2 mutant sequences. JID. 108(1):30-34, 1997.

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Evidence to suggest that expression of MITF induces melanocyte differentiation and haploinsufficiency of MITF causes Waardenburg-syndrome type 2A. Pigment Cell Res. 10(1-2):25-33, 1997.

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A non-epistatic interaction of agouti and extension in the fox, Vulpes vulpes. Nature Genetics. 15(3):311-315, 1997.
Shortened abstract: We have cloned and characterized the MC1R and the agouti gene in coat colour variants of the fox (Vulpes vulpes). A constitutively activating C125R mutation in the MC1R was found specifically in darkly pigmented animals carrying the Alaska Silver allele (E(A)). A deletion in the first coding exon of the agouti gene was found associated with the proposed recessive allele of agouti in the darkly pigmented Standard Silver fox (aa). Thus, as in the mouse, dark pigmentation can be caused by a constitutively active MC1R, or homozygous recessive status at the agouti locus. Our results, demonstrating the presence of dominant extension alleles in foxes with significant red coat colouration, suggest the ability of the fox agouti protein to counteract the signalling activity of a constitutively active fox MC1R.

- Xu YM, Stokes AH, Freeman WM, Kumer SC, Vogt BA, Vrana KE.
Tyrosinase mRNA is expressed in human substantia nigra. Mol Brain Res. 45(1):159-162, 1997.
Summary: It is commonly assumed that neuromelanin in substantia nigra is due to spontaneous autooxidation of dopamine, and that tyrosinase is not involved in these processes since not expressed in nervous system. However, this issue is not completely solved. The results presented here further support the idea of tyrosinase expression in brain. The authors used RT-PCR on RNA isolated from human brain tissue. Using tyrosinase-specific primers, they detect specific amplification of tyrosinase in all (four) samples of the substantia nigra, and no amplification (control) in RNA of cerebellum.

7. Tyrosinase, TRP1, TRP2 and other enzymes

(Comments by Prof. J.C. Garcia-Borron)

Several papers referenced in this issue comment on the production and metabolism of reactive oxygen species (ROS) during melanogenesis or within the melanocytes. Nappi and Vass (Melanoma Res. 6, 341-9) provide evidence for the "in vitro" generation of hydrogen peroxide during spontaneous and tyrosinase-catalyzed oxidation of the melanogenic intermediates 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid. As pointed out by the authors (and also by Wittbjer et al., Pigment Cell Res., 9, 92-5), hydrogen peroxide generation most likely affects the rate, final outcome and cytotoxicity of the melanogenic pathway. In two related papers, Valverde et al report on the possible use of another ROS, the superoxide anion, as a tyrosinase substrate. This would account for a protective effect of tyrosinase (and hence melanin formation) against oxidative stress, both in mouse (Valverde et al., Pigment Cell Res., 9, 77-84) and in human melanocytes (Valverde et al., Exp. Dermatol., 5, 247-253). According to the authors, tyrosinase, by using superoxide anion as a substrate, would lower the intracellular levels of this highly reactive species, thus providing a detoxification mechanism. The idea that superoxide anion might be a substrate for tyrosinase has been also supported by previous studies, but this point of view is still a matter of discussion. For example Wittbjer et al., (Pigment Cell Res., 9, 92-5) do not find convincing evidence in favour of a possible role of superoxide as a tyrosinase substrate, in an experimental system much simpler than the one used by Valverde et al. Moreover, these authors provide a reasonable mechanism to account for dopa formation in the presence of tyrosine and superoxide. The possible role of the superoxide anion in the melanogenic pathway remains therefore open to discussion, and its elucidation will probably call for "in vitro" studies using experimental setups as simple as possible and highly purified enzymatic preparations.
The regulatory roles of autocrine and paracrine signaling molecules is the subject of two papers referenced in this issue. Martínez-Esparze et al. (J. Biol. Chem. 272, 3967-72) describe a potent inhibitory effect of TGF beta on melanin formation in B16 mouse melanoma cells. TGF beta is produced by keratinocytes, and possibly also by melanocytes, and is therefore a potentially important regulatory molecule, although further studies are needed to examine whether or not the findings in the B16 mouse melanoma model can be extrapolated to normal human melanocytes. As opposed to the hypopigmenting role of TGF beta, nitric oxide synthesized by keratinocytes in response to UV irradiation is reported to stimulate melanogenesis (Romero-Graillet et al., J. Clin. Invest., 99, 635-42). The same research team prevously reported a similar effect of nitric oxide synthesized by melanocytes themselves. In addition to these recent data, the number of reports on the production of proopiomelanocortin-derived melanogenic peptides is raising steadily. A local regulation of melanogenesis is therefore more than likely, and the relative importance of local signaling molecules, as compared to endocrine factors, will have to be assessed.

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On the melanization of the rat's eye. Pigment Cell Res. 9:142-147, 1996.

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Inhibition of the phosphatidylinositol 3-kinase/p70(S6)-kinase pathway induces B16 melanoma cell differentiation. J Biol Chem. 271:31824-31830, 1996.

- Hedley S, Gawkrodger D, Weetman A, MacNeil S.
Investigation of the influence of extracellular matrix proteins on normal human melanocyte morphology and melanogenic activity. Br J Dermatol. 888-897, 1996.

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Demonstration of cytoplasmic tyrosinase mRNA in tissue-cultured cells by reverse transcriptio (RT) in situ polymerase chain reaction (PCR) and RT PCR in situ hybridization. Diagn Mol Pathol. 6:26-33, 1997.

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Tyrosinase may protect human melanocytes from the cytotoxic effects of the superoxide anion. Exp Dermatol. 5:247-253, 1996.

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8. Melanoma and other pigmented tumours

(Comments by Dr N. Smit)

Melanoma Therapy

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Direct transfer of a foreign MHC gene into human melanoma alters T cell receptor V beta usage by tumor-infiltrating lymphocytes. Cancer Immunol Immunother. 43(1):49-58, 1996.

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Adoptive immunotherapy with vaccine-primed lymph node cells secondarily activated with anti-CD3 and interleukin-2. J Clin Oncol. 15(2):796-807, 1997.

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Melanoma Experimental Therapy

In the papers by Cooksey et al and Riley et al the development and cytotoxicity of anti-melanoma prodrugs is described. A set of 26 substituted phenols were tested in a cytotoxicity assay in which tyrosinase was added to generate the corresponding o-quinones (Riley). In the paper by Cooksey six new 4-substituted phenols were described and by extrapolation of the quantitative structure activity relationship (QSAR)of compounds studied earlier (Cooksey et al, 1995) Anti-Cancer Drug Design 10, 119) the reactivities of the o-quinones towards thiols could be predicted. In the paper by Riley it is shown that in the cytotoxicy assay the cytotoxicy of the compounds was abrogated by the addition of glutathione. This effect could be ascribed to prevention of cellular thiol depletion. The work is an indication of the importance of thiol compounds in the protection against o-quinones in the cell. It also shows that QSAR can be helpfull in the design of anti-melanoma pro-drugs. The work of ! Pendyala et al shows that reduction of glutathione in melanoma tumour cells can strongly increase the cytotoxicity of agents such as iproplatin.

Fosternora and Siden have reviewed the use of amifostine as a pro-drug in general cancer treatment in order to protect normal tissues against the toxicity of antineoplastic drugs. Amifostine is converted by alkaline phosphatase to yield WR-1065, an active sulphydryl compound which protects against oxidative damage. Among other reasons, a decreased activity of alkaline phosphatase in tumour cells was suggested as an explanation for the selectivity of amifostine for normal tissues. Johansson and coworkers studied the infiltration of NK cells into B16-F10 melanoma tumour models. The infiltration of NK cells into substratum-bound microtumours (MTs) resulted in the disintegration of the MTs and also when the melanoma cells were grown on macroporous gelatinous microcarriers the NK cells displaced the tumour cells effectively. The model systems may be used to further study of the interaction of NK-cells and their targets.

The papers by Setiawan et al, Sharma et al and Szala et al make use of different forms of drug delivery. Setiawan used diet supplementations to reduce low-density lipoprotein (LDL) receptors in the liver. Addition of boronated LDL

resulted in a 5:1 ratio of the tumour-blood boron concentrations. Sharma et al used polivinylpyrrolidone nanoparticles which contained Taxol which resulted in significant increased survival times of B16F10 melanoma bearing mice. Szala and co-authors have used cationic liposome complexes of different compositions for transfer of the 'suicide gene' cytosine deaminase which converts 5-fluorocytosine to 5-fluorouracil. Injections of the DNA-liposome complexes followed by 5-fluorocytosine caused an inhibition of tumour growth and increased survival of the melanoma bearing mice.

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ANNOUNCEMENTS & RELATED ACTIVITIES

Also available in more details from address: http://www.ulb.ac.be/medecine/loce/espcr.htm

1997 **7th ESPCR Meeting: Bordeaux, France, 9 - 11 October**

Contact: Dr Alain TAEIB
Unité de Dermatologie Pédiatrique
Hôpital Pellegrin - Enfants
F- 33076 Bordeaux Cedex
E-mail: Alain.Taieb@dermatol.u-bordeaux2.fr

1998 **8th ESPCR Meeting: Prague**

Contact: Dr J. Borovansky

1999 **XVIIth International Pigment Cell Conference: Nagoya Congress Center, Japan, October 30 - November 3**

Contact: Fujita Health University School of Health Sciences
J- Toyoake, Aichi 470-11
Phone: +81-562-93-2595
Fax +81-562-93-4595
E-mail: sito@fujita-hu.ac.jp

2000 **9th ESPCR Meeting: Krakow**

Contact: Dr T. Sarna

Postdoctoral position

Ph.D. in molecular biology, biophysics, genetics or biochemistry. Position available to conduct research on molecular mechanisms of cellular response to oxidative stress in human melanocytes and melanoma cells and its regulation for preventive and therapeutic indications.

Contact: Dr. Frank L. Meyskens Jr., Director, University of California-Irvine, Chao Family Clinical Cancer Research Center, 101 The City Drive, Orange, CA 92668, USA. Fax (714) 456-5039 Email flmeyske@uci-edu

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G. Ghanem, ESPCR Bulletin Editor
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Message from the President

The ESPCR would like to thank and acknowledge Pharmacia & Upjohn for generous donations in support of the 7th Scientific ESPCR Meeting in Bordeaux and the invitation of the Council of the International Federation of Pigment Cell Societies (IFPCS) to their annual Board meeting, which will be hosted by the ESPCR in Bordeaux. We expect these financial contributions to be of great value for the organization of the meeting in all respects.

IFPCS Travel Stipends

As part of our initiatives to promote international interactions, the International Federation of Pigment Cell Societies (IFPCS) has established IFPCS-sponsored travel grants for young scientists to visit and train in laboratories outside of their own country.

These travel grants will be awarded each year to highly qualified young scientists from each of the three regional Societies to visit other laboratories to learn specialized techniques and establish collaborations that will facilitate their own future research objectives. In addition to the importance of such training to the young investigators who receive these awards, this program should stimulate and foster interactions and collaborations between the international laboratories involved.

Each stipend will be for a maximum of $3,000 to support a visit for 2-3 months, and they will be competitive on an annual basis. Each regional Society will be entitled to award one IFPCS Travel Stipend per year.

We now invite young scientists from the ESPCR to apply for the IFPCS Travel Stipend 1997. The applicant should forward a single page, single spaced letter which states their laboratory of origin, the laboratory they wish to visit, the project overview, the specific reason for the visit, and the projected expenses (not to exceed $3,000). We also request a letter from the laboratory to be visited that they will accept the candidate if the application is successful.

The application should be sent to the Secretary of the ESPCR (Dr Stan Pavel) not later than November 1, 1997. The final designation of the Awardee will be made by an ad hoc Committee of the ESPCR Council.

Travel must begin in the calender year within which the IFPCS Travel Stipend is awarded but may continue into the following year. Awardees must send a summary of their expenses with appropriate receipts to the IFPCS Secretary/Treasurer (Dr Bengt S. Larsson) within two months following the conclusion of their travel.

Book announcement

CANCER NEUTRON CAPTURE THERAPY

Edited by Yutaka Mishima

Plenum Press, New York, 1996

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