Professor P. Riley presenting a commemorative plaque to Professor G. Prota on behalf of the Society on his retirement from the Presidency of the ESPCR. 5th Meeting of the ESPCR, October 22nd 1994, Hofburg Congress Centre, Vienna. (From left to right: P. Riley, G. Prota and B. Larsson the newly elected President)
RETIREMENT OF PROFESSOR GIUSEPPE PROTA
FROM THE PRESIDENCY OF THE ESPCR:
THE END OF AN ERA

Professor P.A. Riley

Following the Elections last summer the new Officers and Council members were instated at the ESPCR Annual General Meeting held during the 5th Scientific Meeting at the Hofburg Congress Centre in Vienna organized by Professor Hubert Pechhamberger and Professor Klaus Wolff. The General Assembly took place on Saturday, October the 22nd 1994 and, when Professor Bengt Larsson took over the Presidency of the ESPCR from Professor Giuseppe Prota, it marked the end of an era begun nearly a decade earlier.

Giuseppe Prota had held the position of President for the first ten years of the Society with great distinction. The Society was formed in 1985 in response to widespread support for a proposal that had been raised and debated at the European Workshop on Melanin Pigmentation meeting held in Murcia. In the wake of these discussions a constitution was drawn up and an Italian version prepared by Marco D’Inchia was duly sworn before the official notary in Naples on (11 December 1985) by a triumvirate consisting of Giuseppe Prota, Patrick Riley and Natale (Pino) Casinelli as representatives of the transitional Executive Council of which Giuseppe Prota was elected Chairman and Patrick Riley Secretary/Treasurer. Giuseppe Prota immediately set to work to arrange the highly successful 1st Scientific Meeting of the ESPCR which was held in Sorrento on (11 October 1987) at which he has elected to the Presidency, a post which he occupied until October last year.

His Presidency spanned a most interesting and successful period in the history of the ESPCR. It has included not only the infancy of the Society with its rapid growth and extending influence but also the sometimes turbulent years which have seen the birth of our societies which followed our lead - the JSPCR and PASPCR - and the subsequent formation of the International Federation of Pigment Cell Societies of which Giuseppe Prota is the current President. We now possess truly international and democratic organizations devoted to pigment cell research which have continued and extended the highest traditions espoused by that distinguished vanguard of pigment cell researchers including Myron Gordon, Vernon Riley, Tom Fitzpatrick, Aaron Lerner and many other giants in the field.

Many of these achievements have sprung from the energy and enthusiasm infused into the ESPCR by Giuseppe Prota and as token of the appreciation of the Society for his long and distinguished service as President a commemorative plaque was presented to him on behalf of the Society on his retirement from the Presidency of the ESPCR. In making the presentation, at the General Assembly, Patrick Riley praised Professor Prota’s vision, inspiration, commitment and dedication and his boundless energy in founding the Society and in steering it safely into calm waters; the very existence of the ESPCR will be his enduring monument.

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P. Riley’s short speech at the ESPCR General Assembly in Vienna. From left to right: P. Riley, G. Prota, S. Pavel (the newly elected Secretary) and B. Larsson.

A moment of emotion ...
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Discussion

THE RECONSTRUCTION OF THE EPIDERMIS WITH MELANOCYTES EX Vivo: HISTORY AND PERSPECTIVES

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Human skin is the first organ for which attempts of ex-vivo reconstruction have been made over the last two decades. A landmark was the discovery by Rheinwald & Green that irradiated 3T3 murine fibroblasts could be efficiently used to grow keratinocytes from epidermal cell suspensions (1). Epidermal sheets were obtained with incomplete differentiation and fibroblast contamination was frequent, but variants of this technique remained as a reference for human therapy especially in the burn patient, because of the selection of cells of high clonogenic potential.

A better differentiation of the reconstructed of the epidermis was noted by Prunieras et al (2) when keratinocytes were cultured on a dead deepdermized dermis and lifted at the air-liquid interface (3-4). The presence of living fibroblasts in a dermal equivalent, like in Bell’s model (5), has been considered by some as an important factor to reconstruct an epidermis with better differentiation.

A further advance in epidermal culture was the development of a serum-free medium in Ham’s laboratory (6). The first generation media were based on a mixture of aminoacids, vitamins and minerals completed by fetal calf serum, EGF and cholera toxin (CT). Boyce & Ham adjusted by a careful stepwise approach the basic medium composition to the specific metabolic needs of keratinocytes. In this low calcium medium feeder layers, the cAMP agonist cholera toxin and serum were no more required. The addition of bovine pituitary extract (BPE) and aminoacid supplementation allowed to grow primary cultures to confluency and to passage epidermal monolayers.

The switch to high calcium generated multilayers much similar to those obtained in Green’s system (1). Since the early eighties, melanocytes had been grown in a few laboratories using Eisinger and Marko’s (7) or Halaban’s (8) techniques with serum, cAMP agonists (cholera toxin or IBMX) and phorbol esters (PKC agonists). Gilchrest’s system using a pituitary extract(BPE), cholera toxin and a basic medium without serum showed the way towards more physiological culture condition of melanocytes, but cell growth and differentiation were poor (9).

In these techniques phorbol esters increased the mitotic activity of melanocytes and their morphology, and cAMP agonists like CT increased dendricity and melanogenesis.

Using Boyce & Ham’s technique for keratinocyte culture (MCDR 153), preliminary observations were made in several laboratories about “melanocyte contamination” of cultures, fibroblasts being eliminated by the low calcium concentration and absence of serum.

Pittelkow et al introduced in the system the phorbol ester TPA and were able to grow pure...
melanocyte cultures (10). Modifications to this system have been made at several institution and this technique is now used with variants worldwide.

In our lab, Donatien has successfully grown well-differentiated melanocytes without CT or phorbol esters using MCDB153 supplemented with BPE, insulin, hydrocortisone, 3% serum and calcium (11). This physiological modified medium allowed to answer the controversial question of α-MSH receptors on human melanocytes (12).

The two dimensional culture systems so far described are however poor models for studying melanocyte biology ex-vivo. The in-vivo epidermal melanization unit is a well-organized three dimensional structure in which melanocytes are polarized to the basal layers and maintain close contacts with both the basal lamina and neighbouring keratinocytes. The influence of keratinocyte factors to maintain the growth and differentiation of melanocytes ex-vivo either by contact or by diffusible factors, is now well-established (13-14-15).

The reconstruction of the epidermal melanization unit ex vivo has been achieved recently. Bertaux et al have obtained epidermal explants and stimulated melanogenesis with UVB ex vivo (16). De Luca et al have obtained a coculture of keratinocytes and melanocytes resulting in a moderately differentiated epidermis with melanocytes polarized in basal position when epidermal keratinocytes were used but only in basal and suprabasal position when non keratinizing oral epithelial cells were used (17-18). Similarly, the polarisation of neonatal and fetal melanocytes and the ratio keratinocytes/melanocytes was studied by Haake & Scott in a epidermis reconstructed on a collagen lattice. The role of keratinocytes in stereologic and ratio regulation was confirmed (19-20).

All these experiments show that a mature epidermis is necessary to regulate the epidermal melanization unit.

Other experiments in cocultures of keratinocytes have been conducted at various institutions [Topol (21), Stainao (22), Valyi (23)].

More recently, Todd et al (24) have stimulated with UVB an epidermis reconstructed ex vivio from pure cultures of melanocytes and keratinocytes on a dead deepedermized dermis with at least 10% melanocytes. The cells came from non caucasian donors and melanosome transfer was not demonstrated. Our experience with keratinocyte and melanocyte culture led us to set up experiments of epidermal reconstruction with melanocytes ex vivio which have been reported at the last ESPCR meeting in Vienna (25). Using pure cultures of keratinocytes and melanocytes of the same donors, we have been successful in obtaining a well differentiated epidermis with basal functional melanocytes. The system was basically a modification of the Pruni_ras model using 5% melanocytes in the seeding suspension and a novel culture medium.

UVB irradiation induced a stimulation of melanogenesis macroscopically, microscopically and increased melanin concentrations. These results have been reproduced with skin of various phototypes and melanosome transfer to keratinocytes was demonstrated by electron microscopy.

Despite all these exciting new developments, the complete reconstruction of the human skin remains a challenge, since other epidermal components like Langerhans cells are not yet amenable to long-term cultivation, and that dermal reconstruction has not made real breakthroughs over the last decade.

However, the European Center for the Validation of Alternative Methods (ECVAM) setup by the European Union in Ispra, Italy, has considered skin reconstruction among the most advanced projects for the development of in vitro pharmacotoxicology and research programs in this field should be stimulated.

The future of the models of epidermal reconstruction with melanocytes looks promising. First, with better standardization, assays for pharmacologic and cosmetic agents suspected to interfere with pigmentation may be developed to screen interesting molecules, with a better relevance than monolayers of melanocytes. Second, physiologic and therapeutic studies in human pigimentary disorders may be approached directly ex-vivo or after grafting the reconstructed epidermis on the nude mouse.

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References


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1. Melanins and other pigments chemistry

(Comments by Prof. M. Peter)

Solid state $^{13}$C spectroscopy and matrix assisted laser desorption ionization (MALDI) mass spectrometry have emerged in recent years as valuable methodologies for the physicochemical analysis of melanins and continue to yield important structural informations. Two papers have appeared on NMR investigations; Herve et al. describe methods for the identification of carbon bonds (quaternary, C-H bonded) and their quantification. Crescenzi et al. trace unexpected oxidation patterns in dopamine melanins by analysis of samples prepared from side chain $^{13}$C-enriched dopamines. Information on the molecular weight distribution in various melanins, including natural melanins as well as samples prepared from serotonin, tyrosine, DOPA and further catechols, are obtained by Seraglia et al., and Bertazzi et al. cluster size (M, up to 30,000 are detected) varies not only with the precursor but also with the source of the phenoloxidase used in enzyme catalysed preparations. Other approaches involve photometric and photothermical methods (Vükis et al.). Scanning tunneling electron microscopy (STM), supplemented by molecular mechanics and orbital calculations suggest a stacking model for oligomeric indolequinones in tyrosine melanin that is in accordance with previous x-ray data (Zajic et al.).

Further papers deal with drug binding of melanins, the chemistry of reactive intermediates of melanogenesis, and the synthesis of precursor analogs. Two types of binding sites (high and low affinity) exist in melanin for the cationic N-methylalansilinium ion, as observed by Naoi et al. The binding is enhanced by Fe$^{3+}$ but inhibited by Fe$^{2+}$ and MPP. This could have important implications with respect to the accumulation of N-methylalansilinimum in dopaminergic neurons. Homogentisic acid is shown to give melanin-like polymers in human blood plasma. Correlation with the symptoms of alkaptonuria and reactivities of intermediates of catechol oxidation suggest that oxidative metabolism of catechols may be involved in ageing. With the aim to study structure-activity relationships of o- and m-boronoxytrosine in comparison with p-borono-phenylalanine which is used in boron-neuron capture therapy of melanoma, Takahashi et al. report on the synthesis and enantiomeric resolution of the two former compounds. It will be interesting to learn the results of the chemically oriented work with respect to the biological behaviour of the scions.


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2. Biology of pigment cells and pigmentation disorders

Melanocytic cultures
(Comments by Dr. N. Smit)

The work on DNA-damage as the trigger for UV-induced melanogenesis by Eller et al may contribute to clarify a phenomenon that is not understood so far. Additional work such as the investigation of Carsberg et al may answer the question which intracellular signals are necessary next to translate the UV-damage to an increase in melanin production.

After their paper in Science 263 Schallreuter et al showed once more that control of melanogenesis can start already before the conversion of L-tyrosine to L-DOPA and that intermediate products leading to L-tyrosine can be harmful to cultured melanocytes.

Zhuo and coworkers describe a very nice model for investigation by growing melanocytes from Chediak-Higashi syndrome patients. The defects of melanogenesis in this disease may add to our knowledge of melanosome formation in the normal melanocyte.

Shih et al have shown that in melanocyte and keratinocyte co-cultures expression of Mel-CAM/MUC18 is down regulated. For melanoma cells from primary and metastatic lesions this is not the case. The possibility is suggested that control of antigen expression by keratinocytes may influence melanoma development.

- Shih IM, Elder DE, Hsu MY, Hery M. Regulation of Mel-CAM/MUC18 expression in melanocytes of different stages of tumor progression by normal


3. MSH, MCH, other hormones, differentiation


Abstract: In malignant melanoma, melanocyte-stimulating hormone (alpha-MSH) has been found to influence the cellular metabolism of melanoma cells (c-AMP production, protein and RNA synthesis, and tyrosinase activation). In some publications elevated alpha-MSH levels have been described in melanoma patients. In the present study we used a commercially available radioimmunoassay to examine the alpha-MSH levels in patients with malignant melanoma and a control group consisting of apparently healthy volunteers (laboratory assistants) and dermatological patients without malignant tumours. The plasma alpha-MSH levels were (mean ± SD) 12.2 ± 3.5 pmol/l for 38 control persons (18 female, 20 male). The difference is significant according to the distribution-free U-test of Mann and Whitney. It is 13 (35%) of the melanoma patients values were above the normal range defined by the 95.3% confidence limit. alpha-MSH cannot be classified as a typical tumour marker. Nonetheless, in our opinion alpha-MSH levels may be useful in monitoring melanoma patients with reference to prognosis and follow up during and after therapy.

MSH biology:


MSH Receptors:


MSH analogues:

MSH effect:
- Scheenen WJ, Jekas BG, Wilkens PS, Roubaux EW. Action of stimulators and inhibitory alpha-MSH secretagogues on spontaneous calcium oscillations in melanotrope cells of Xenopus laevis. Pflugers Arch. 427(3-4): 244-51, 1994. Abstract: The secretion of alpha-melanophore-stimulating hormone (alpha-MSH) from melanotrope cells in the pituitary gland of Xenopus laevis is regulated by various neural factors, both classical neurotransmitters and neuropeptides. The majority of these cells (80%) display spontaneous Ca2+ oscillations. In order to gain a better understanding of the external regulation of intracellular Ca2+ ([Ca2+]:i) in the melanotrope cell, we have examined the action of well known alpha-MSH secretagogues on the Ca2+ oscillations. It is shown that all secretagogues tested also control the oscillatory state of Xenopus melanotropes, that is, the secretory-inhibitors dopamine, norepinephrine (gamma-amino butyric acid, GABA agonist), baclofen (GABAB agonist) and neurotensin evoked a rapid quenching of the spontaneous Ca2+ oscillations, whereas the secretory-stimulant suvagine, an amphibian peptide related to corticotropin releasing hormone, induced oscillatory activity in non-oscillating cells. Supporting evidence is given for the idea that the regulation of Ca2+ oscillations is a focal point in the regulation of secretory activity of melanotrope cells. There was considerable heterogeneity among melanotrope cells in the threshold of their Ca2+ response to secretagogue treatment. This heterogeneity may be the basis for melanotrope cell recruitment observed during physiological adaptations of the animal to the light intensity of its background.

Drug targeting:

4. Photobiology and photochemistry
(Comments by Dr M. Picardo)
- Lever LR, Farr PM. Skin cancer or premalignant lesions occur in half of high-dose PUVA patients. Br. J. Dermatol. 131(2):215-9, 1994. Commentary: The incidence of skin cancer and pre-malignant pigmented lesions in patients treated with PUVA therapy has been evaluated by different Authors. Now, Lever and Farr, correctly, underline the risk of the use of high doses PUVA.

Commentary: The possibility of in vitro induction of differentiation of neoplastic cells is an interesting aspect of the biology of malignant cells. The intrinsic mechanisms of this process, which can be induced by different chemicals, is not completely clear. The authors report the modifications induced in different melanoma cell lines by two aromatic fatty acids. The potential of melanoma cells to not, by an intrinsic mechanism, has been induced by different physico-chemical and biochemical mechanisms. The results of these in vitro experiments and the possible clinical implications of these findings are discussed. The authors suggest a potential clinical use for these substances in the treatment of melanoma together with currently used drugs.


Commentary: The peculiar enzymatic properties of melanocytes, the hydroxylation and sulfation activity of tyrosinase, has been considered as a possible specific target for a selective chemotherapy for malignant melanomas by several authors. Prota and coworkers present a well-designed review on the subject with a large bibliography underlying the chemical and biochemical mechanisms probably involved in the melanocytotoxicity of different phenolprodrugs which have been experimentally used. The field is probably one of the most intriguing ones, either for the interest the goal of the discoveries and for the misleading that some methods used and some results have produced. A clear up to date on the subject was probably necessary.


Commentary: The interaction between melanocytes and cytokines have been investigated by different point of view both studying the effects of cytokines on melanocytes and the cytokine produced by melanocytes. Now, Mc Kinzie et al., evaluating the possible mechanisms by which UVB can induce or stimulate melanoma development and growth, report a possible effect through the induction of cytokines production by keratinocytes. In particular IL1 was able, when injected in animals before B16 melanoma cells, to increase the tumor growth and size, whereas it was ineffective when administered after the formation of visible tumors. The result indicate that UVB-mediated damage of keratinocytes with the consequent production of IL1 may be one of the factors facilitating the early growth of melanoma. Different effects, however, have been observed for TNFα and IL6 between in vitro and in vivo experiments. Schultz and coworkers report that the lysis induced by IL2, released by stimulated PMC, on melanoma cell lines was significantly modified by the presence of other cytokines. However the optimal cytokine combination was different for the two cell lines used, indicating an high variability in the biological response of the melanoma cells in vitro and possibly also in vivo. Reading both papers it can be suggested that before transferring the in vitro results to in vivo pathological conditions the biological effect of cytokines has to be carefully evaluated.


Commentary: The authors present data on some in vitro biological properties of vitiligo melanocytes in respect to normal melanocytes. Different from some other papers, the results showed similar biological properties between normal and pathological cells, including the cell growth characteristics, tyrosinase activity and the response to UVB. Some ultrastructural changes in the KER of vitiligo melanocytes, which have been considered not directly related to the biological characteristics of the cells, have been observed. Unfortunately, the authors did not discuss the possible reason of the difference with other reports on the same topic, and conclude, and we completely agree, that more extended (and possibly better defined) studies should be focused on the defects of melanocytes in the pathogenesis of vitiligo.


5. Neurermelansins

(Commentary by Dr. M. d'Iscia)

The literature papers relating to neurermelansins and its implications in neurological disorders surveyed in this issue offer an opportunity for a brief comment on two main aspects of great current concern. The first one deals with the role of iron as a possible etiological factor in Parkinson's disease. Discussion of this topic under
the leading Neuromelanin is justified by the apparent ability of the pigment to favour accumulation of metal ions, and particularly iron, in those areas of the Substantia nigra, such as the zona compacta, which are more vulnerable and prone to degeneration. There is now a substantial body of evidence, reinforced by the papers by Youdim and Riederer (J. Neural Transm. Suppl. 1993; 40: 57-67) and Temel et al. (J. Neurochem. 1994, 62: 134-146), to suggest that neuromelanin, just like cutaneous melamin, may serve as a deposit of metal ions and, as such, may act as a pro-oxidant, catalysing generation of toxic oxygenated free radicals and leading eventually to cell death. The detailed mechanisms by which iron-melanin interaction may promote neuronal degeneration are still not fully understood, and are the focus of intense research activity in several laboratories. It will be interesting to see whether metal accumulation is indeed a consequence of neuromelanin formation, or rather it is an alteration of iron metabolism that causes aberrant dopamine oxidation and hence pigment deposition.

The second point, emerging from the papers by Glownow (Mov. Dis. 1993, 8, suppl. 1, SI-7) and Knoll (J. Nerv. Transm. Suppl. 1993, 40: 69-91), concerns the potential of monoamine oxidase inhibitors, such as deprenyl, as symptomatic and neuroprotective agents in Parkinson's disease. The rationale of this approach revolves around prevention of excessive generation of hydrogen peroxide, a most likely mediator of cellular toxicity under conditions of oxidative stress. Here again, neuromelanin appears to be involved both as a direct product of the action of hydrogen peroxide and reactive oxygen radicals on the catecholamine pool within dopaminergic neurons, and as an iron-complexing agent capable of exacerbating peroxide-induced neurotoxicity through Fenton-type processes. Further work in this area is likely to offer new promising options for an effective treatment of neurodegenerative disorders.


- Comment. A new aspect that may provide a new treatment for Parkinson's disease.

6. GeneSys, molecular biology
(Comments by Dr T. Beemus)

- Adema G, de Boer AJ, Vogel AM, Lommen WA, Figdor CG. Molecular characterization of the melanocyte lineage-specific antigen gp100. J Biol Chem. 269(31):20126-33, 1994. Commentary: The cDNA for gp 100 (clone termed gp100-c) was isolated and characterized. It encodes glycoproteins of 100kDa (gp100) and 100kDa (gp100) which are recognized by the corresponding antibodies. The product and the sequence are highly homologous to the melanocyte-specific protein Pmel17 (which has been mapped in the mouse to the silver locus. It is suggested that the transcripts corresponding to gp100 and Pmel17 cDNAs originate from a single gene via alternative splicing. In all normal and malignant melanocytic cells analyzed, gp100 and Pmel17 RNAs are simultaneously expressed.


- Cassady JL, Sturm RA.


Commentary: A brief report on the effect of d Nicotinamide on melanogenesis. Tyrosinase mRNA, melanin production and tanning are increased following treatment with dipyrnol dine dithymydic acid.


Commentary: This study aimed at the search for upstream elements of the mouse tyrosinase gene which confer copy number dependent and position independent expression in transgenic mice. A melanoma cell-specific DNaase I hypersensitive site was identified at -12kb upstream of the tyrosinase gene. Functional analysis of the corresponding cisacting element in transgenic mice and transient transfection assays revealed properties of a strong cell-specific enhancer. RNA expression levels of the transgene correlate with copy number, which is reflected in coat colour and eye pigmentation of transgenic mice. Full enhancer activity in transient transfections is obtained with a minimal sequence of 200 bp. Protein binding analysis reveals the presence of a melanoma cell-specific complex which might contribute to the faithful expression of the tyrosinase gene. Similar results have been obtained in a paper by Porter and Mayer (Development 120, 2103-11, 1994).


Commentary: In albino (c) mice the pattern of connections between the eye and brain is disrupted at the optic chiasm. This paper demonstrates that the abnormality is corrected in tyrosinase transgenic mice.


Commentary: The human agouti gene is 85% identical to the mouse gene and maps to chromosome band 20q11.2. In humans, the gene is mainly expressed in adipose tissues and testis.


Commentary: Monkey kidney COS-7 cells and human amelanotic and melanotic melanoma cells were transfected with
tyrosinase, TRP-1 or both. Degeneration and premature death of melanocytes occurred in tyrosinase transfectants, but not in TRP-1 transfectants or double transfectant cells. In these cotransfected cells expression of the tyrosinase protein Lamp-1 is increased. Antisense oligodeoxynucleotides against Lamp-1 resulted in a decreased expression of TRP-1 protein. The authors postulate that tyrosinase, TRP-1, and Lamp-1 gene products may function together, being expressed as a multigene complex within the melanosomal compartment. Specifically, tyrosinase and TRP-1 may function together via Lamp-1 by stabilizing the enzyme-protein complex within the melanosome and prevent the premature death of melanocytes due to tyrosinase-mediated cytotoxicity.


**Commentary:** The authors have studied different alleles of the agouti locus. Based on the finding that some agouti alleles affect the dorsal and ventral independently they report that alternative isoforms of the agouti gene exist, which contain different 5' untranslatable exons up to 100 kb apart. These different forms are expressed in a region- and temporal- specific fashion.


Commentary: This study is a continuation of a previous one (Lowings et al., Mol Cell Biol 12:3653-62, 1992) on the melanocytic-specific expression of the TRP-1 promoter. Positive regulation of TRP-1 expression is mediated by both an octamer-binding motif and an 11 bp element, termed the M box, which is conserved between the TRP-1 and other melanocytic-specific promoters. Additional elements involved in regulating TRP-1 expression were identified which include the TATA region, which appears to contribute to the melanocytic specificity of the TRP-1 promoter. Mutational analysis also identified two repressor elements, one at the start site, the other located at -240, which function both in melanoma and nonmelanoma cells. In addition, a melanocytic-specific factor, MSF, binds to sites which overlap both repressor elements and can possibly function as an antirepressor.


Commentary by Dr N. Smit:

Three of the papers by Vile et al describe the possibility of targeted gene therapy using the 3'flanking region of the tyrosinase gene for specific expression of different genes in the melanoma cells. Also currently available gene delivery systems for cancer treatment are reviewed by Vile and Racil in Gene Therapy 1. In the Eur. J. Cancer 30A:1609-1201, 1994, many different aspects of gene therapy are described in eight papers on this topic.

According to the work of Ohira et al II-4 may be useful in melanoma cytokine gene therapy.


Vile R, Miller N, Chernajovsky Y, Hart I. A comparison of the properties of different retroviral vectors containing the murine tyrosinase promoter to achieve transcriptionally targeted expression of the HSVtk or IL-2 genes. Gene Therapy 1:3;307-316, 1994. See comments.


7. Tyrosinase, TRP-1, TRP-2 and other enzymes

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

As the complexity of the enzymatic melanogenic machinery in mammals becomes evident, new challenging questions are being addressed by pigment cell researchers. Clearly, the times of the 'single enzyme pathway' concept are over. However, it is also obvious that the realization that the tyrosinase family proteins are involved in the control of the pathway raises more questions, for the time being, than it does answer.

Several recent papers deal with the function of TRP-2/DCT and TRP-1. The report by Palumbo et al. (Biochem. J. 299, 839-844) on the characterization of a new dopamine rearranging enzyme in the ink of the cuttlefish suggests that the occurrence of dopamine converting enzymes might be a rather general and widespread phenomenon. The new enzyme, described by the Italian group catalyzes the conversion of dopamine into dihydroyxindole, and should therefore accelerate melanin synthesis. Conversely, melanosome DCT efficiently catalyzes dopamine rearrangement to DHICA, a very stable intermediate, thus raising the question of the incorporation of this dipeptide into the melanin polymer. The paper by Jimenez-Cervantes et al. (J. Biol. Chem. 269, .7993-18000) gives a reasonable explanation for the metabolism of DHICA, and at the same time, accounts for the importance of TRP-1 in normal melanogenesis, noted by others (see, for example, Haro et al., J. Invest. Dermatol. 102, 495-500). However, the question of the enzymatic function of TRP-1 remains open, especially if one takes into account the existence of a melanogenic complex such as the one described by Orlow and coworkers (J. Invest. Dermatol. 103, 196-201). This multienzyme activity was suspected by many of us, but it had never been proven so
unequivocally. It could well be that the actual "in vivo" activities of the melanogenic enzymes are different in the frame of this complex than in purified preparations. It can be anticipated that the investigation of the nature of this complex and of the modulation of the enzymatic capabilities of its components by the interaction with their counterparts will be an active and fruitful area of research in the near future.

It is obvious that our knowledge of the melanogenic enzymes is more complete in mouse than in human melanocytes. Therefore, the report by Bouchard et al. (J. Invest. Dermatol., 102, 291-295) on the production, by an elegant method, of specific anti human tyrosinase antibodies is very stimulating. The antibodies against the mouse enzymes developed in Vince Hearing's laboratory, and so generously made available to the pigment cell community, have been instrumental in the development of most of the current concepts in the enzymology of the pathway. A similar role can be predicted for the specific antisera against the human enzymes.


- Wang J, Fang L, Lopez D. 597

- Winder AJ.

- Wolff T, Van Peh A, Brichard V, Schneider I, Seliger B, Meyer zum Buschenfelde KH, Boon T

8. Melanoma and other pigmented tumours
(Comments by Dr R. Peter)
- Cannistra SA, Myers LE, Goldger DE, Lewis CM, McWorter WP, Just M, Hennon D, Anderson DE, Zone JJ, Skolnick MH.
Commentary: A susceptibility locus for familial melanoma has been localized to the short arm of chromosome 9. The penetrance was estimated to be 53% by age 80. Skin type, number of moles and sun exposure were analysed in association with this chromosomal locus. Gene carriers have higher numbers of moles. Gene carriers with melanoma were found to have a history with more sun exposure within each skin type than gene carriers without melanoma. The 9p melanoma susceptibility might be related to the number of moles and might interact with environmental factors. This is an interesting study designed to combine both experimental and clinical approaches to define a possible gene locus for familial malignant melanoma.

- Kamb A, Gruss NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavignian SV, Stockert E, Day RS, Johnson BE, Skolnick MH.
Commentary: A putative new tumor suppressor gene was isolated in a melanoma cell line, the so called MTS1 for multiple tumour suppressor gene. It encodes a p21-related protein, p16, which is able to regulate cyclin-dependent kinase 4. p16 binds to CDK4, thereby inhibiting the ability of CDK4 to interact with cyclin D and hence stimulate passage through G1. MTS1 therefore encodes for an important cell cycle regulator. Since MTS1 maps to chromosome 9q21, it might be the familial melanoma gene. MTS1 was found in 75% of tested melanoma cell lines. This newly identified tumor suppressor gene is a promising candidate for the human familial melanoma gene.

Commentary: Dermoscopy is a practical and easy to learn method, which helps to diagnose pigmented skin lesions. To improve and facilitate the differentiation between benign melanocytic nevi and malignant melanoma, a new scoring system has been developed. Multivariate analysis revealed that scores of four features, asymmetry (A), border (B), colour (C), and dermoscopic structure (D) combined in a formula provide a final score, with a diagnostic accuracy of 94%, specificity of 90.3% and sensitivity of 100% in the training set. With experience in dermatoscopy a diagnostic accuracy of 92.2%, specificity of 97.9% and sensitivity of 90.3% can be achieved. This is a helpful, reliable and valuable tool for distinguishing benign nevi from malignant melanomas.

Melanoma Radiotherapy
(Comments by Dr N. Smit)

- Bagusti C, Stolt B, Albert B, Braas C, Plas J, Eerle AN.
Commentary: In the paper by Abels et al it is shown that the 5-aminovaleric acid is accumulate selectively in an amelanotic melanoma tumour and optimal times for photodynamic diagnosis and therapy with this compound of 90 and 150 min, respectively were established. Although not specific for melanoma a good binding of two carbonates to 516 melanoma cells was found and a decrease in survival was observed after neutron irradiation when tals were treated with the boron containing L-carboranylalanine. The carbonates are therefore considered to be of interest for boron neutron capture therapy (Pottersson et al).

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Melanoma (Experimental) Drug Therapy.

(Commentary by Dr N. Smit)

- Atkins MB, Oboye KR, Sossman JA, Weiss OR, Margolin KA, Ernest ML, Kapppler K, Mier JW, Sparano JA, Fisher RI, Eckardt JF, Penrice C, Averson FR. Multinstitutional phase II trial of intensive combination chemotherapy for metastatic melanoma. Journal of Clinical Oncology 12, 8:1553-1560, 1994. Commentary: According to the letter of Shonanski and Pass in the J Invest Dermatol 103: p742, melanogenesis should be inhibited via L-tyrosine and L-DOPA restricted diets or with tyrosinase inhibitors. As they describe, upregulation of melanogenesis may result in melanocytotoxicity in vitro and this may not be the case in vivo as a result of immune-suppressive or immune-modulatory action of L-DOPA. In contrast we have reported (Smit et al) on some preliminary investigations which were aimed at inducing selective cytotoxicity making use of melanogenesis via inhibition of the enzyme catechol-O-methyltransferase. Retinoic acid induces differentiation in B16 mouse melanoma (Niles). This also effects the expression of Vimentin which was suggested as a basis for retinoic acid therapy of melanoma (Santos et al). Since two synthetic retinoids were shown to have antiproliferative effects on four melanoma lines tested (schadendorf et al) this could be an interesting line of investigation.

- Siwek B, Bahbouth E, Serra MA, Sabbioni E, de Paauw-Gille MC, Bassler R. Effect of selenium compounds on murine
Melanocortin Receptor Structure and Signalling: Neural, Pigmentary and Immunomodulatory Functions
April 12
at the Experimental Biology '95 Meeting in Atlanta, Georgia
April 9-13, 1995

The presentations will include:

- Jeffrey B. Tatro, Functional organization of the melanocortin hormonal and neural systems.
- Victor J. Hruby, Univ. Arizona, Design of synthetic and receptor subclass-specific melanocortins.
- James M. Lipton, Univ. Texas, Southwestern Med. Ctr. Central and peripheral immunomodulatory actions of melanocortins.

Contact:
American Physiological Society office
tel: (301) 530-7118  fax: (301) 571-8305

Session on Melanocortin Receptors
June 14
at the 1995 Annual Meeting of the Endocrine Society
Washington, D.C. June 14-17, 1995

The speakers will include Roger Cone, Ira Gantz and Jeffrey B. Tatro.

Contact:
Endocrine Society office
tel: (301) 571-1800  fax: (301) 571-1869
PROGRAMME

WEDNESDAY MAY 10th
10.30-12.30 Plenary Session

The Prevention of Melanoma
Epideimiological basis
Dr Anne Osterlind
UV light and the skin
Dr Brian Difffy
Public education
Dr Robin Graham-Brown

The Australian experience
Dr Robin Marks
Smoking, children & skin cancer
Ms Anne Charlton
Children in the sun
Dr Julia Newton Bishop

LUNCH
Opening of Exhibition
14.00-16.00 Parallel Sessions

The Health of the Nation
Dr Margaret Price & Ms Dorothy Pizzala
The Surgery of metastatic disease
Dr David Robin
Pathology - When is a risk factor a precursor?
Prof David Elder & Dr Nigel Kirkham
TEA
16.30-17.30 Guest Lecture
Prof Ian Hart

18.30 Civic reception with the Mayor of Brighton

THURSDAY MAY 11th
08.30-10.30 Plenary Session
The Surgery of Primary Melanoma
Dr Judy Evans & Mr Per Hall
11.00-12.30 Parallel Sessions
Nursing management soon after diagnosis
Ms Jacquelyn Moore & Ms Polly Perkins
What do you tell the patient when the doctor has a bad news?
Dr Lesley Fallowfield

Screening for melanoma
Options and cost effectiveness
Dr Jack Curzick
Pigmented lesion clinics
Dr Jane Melia & Dr Robin Graham-Brown
Screening on the beach
Dr Judy Evans
Screening in the workplace
Dr Regina Curley
Screening of at risk groups
Dr Julia Newton Bishop

Pathology - What is sufficient for melanoma?
Dr Dennis Cotton & Dr Nigel Kirkham

LUNCH
14.00-16.00 Parallel Sessions

Nursing issues in the management of patients
Ms Jacquelyn Moore & Ms Polly Perkins
The European Society for Pigment Cell Research
Dr B Larson
Pathology - When is a naevus not a naevus?
Dr Neil Smith & Dr Nigel Kirkham
TEA
16.30-17.30 Guest Lecture
The surgical treatment of melanoma
Dr Natalie Caccinelli

Evening Conference Banquet
FRIDAY MAY 12th
08.30-10.30 Plenary Session
Epidemiology & Genetics of Melanoma
Trends in incidence & mortality
Prof Rena MacKie
Atypical moles and melanoma
Dr Veronique Basaile
The genetics of melanoma
Dr Tim Bishop
Familial melanoma
Prof Dr Wilma Bergman

11.00-12.10 Parallel Sessions
Ocular melanoma
Mr John Hungerford

Not all tumours are benign or malignant?
Mr Morion Thomas & Dr Nigel Kirkham
Systemic treatment of melanoma
Chemotherapy
Dr N Thacher
Cytokine therapy
Dr J Radford
Vaccines: past, present & future
Dr M Moore

LUNCH
14.00-16.00 Plenary Session
Systemic Treatment for Malignant Melanoma

The role of adjuvant therapy
Guess Lecture
Prof John Kirkwood
Monoclonal antibody therapy
Gene therapy
Prof R Begent
Dr ME Gire

TEA

Contact
Dr Nigel KIRKHAM
MELANOMA '95
CO-ORDINATORS OFFICE
14A SHIP STREET
BRIGHTON
EAST SUSSEX
ENGLAND
BN1 1AD
TEL: 44 (0) 773 32515
FAX: 44 (0) 773 33882
6th Meeting of the European Society for Pigment Cell Research
Centre Hospitalier Universitaire Vaudois
Lausanne, Switzerland
October 19-21, 1995

SCIENTIFIC PROGRAM

Guest lectures
Scientific sessions, with selected papers:

The final program depends on the submitted abstracts, which will be expected to cover the whole field of pigment cell research in man and animals. In particular it will include:
- Biochemistry and biophysics of melanins.
- Photobiology
- Genetics of pigmentation disorders in humans
- Melanin pigmentation in lower animals
- Extracutaneous melanos
- Newer approaches to melanoma treatment

Poster Sessions

SOCIAL EVENTS
Welcome party on Thursday October 19, 1995
Banquet in a historic setting on Friday October 20 1995
Possibilities for accompanying persons: Tours to Montreux and Chillon castle, the medieval town of Gruyere, City of Lausanne, etc.

SCIENTIFIC COMMITTEE
F. Beerman (CH)
A. Eberle (CH)
E. Frenk (CH) (Co-chairman)
J. Garcia-Borrón (ES)
B.S. Larson (S)
F. Lejeune (CH) (Co-chairman)
S. Pavel (NL)
G. Prota (I)
P. Romero (CH)
R. Tyrrell (CH)

CONTACT
2SPCR ‘95
Centre Pluridisciplinaire d’Oncologie
CHUV - BH 06
CH-1011 Lausanne - Switzerland

Tel: 41/21/314.39.58
Fax: 41/21/314.39.57

DEADLINE FOR ABSTRACT SUBMISSION: June 30, 1995
XVI INTERNATIONAL PIGMENT CELL CONFERENCE

DISNEYLAND HOTEL
ANAHEIM, CALIFORNIA

OCTOBER 29-NOVEMBER 3, 1996

Contact

XVI INTERNATIONAL PIGMENT CELL CONFERENCE
MM/CUCI Centre for Health Education
P.O. Box 1428, Long Beach, California 90801-1428
Information about the XVIth IPCC

1. **Satellite Conferences:** No satellite conference will be supported by the local organizing committee that are held within the time frame of the XVIth International Pigment Cell Conference, Tuesday, October 29, 1996; 6:00 p.m. to Sunday, November 3, 1996; 8:00 a.m. There are a wide number of venues possible to hold small or large satellite conferences either before or after the main pigment cell meeting. Our Memorial/UCI Educational Foundations will be happy to work with you in planning, for a small fee, and we request that we be notified of the intent of any satellite conference no later than June 1, 1995. If we are notified later than this date, accommodations and planning availability cannot be guaranteed.

2. **Competitive Stipенд for Travel Support:** The Organizing Committee will provide funds in a competitive manner for graduate students, post doctoral fellows and those within five years of formal academic appointment. The number of stipends will depend on the availability of funds and further information will become available during the second and subsequent informational mailings.

Frank L. MEYSKENS, Jr., M.D., F.A.C.P.
President, XVIth International Pigment Cell Conference
Clinical Cancer Center
University of California Irvine Medical Centre
101 The City Drive, R1, 81, Bldg. 23
Orange, California 92668

Tel: 714 456 5081
Fax: 714 456 5039
Report on the General Assembly of the ESPCR in Vienna

A General Assembly of the ESPCR took place on Saturday, October the 22nd 1994, at the Congress Center Hofburg, Vienna, in conjunction with the 5th Meeting of the ESPCR.

It was reported that the membership of the ESPCR officially stood at 173, including 13 new members of the year. The previous membership list had been excluded. The Treasurer reported that the financial statement of the Society was relatively strong; the annual balance of 1993 was +£4,339, and the statement of account in October 1994 was +6,781. It was agreed to keep the subscription rate for 1995 unaltered at £40 p.a., including US$23 contribution to the IPPCS, and also to reduce the subscription rate for PhD students to £20.

The Secretary informed that an International Colloquium on Neuromelanin and Parkinson's Disease had taken place in Sorrento, May 1993, under the auspices of the ESPCR and the leadership of Prof. Prota and Prof. d’Ischia and their able team. The Colloquium provided multidisciplinary discussions on the controversial role of neuromelanin in Parkinson's disease and related neurodegenerative disorders. The Secretary also reported that the ESPCR had scientifically supported the International Melanoma Conference in Brisbane, held in April 1994.

The results from the postal ballot were announced and the following were declared duly elected: Officers: Bengt S. Larsson (President), Stan Pavel (Secretary), and Martin G. Peter (Treasurer); Council Members: Jan Borovansky, Marco d’Ischia, José-C. Garcia-Borron and Tadeusz Sarna.

The incoming President, Professor Giuseppe Prota, was thanked by Professor Patrick Riley, on behalf of the Society, for all his important work in founding and consolidating the Society. Professor Prota was presented a plaque with the inscription: Presented to Professor Giuseppe Prota, Founder and President (1985-1994) of the ESPCR, in recognition of his inspiration, leadership and devoted service to the Society. Professor Prota was also appointed Honorary Member of the ESPCR, which was unanimously approved by the Assembly and carried with acclaim.

Before adjourning the Assembly Meeting, the Secretary took the opportunity to thank Professor Klaus Wolff and Professor Hubert Pehamberg for the very well organized and conducted ESPCR Meeting in Vienna.

B. Larsson
President
Aravind Menon

1933 - September 12, 1994

Summary

Dr I. Aravind Menon was born in Kerala in 1933. He received his Ph.D. from Bombay University. Following this, he spent a number of years doing work with Dr Shemin at Columbia University and then with Dr Quastel at McGill University.

He came to Toronto in 1966 and established a very productive research laboratory. He wrote numerous articles, had over 100 publications and made numerous invited presentations. Aravind had a well-deserved and well-respected reputation in the fields of melanin, porphyrins, and reactive oxygen species. He was highly respected by colleagues in many countries.

The above tells little about the real man, for Dr Menon had a truly beautiful nature. He maintained this nature throughout his whole life, even to the end of his debilitating illness.

He was a kind, gentle and caring individual. He was deeply religious and believed in the oneness of all mankind and all religions. Dr Menon was deeply involved with interfaith meetings and communications. He had an openness of mind and enjoyed philosophical, ethical and religious discussions. He was a very moral individual and scrupulously honest in all his professional and personal life.

Dr Menon had a brilliant mind. In addition to his scientific work, he had a philosophical nature. He was a scholar of Sanskrit and of the Upanishads. He wrote manuscripts on philosophy and poetry. He was a scientific, ethical and religious mentor to many. Aravind was a devoted father and husband and a wonderful friend. He is survived by his two daughters, Ananya and Natasha and his wife, Hansa.

He will be greatly missed by all who knew him.

Written by Dr H.F. Haverman
October 7, 1994
NEWS FROM THE IFPCS

International Federation of Pigment Cell Societies Database

As one of the new initiatives of the IFPCS, a Committee has been established to collect data on resources available to the pigment cell community; that Committee includes a member from each constituent Society of the IFPCS and consists of V.J. Hearing (Chair), P.A. Riley and T. Takeuchi. The Committee is establishing an interactive data base of available resources (from research and commercial sources), to be known as the “International Database of Pigment Cell Research Resources” (to be abbreviated INTERPIG on the Internet). This Database will be available to all researchers, especially to members of the IFPCS. Listed in the Database will be all reagents, probes and other materials available and useful in research targeted towards pigment cells, including those for chemical, biochemical, immunological, molecular biology and other types of studies. The data collection form is attached (cf instructions below) and we encourage everyone to complete these forms and return them as soon as possible. Listing of resources will be free for nonprofit research laboratories; commercial suppliers must enclose a check for $25 USD for each item (made payable to the IFPCS). We will attempt to have the IFPCS INTERPIG Database available within the next year and IFPCS members will be advised as soon as it is ready; it will be accessible on the Internet, or by request on computer diskette in a number of different database and word processing formats. Of course, the ultimate success of the IFPCS INTERPIG Database will depend on our members to complete the forms as thoroughly and clearly as possible and to return them in as timely a fashion as feasible. We thank you in advance for your help and cooperation and we feel that the INTERPIG Database will be a valuable resource for all of us within the near future.

Instructions for Completing the IFPCS ‘INTERPIG’ Database Entry Form:

Please Type or Print Clearly

The bottom part of the Database form contains information specific to each contributor. Please fill out the bottom part of the form first with your name and the other relevant information requested, as completely as possible, and then make as many photocopies of that completed form as necessary (a separate copy will be required for each item to be listed in the Database).

Complete a separate form for each distinct item to be entered into the IFPCS Database.

Item - enter the specific name of the reagent, antibody, probe, etc.
Description - enter a brief description of the material.
Method - list the most relevant method for the material - please choose from one of the following categories:

- Biochemistry
- Cell Biology
- Cell Culture
- Chemistry
- Dermatology
- Electron Microscopy
- Immunology
- Melanoma
- Molecular Biology
- Physics
- Other: (specify)

Quantity - indicate the quantity of the reagent supplied in a single request.
Reference - list the relevant citation for the reagent (if any).
Comment - list any further comment on the reagent.

Please return all forms to: Dr. Vincent J Hearing, Laboratory of Cell Biology, Bldg 37 Rm 1B25, National Institutes of Health, Bethesda, MD 20892 USA or FAX to: 1-301-402-8787

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ANNOUNCEMENTS
I wish to announce the following :

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