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EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH BULLETIN

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CONTENTS

MEETING REPORT

VIII[TH] ANNUAL MEETING OF THE PASPCR
Aug 15-18, 1998 - Snowmass, CO, USA

(with the Bulletin's Editor apologies for the delay)

The PASPCR meeting this year was held in Snowmass, CO under the chairmanship of Dr. David Norris. The Editor would like to thank the following authors (as noted) for contributing commentaries on each of the sessions held.

Sunday morning Sessions by Zalfa Abdel-Malek

The Gelb lecture for the eighth meeting for the PASPCR was given by John Pawelek, Ph.D., and was entitled "Melanoma/macrophage hybrids and the development of metastases in melanoma". In his presentation, Dr. Pawelek provided a historical overview of reports by various investigators stating that such hybrids indeed exist and are associated with metastatic disease. Dr. Pawelek presented results from his laboratory, using mouse Cloudman melanoma cells that are known to have a poor metastatic potential. He reported that Cloudman melanoma X macrophage hybrids greatly enhanced the metastatic ability of tumor cells. It was observed that these hybrids became significantly more melanotic than the original melanoma cells. The proposed mechanism for the enhancement of metastasis and melanization is increased N-glycosylation of proteins, that included several melanogenic proteins. The melanoma/macrophage hybrids acquired a macrophage-like glycosylation system that resulted in enhancement of N-glycosylation. This mechanism offers one explanation for increased aggressiveness of melanoma tumors during disease progression.

The first plenary session was entitled "Control of Melanocyte Development and Differentiation". The abstract by Southard-Smith et al. was presented by Bill Pavan. He stated that premature termination of SOX 10, one of the SRY-like HMG box transcription factors resulted in the absence of neural crest derivatives in Dom mice, a model of Waardenburg-Hirschprung disease. SOX 10 was found to function intrinsic to melanocytes and to be expressed in early melanoblasts. The potential target genes for SOX 10 are to be identified using cDNA expression microarrays. The abstract by Donatien and Bennett was presented by Dorothy Bennett who described the procedure for establishing long-term culture of human fetal melanoblasts. For this, murine keratinocyte feeder cells were used, and the growth medium consisted of RPMI 1640 supplemented with 10% fetal calf serum, 1.5 μM hydrocortisone, 20 pM thyroxine, 40 pM basic fibroblast growth factor, 20 ng/ml stem cell factor, 20 pM cholera toxin, 100nM endothelin-3, and 20 nM TPA. In this medium, the cells doubled every 3-5 days, and expressed TRP-2 and Pmel-17, but not P protein, and were DOPA-negative. Growth could be further enhanced by the addition of NDP-MSH, and melanin content could be increased by the addition of oleoyl acetyl glycerol or cAMP inducers. The abstract by Xu et al., was presented by Estela Medrano. The authors used the yeast two-hybrid system to identify human proteins that interact with the transcription factor MITF. By screening the melanoma cell line IIB-Mel-J cDNA library, they found that most clones with a positive interaction with MITF encoded the human ubiquitin-conjugation enzyme hUBC9 (UBE21). This was confirmed by in vitro GST "pull down" assay, using 6 x His-tagged MITF, by co-localization of MITF and hUBC9 in the nucleus, and by increased degradation of MITF upon co-transfection of MITF and hUBC9 into Cos 7 cells. Dong and Vijayasaradhi investigated the role of the transcription factor MITF in the coordinated regulation of tyrosinase and TRP-1 in human melanocytes and melanoma cells. Upon treatment of these cells with

the differentiation inducer hexamethylene bis-acetamide (HMBA), the level of TRP-1 mRNA was drastically reduced, an effect that was not due to increased rate of mRNA degradation, and could be abolished by inhibition of de novo protein synthesis. The protein level of TRP-1 correlated with the level of its mRNA. On the other hand, tyrosinase and MITF mRNA levels were up regulated, with no change in the protein level of MITF. It was concluded that while expression of tyrosinase and other pigmentary genes correlated with MITF expression, TRP-1 expression was regulated independently of MITF and required other transcriptional factors.

The second Plenary Session was entitled "Control of Pigmentation by MSH, The Melanocortin Receptor and The Agouti Signaling Protein". Suzuki et al. reported on the regulation of the human MC1R expressed on human epidermal melanocytes. Brief treatment of melanocytes with α-MSH, ACTH or endothelin-1 resulted in increased MC1R mRNA level. This might account for lack of desensitization of the receptor following prolonged treatment with its ligands. Treatment of melanocytes with agouti signaling protein (ASP) or with UV radiation down regulated MC1R mRNA level. Unlike melanocytes, human keratinocytes did not express functional MC1R, as determined by Northern blot analysis, receptor binding assay, and cAMP radioimmunoassay. Matsunaga et al.used αPEP 16, a rabbit polyclonal antiserum generated against a synthetic peptide that corresponds to the carboxy teminus of mouse agouti protein, to characterize the expression of this protein in 3, 6, and 9 day old non-agouti black, agouti, and lethal yellow mouse skin specimens. Expression of agouti protein was very low in black mice, was noticeable and increased with age in hair matrix cells adjacent to melanocytes in lethal yellow mice, and was observed during the pheomelanogenic phase in agouti mice. Abdel-Malek et al. addressed the question whether ASP antagonizes the effects of α-MSH by exclusively binding to the MC1R, or by additionally binding to another unknown receptor. Melanocytes cultured from C57 BL6J E+ /E+ mice, congenic e/e or Eso /Eso mice were compared for their responsiveness to ASP. Only E+ /E+ responded to ASP with inhibition of basal and α-MSH stimulated tyrosinase activity, significant reduction of tyrosinase, TRP-1 and TRP-2 protein levels, and inhibition of α-MSH induced cAMP level. This demonstrates that expression of normal MC1R is pivotal for the responsiveness of mammalian melanocytes to ASP. Miltenberg et al. examined the biological significance of the highly conserved basic domain, adjacent to the cysteine-rich agouti C-terminal. Deletion of this entire region and expression of the mutant gene in transgenic mice resulted in mice with yellow coat color, but without hypoglycemia, insulinemia, or obesity. This mutation rendered the protein inactive in neural tissues, but functional in regulating pigmentation, with a reduced capacity to inhibit MC1R-induced stimulation of cAMP level. This suggests that the basic domain in the agouti gene is involved in the proteolytic processing of agouti signaling protein, affects the secretory pathway, and/or facilitates melanocortin receptor binding. Virador et al. attempted to identify bioactive domains of ASP by synthesizing overlapping 15 mer peptides that encompass its entire sequence. The biological effects of these peptides on melan-a cells were investigated by determining total melanin synthesis, and the expression of tyrosinase and TRP-1 mRNA. Peptides in the regions 30-52 and 57-91 generated about 10% reduction in total melanin production, compared to 30% reduction generated by recombinant ASP. The functional agouti region was narrowed down to 5 amino acids, Lys82Pro86, which resulted in significant reduction in melanin formation, tyrosinase expression and function. Johansen et al. described the isolation and sequencing of the bovine and porcine agouti genes, and the expression of this gene in bovine tissues. Using murine agouti cDNA as a probe, they found that cattle and pigs possess an agouti gene with 161 bp products that hybridize to murine agouti exon 2. This bovine and porcine agouti region was 75 and 76% similar to murine, 82 and 79% similar to human, respectively, and 88% similar to each other. The predicted amino acid sequences showed about the same percent homology. Furumura et al. reported the results of differential display study in which they identified three genes that were up regulated during the switch from eumelanin to pheomelanin synthesis. One of these genes is ITF2, an E type basic helix loop helix transcription factor. Upon transfection of Melan-a cells with the murine ITF2 gene, ITF2 trans-activated the TRP-1 promoter to the same extent as MITF, was less efficient than MITF in trans-activating the tyrosinase promoter, and had no trans-activating effect on the TRP-2 promoter. ITF2 over expression up regulated TRP-1 level, but down regulated the levels of tyrosinase and TRP-2, suggesting that ITF2 regulates melanogenesis by acting as an E-box binding protein. Abdel-Malek et al reported on the differential responsiveness of cultured melanocytes established from different skin types to α-MSH. Five out of five cultures with high constitutive melanin contents, and four out of five cultures with very low melanin contents exhibited the typical dose-dependent responses to α-MSH, evidenced by stimulation of cAMP formation, proliferation, and tyrosinase activity, beginning at a

dose of 0.1 nM. One of the five cultures with a very low melanin content demonstrated a significantly reduced response to α-MSH, evident as a shift to the right in the dose-response curves for cAMP formation and tyrosinase activity, with a minimal effect dose of α-MSH equal to 10 nM. RT-PCR and sequencing of the entire coding region of the MC1R gene revealed the presence of these point mutations in this culture: Phe147Leu, Ile155Leu, Arg160Trp,Thr177Arg, and Ile264Met. The significance of these mutations on the loss of function of the MC1R is being investigated.

Sunday afternoon Sessions by John Pawelek

The afternoon Plenary Session was on the Biochemical Control of Pigmentation.

1) Keynote Lecture: Perspectives on biochemical control of pigmentation and pigment cell survival, K. Schallreuter. Dr. Schallreuter discussed the hypothesis, proposed by Dr. John Wood and herself, that (6R)-L-erythro 5,6,7,8 tetrahydrobiopterin (6BH4) through regulation of phenylalanine hydroxylase (PAH) and tyrosinase activities is a key, rate-limiting factor in the control of melanogenesis, wherein the availability of L-tyr is regulated by PAH and 6BH4. The proposal is based in part on their observations that L-Phe is actively transported by human melanocytes, whereas L-tyr enters cells much more slowly, through passive diffusion. 6BH4 regulates melanogenesis through specific binding sites on both tyrosinase and MSH. MSH activates tyrosinase by removing 6BH4 from a tyrosinase: 6BH4 inhibitory complex. UVB, through photooxidation of 6BH4, activates both PAH and tyrosinase.

2) Macrophage migration inhibitory factor (MIF) has enzyme activity towards oxidized catecholamines and recues cells from dopaminechrome induced death. J. Matsunaga et al. Evidence was presented that MIF is able to catalyze the conversion of DOPaminechrome and norepinephrinecrome, toxic qinone neurotransmitter by-products, to indole-quinone derivatives that may serve as precursors of neuromelanin. Cytotoxicity experiments showed that MIF can rescue cells from DOPaminechrome induced death in culture. Since MIF is highly expressed in brain, the possibility is raised that MIF detoxifies catecholamine products and therefore could have a protective role for neural tissues.

3) The function of the pink-eyed dilution protein. N. Puri and M.H. Brilliant. Using immunohistochemistry and confocal microscopy, the authors showed that at least one function of the pink-eyed dilution protein (p protein) is to regulate melanosomal pH. Acidic compartments of melanocytes were detected by DAMP incorporation and indirectly visualized using fluorescein-conjugated antibodies. In wild type melanocytes, virtually all melanosomes were acidic and virtually all acidic compartments were melanosomes. In contrast, melanosomes of p-deficient cell lines were almost never acidic. As tyrosinase activity in melanosomes is dependent on a low pH, the authors postulated that the minimal melanin synthesis observed in p-deficient mutants is due to insufficiently low pH.

4) Cell density-mediated induction of tyrosinase-related protein gene expression and differential regulation of TRP-2 glycoforms. T.J. Hornyak et al. The effects of cell density on the expression of TRP-1 and TRP-2 in cultured melan-a mouse melanocytes were investigated. The relative levels of mRNA for both proteins increased with increasing cell density and decreased when confluent cells were replated at low density. Compared to TRP-2, TRP-1 both decreased more rapidly with low density and increased more rapidly as density increased. Western blotting showed that TRP-2 existed in two distinct glycoforms under separate regulation with cell density changes. The results showed the potential importance of cell density or cell contact in determining the extent melanogenesis.

5) Tyrosinase-related proteins (TRPs) modulate tyrosine hybroxylase activity of tyrosinase in genetically defined mouse melanocytes. R. Sarangarajan et al. Tyrosine hydroxylase (TH) activity of cultured mouse melanocytes from wild type B/B mice was compared to mice with mutations in TRP-1 and TRP-2. Using ^{3}H-L-tyrosine in the Pomerantz assay, it was determined that TH activity was higher in wild type over mutant cells, even though tyrosinase itself was wild type in all cases except for albino (c/c) negative controls. Conclusion: TRPs are positive regulators of TH activity.

6) The regulation of pigmentation by serine proteases and their inhibitors. M. Seiberg and S.S. Shapiro. Multilayered epidermal equivalents expressing UVB-inducible melanogenesis were used to study the effect of protease inhibitors on melanogenesis. Several serine protease inhibitors were also effective melanogenesis inhibitors. The pigmented Yucatan swine treated with one of the protease inhibitors showed a visible lightening effect. Data suggested that the protease inhibitors interfered with melanosomal transfer from melanocytes to keratinocytes.

7) Supermelanotic and metastatic melanoma x macrophage fusion hybrids: Altered N-glycosylation

as an underlying mechanism. S. Sodi et al. A number of fusion hybrids between weakly metastatic Cloudman S91 cells and peritoneal macrophages were shown to have increased metastatic potential and this correlated with a dramatic increase in both basal and MSH-inducible pigmentation. Using Western Blotting of LAMP-1 and TRPs, incorporation of ^{3}H-glucoseamine, and glycosylation inhibitors, it was determined that the increased pigmentation was likely to be a result of a macrophage-like N-glycosylation system expressed in the metastatic hybrids, and that this glycosylation system might be an underlying cause for increased metastasis as well. Further, the data revealed for the first time that N-glycosylation may be an important pathway for MSH-induced melanogenesis.

8) Chemical characterization of dopamine-melanin: Application to identification of melanins in *Cryptococcus neoformans*. S. Ito, et al. Melanin has long been associated with virulence in *C. neoformans* and is believed to be produced by laccase. Several isolates of C. neoformans were incubated with dopamine or DOPA and subjected to improved melanin analyses using alkaline H_2O_2 oxidation and HI hybrolysis. The results indicated that laccase from *C. neoformans* indeed oxidizes catecholamines to produce eumelanic pigments.

9) Molecular characterization of c-kit from the Mexican axolotl. K.A. Mason et al. Using alignments of known c-kit sequences, degenerate PCR primers were designed and used to amplify a small fragment of c-kit from axolotl RNA by RT-PCR. With this fragment, 3 positive clones were identified in an axolotl cDNA library. Two clones were identical and appeared to encode a complete cDNA sequence for axolotl c-kit. Genetic mapping excluded c-kit as a candidate for the axolotl white mutant, but showed that c-kit is tightly linked to PDGFα, seen in other vertebrates, and additionally confirming that this represented at least one form of axolotl c-kit.

10) An analysis of pigment patterns in leopard and golden mutant zebrafish and related taxa of danios. R. Morrison and K. Nagashima. The zebrafish, *Danio rerio*, was studied as a model organism for pigment pattern formation. There are at least four types of chromatophores in zebrafish: xanthophores, iridophores, leucophores, and melanophores. There is a distinct embryonic pigment pattern composed of four melanophore stripes that transform into the adult pattern of alternating blue-black and silver yellow stripes. The golden mutant, which lacks melanophores and iridophores as adults, and the leopard mutant, which shows a spotted phenotype were studied, along with the pearl danios, which lacks alternating striping elements as an adult but contains a fifth type of chromatophore, the erythophore, and the giant danios that has a different arrangement of chromatophores than that seen in wild-type zebrafish. It was proposed that analyses of these fish should further clarify the critical events and time-points in zebrafish pigment pattern formation.

11) LiCl is involved in the pigmentation of the embryonic zebrafish (*Brachydanio rerio*). E-J Jin and G. Thibaudeau. Zebrafish embryos were treated with various signalling-related molecules. LiCl and LiCl/forskolin treatments each increased pigmentation. The LiCl/forskolin-induced pigmentation was not accompanied by an increase in melanophore number, but did result in increased tyrosinase activity and increased expression of MSH-1, a pigment specific protein, and TRP-2 as assessed by immunoblotting.

Monday morning Sessions by Raymond Boissy

The morning began with a sunrise session entitled "New Perspectives on the Treatment of Human Vitiligo". David Norris presented a review of the processes of programmed cell death (i.e., apoptosis) and necrosis and discussed the balance between these two mechanisms as they pertain to cell survival. Molecular regulators of apoptosis (i.e., the bcl family of proteins, FAS and the caspases) were reviewed. It was proposed that melanocyte destruction in vitiligo occurs via apoptosis and intervention of this may provide potential therapeutic opportunities. Raymond Boissy discussed occupational/contact vitiligo resulting from exposure to phenolic/catecholic agents. The phenolic agent, 4-tertiary butyl phenol, is cytotoxic to melanocytes via an apoptotic process. The antioxidant catalase could provide some protection against this form of melanocyte destruction. Pranab Das reviewed the immunological components of vitiligo. The role of T-cell autoreactive clones, conducting a hit and run affect on melanocytes in vitiligo was discussed. Immunomodulary therapy was proposed as a perspective for treatment for vitiligo. Finally, Karin Schallreuter discussed the biochemical aberration in skin of patients with vitiligo. The role of biopterin in the regulation of tyrosinase activity was reviewed. Successful results of the daily topical application of pseudocatalase on repigmentation in vitiligo was presented and discussed. It was concluded in this sunrise session that the etiology of vitiligo is both complex and diverse and that multiple theraputic regimes will have to be developed to successfully treat this disease.

A keynote lecture was then presented by Richard Spritz entitled "New Approaches in Genetics and Their Application to Pigment Cell Research" and subtitled "How to find a gene". Functional versus Positional Cloning methods were presented and the difference between discussed. Mapping a gene directly to a chromosome was first discussed and techniques using autoradiography, FISH, and chromosome abnormality was presented and the c-kit associated Waardenberg Syndrome and OCA2 were provided as examples. Genomic analysis (i.e., linkage) was discussed next. Examples of using RFLP assessment, Simple Tandem Repeats, and the utilization of chromosome site markers were presented. Finally, current methods for gene mapping were described. This included the need for large family trees, Yeast Artificial Chromosomes, Sequence-tagged-site, homozygosity mapping, linkage disequilibrium mapping, and the generation of a physical map.

A session focussing on the Hermansky-Pudlak Syndrome followed. Richard Spritz presented genetic and functional studies of Hermansky-Pudlak syndrome. The characteristics of this syndrome consist of oculocutaneous albinism, a platelet aggregation dysfunction, and the development of a ceroid like material in the lungs resulting in pulmonary fibrosis. The cloning of the gene (and the murine counterpart) and the identification of various protein-null mutations was presented. The HPS gene product appears to be soluble and unglycosylated. It is predominantly unassociated with organelles or granules in fibroblasts and lymphoblastoid cells and loosely associated with large granules in melanoma cells. The HPS gene product demonstrated some co-localization with tyrosinase, LAMP-1, and Myo5A (the product of the *dilute* locus). Richard King next presented the identification of an alternative 1.5 kb transcript (in addition to the 3.6 kb full length transcript) also present in bone marrow and a melanoma cell line. Raymond Boissy next discussed cytological aberrations in melanocytes cultured from patients with mutations in the HPS gene resulting in the lack of transcript expression. These hypopigmented melanocytes demonstrated muted tyrosinase activity, large membranous complexes, and DOPA positive 50 nm vesicles distributed throughout the cell. Tyrosinase, TRP-1 and ME491 were not efficiently trafficked to melanosomes in the mutant cells. Localization studies suggested that the HPS gene product was associated with the endoplasmic reticulum and melanosomes in the normal melanocytes.

The final session of the morning was entitled "Vitiligo: mechanisms of depigmentation and repigmentation". Pranab Das presented data demonstrating the appearance of immunocytes at the border of a vitiligo lesion and the associated development of apoptosis in some melanocytes. In addition, studies demonstrating that immune cells can lead to *de novo* generation of nitric oxide in melanocytes resulting in apoptosis. Caroline LePoole presented the immortalization of a line of vitiligo derived melanocytes using the E6 and E7 gene of HPV. These cells were passaged over 60 generations, demonstrated dilated RER, and exhibited alterations in proteins identified in fractionated RER. Marna Ericson presented data in which biopsies both pre and post treatment with topical steroids were immunocytochemically processed for the identification of melanocytes, Langerhans cells and nerve cells, and viewed with confocal microscopy and computer reconstruction. Demonstration of a decrease in the appearance of nerve fibers in the epidermis after successful treatment was provided. Fan Yang presented data demonstrating the 4-tertiary butylphenol (4-TBP) acts as a specific competitive inhibitor of tyrosinase at concentrations well below the threshold that generated a cytotoxic response in normal melanocytes. Finally, Caroline LePoole demonstrated that shortly after exposure of melanocytes to 4-TBP several transcripts are differentially expressed. One of these upregulated proteins was an adenosine receptor that has been implicated in the activation of apoptosis.

Monday afternoon Sessions by Frank Meyskens

The session on malignant melanoma covered a diversity of topics. Meenhard Herlyn started the session with an eloquent keynote presentation of their skin reconstruction model, which is used to study progression. Using the model, Hsu has shown that adherence of keratinocytes by E-cadherin to melanocytes controls their growth and phenotype and when this adhesion molecule is shut off a cell cluster, a nevus, forms. The overall important point was made that the model more closely resembles the biology in intact skin than that exhibited by cells under typical culture conditions. There were several papers on the role of various proteins in controlling melanocyte growth including its positive regulation by retino-blastoma tumor suppressor protein (R. Halaban, Yale), and negative regulation by p15(INK4B) (T. Pacheco, University of Colorado). In a similar vein Rearden at the University of Colorado used T-cell receptor knock-out mice to show that transduction of B16 F10 melanoma cells with M-CSF increased survival of the inoculated animals suggesting that cells other than T lymphocytes (eg NK or macrophages) are involved in anti-melanoma immunity, at least in this model.

In a fasciniating presentation, Bill Robinson of Australia presented the zebrafish as a powerful developmental model for understading the role of p16 and other regulators in melanocyte proliferation. This model impresses this reviewer as possibly one of the most important new systems to come along in quite some time.

The other Keynote lecture was entertainingly provided by John Cohen of the University of Colorado and apoptosis was reviewed in detail. Two papers were presented dealing with apoptosis in melanoma. Shellman from Colorado convincingly showed that apoptosis was controlled by the dimension status of tumor growth; i.e.under monolayer growth conditions, ras-alterated cells underwent apoptosis in response to various stress conditions while under 3-dimensional spheroid growth no or little apoptosis occurred. Since most culture studies are done in monolayer these studies have obvious and important consequences for the interpretation of studies of melanoma growth and drug resistance in the *in vivo* setting. From my own laboratory, Spillane has found that human melanoma cells have high levels of endogenous reactive oxygen species that may induce high levels of constitutive NFKB and a protective stress response. Interestingly, the complex antioxidant PDTC induced apoptosis while classical antioxidants(e.g. α-tocopherol) did not; suprisingly heavy metal chelators could largely mimic the effect PDTC. The most intriguing and interesting study of the session (and perhaps of the conference) was presented by Stan Pavel of the Netherlands. They measured the sulfer and phaeomelanin content of atypical nevi; and found their concentrations to be elevated, as was the concentration of calcium. Normal melanocytes from these same subjects showed no such changes suggesting that an abnormality in melanosomal regulation exists. Since phaemelanin metabolites are considerably more toxic that those of eumelanin. metabolites. One wonders if the generation of atypical nevi results from a simple error of metabolism, e.g. in the Agouti signaling protein. All in all this session was quite stimulating and a lot of new ideas were heard and vigorous discussion ensued.

Tuesday morning Sessions by Joe Bagnara & Vince Hearing

The keynote lecture by Karl H. Pfenninger presented basic insights into cell motility through the use of nerve growth cones as an example. Thus, he set the stage for the subsequent three presentations which were specifically directed toward an understanding of the migration of melanocytes and melanoma cells. Dr. Pfenninger illustrated the importance of pseudopod attachment, release, and reattachment as the basis for cell migration and he considered the means by which these steps are accomplished. The subsequent presentation by Hiroaki Yagi demonstrated through the use of Boyden chamber assays that insulin-like growth factor-1 (IGF-1) is a potent chemoattractant for both human melanocyes and melanoma cells. Endothelin-1 (ET-1) and basic fibroblast growth factor (bFGF) enhanced migration of normal human melanocytes and enhanced the invasion activities of WM35 cells (a human melanoma with low invasiveness). IGF-1 induced maximal movement in both these cell types and this action could be blocked by CDC, a selective 12-lipoxygenase inhibitor. cPLA2, a critical enzyme in pseudopod activation in both cell types is stimulated by ET-1, bFGF and IGF-1. The implications of these findings toward the progression of melanoma were discussed. This presentation was followed by that of Tatsuya Horikawa, et al. who considered the motility and proliferative responses of an H-ras-transfected murine melanocyte cell line, melan-A. In a Boyden chamber assay it was found that H-ras-transfected melanocytes show a higher incidence of migration than do wild type melanocytes. Cell motility was induced by ET-1 and bFGF in wild type melan-A, but not in H-ras-transfectants. TPA was required to grow parental melan-A cells, but the ras-transfectant was TPA-independent and in fact was inhibited by TPA. It was suggested that H-ras plays a key role in the induction of melanocyte locomotion and proliferation. The last talk on acquired MSH-sensitive chemotaxis by highly metastatic melanoma/macrophage fusion hybrids by Rachkovsky et al. was presented by John Pawelek. It was a most fitting follow up to the very first presentation of the conference, John Pawelek's Gelb Lectureship on melanoma/macrophage hybrids and melanoma metastases. In this symposium talk, it was pointed out that of the various fusion hybrids between normal macrophages and Cloudman S91 melanoma cells, the most metastatic also showed dramatically increased motility. Metastatic hybrids demonstrated increased migration in response to 3T3-conditioned medium, lung fibroblast-conditioned media, and lung explants. Treatment of the hybrid cells with MSH/BMX markedly increased migration through enhanced chemotaxis rather than chemokinesis. Evidence was presented suggesting that different glycosylation pathways were expressed in hybrid and parental cells and that motility was probably regulated by means of N-glycosylation. It was suggested "that the enhanced metastatic potential of macrophage x melanoma hybrids may have its basis in a

new, MSH-inducible chemotactic phenotype, with altered N-glycosylation as one of the underlying regulatory mechanisms."

After a short break, Barbara Gilchrest presented a Keynote Lecture on the "Effects of UV on melanocytes: speculative relationship to the epidemiology of melanoma." Melanocyte responses to UV were reviewed with special emphasis on their DNA repair mechanisms. The role these responses play in melanocytes and keratinocytes might explain the differences in malignant transformation in those cell types to produce melanomas and carcinomas, respectively. The importance of sunscreen use to protect from UV damage throughout life was emphasized.

N. Kobayashi then reported on studies examining the photoprotective effects of melanins. Immuno-histochemistry with antibodies specific for different types of DNA photoproducts allows assessment of DNA damage in UV irradiated skin at the cellular level. A direct correlation was found between melanin content in a cell and protection from UV light. Current studies are aimed at examining the efficiency of different types of melanins using mouse tail skins of different genotypes as models. Z. Abdel-Malek then reported on responses of melanocytes from different human skin phototypes to UV light. The sensitivities of melanocytes from different donors to UV light was measured for DNA damage, bcl2 expression and other parameters of cellular injury. There were significant differences in the responses measured depending on skin phototype in melanocytes, keratinocytes and fibroblasts, and the increased sensitivity of keratinocytes and fibroblasts to UV damage was proposed as the reason for the higher incidence of carcinomas in those cell types. Finally, F. Meyskens reported on the response of metastatic melanoma cells to UVB stimulation, particularly with respect to activation of the NFkB transcription factor. There were dramatic differences in activation of 2 NFkB family members (p50 and p75) in malignant melanoma cells and in normal human melanocytes, often by an order of magnitude, suggesting basic differences in response mechanisms of normal and transformed melanocytes. It was suggested that such changes are associated with the metastatic potential of those malignant cells, and that understanding the reason behind such differences may offer novel approaches to the prevention, diagnosis and therapy of melanoma.

Poster Session by Roger Bowers

The poster sessions at this PASPCR meeting were well attended. There were only 12 posters due to the numerous oral presentations but they were of the highest quality. Holder and Thibaudeau showed that increased melanization in the melanoid defect in axolotls may involve aspects in addition to cellular plasticity whereas cellular plasticity is implicated to be involved in enhanced xanthophores from albino axolotl embryos. Parker and Mason demonstrated that extracts from the white axolotl mutant caused a decrease of pigment cells and inhibited their movement in cultured melanoma cells. They are currently working to characterize this extract. Roberson and Thibaudeau showed a two stage time-dependent differentiation of *in vivo* melanophores in zebrafish. Similar results were found in *in vitro* zebrafish melanophores and LiCl increased pigmentation in these same cells. Nurcahyani and Thibaudeau demonstrated cellular parameters influencing ant./post. responses of neural crest-derived pigment cell linages in axolotls. For example, ant. neural crest cells gave rise to more pigment cells whereas post. neural crest cells yielded more melanophores. Gonzalez, Buckner, Ruiz and Bowers, with the use of the glutathione inhibitor BSO, other treatments and parameters, showed the importance of antioxidants in the viability of avian melanocytes. Maxwell, Walsh and Maxwell demonstrated the infection of human melanoma cells by parvoviruses and suggested therapeutic uses of these viruses and their vectors for melanoma. Murakami, Baba, Kawa and Mizoguchi showed that inflammation in atopic dermatitis plays a role in developing acquired dermal melanocytosis along with the hereditary disposition of having immature dermal melanocytes. Sarangarajan, LePoole and Boissy demonstrated that NHE-1 isoform of sodium hydrogen exchanger is expressed in M14 melanoma cells and in keratinocytes but not in human melanocytes. Ahn, Jang, Cho, Lee, Hong and Lee showed that a kojic acid derivative, kojyl caffeic acid, has a greater depigmenting effect than kojic acid. Forest, Nofsinger, Drake and Simon demonstrated that the photochemistry of melanin in the UV is wavelength dependent. Shi, Krauss and Woodward showed evidence for the presence of EP2- and IP- receptors coupled to melanin production via stimulation of tyrosinase activity in S91 Cloudman cells. Butts and Naughten demonstrated that melanocytes decreased with time in new wound closure epidermis in the corneal region of the frog.

1. Melanins and other pigments chemistry

(Prof. M. Peter)

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2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

Members of tyrosinase family share upstream transcriptional regualtory elements suggesting that expression of these genes is regulated by shared mechanisms. Microphtalmia transcription factor (MITF) for example, has been shown to transactivate tyrosinase and TRP-1 genes "in vitro" by binding to a shared regulatory sequence known as Mbox. Fang and Setalury showed earlier that in human melanoma cells TRP1 can be regulated independently of tyrosinase and pigmentation. To investigate the role of MITF in TRP1 regulation the authors studied the effect of pharmacological agents, that modulate transcription of tyrosinase and TRP1 on MITF. Their results have shown that TRP1 gene can be regulated independently of MITF and that both positive factors such as MITF and inactivation of negative regulatory factors can contribute to the TRP1 gene expression during melanocyte differentiation.

The expression patterns of steel factor (SLF) observed in the skin and gonads suggest that SLF mediates a migratory or chemotactic signal for c-Kit-expressing stem cells. Experiments by **Kunisada and co-workers** by using a SLF transgenic mice model, provide direct evidence that SLF stimulate migration of melanocytes "*in vivo*". They also underline that SLF not simply support survival and proliferation of melanocytes but also promotes differentiation of these cells. Unexpectedly melanocyte stem cells independent of the c-Kit signal were maintained in the skin of the SLF transgenic mice. Elimination of c-Kit-dependent melanoblasts by a function-locking anti-c-Kit antibody, these cells continued to proliferate and differentiated into mature melanocytes. The melanoblasts are able to migrate to cover most of the epidermis after several months. The SLF transgenic mice described in this report will be useful in the study of melanocyte biology.

Human MCH I molecules are encoded by three different loci (A-B-C) and in primary human melanomas as well as melanoma cell lines, HLA class I expression is frequently down-regulated in locus B. To study the involvement of

promoter element in HLA-B locus-specific down-regulation, **Griffioen and co-workers** transfected a series of reporter constructs containing 5'-flanking sequences of the HLA-A2 and HLA-B7 genes into melanoma cell lines expressing high and low levels HLA-B antigens. Interferon-Stimulated Response Element (ISRE), known to induce MHC class I expression in response to IFNs and that significantly stimulate constitutive transcription of HLA class I genes, was tested. Although none of the promoter elements considered could be demonstrated to mediate HLA-B locus-specific down regulation, high and low HLA-B expressing melanoma cell lines could differ in ISRE activity as well as in ISRE-binding of nuclear factors.
Brandenburger and co-worker synthesised and tested thirteen oligomeric analogs of alpha-melanocyte-stimulating hormone (alpha-MSH) on melanoma cells for their ability to bind to melanocortin type 1 (MC 1) receptors and stimulate melanin production in the cells. Some constructs showed higher binding activity and higher melanogenesis-inducing activity. The authors discuss these results in terms of possible bridging of neighbouring receptors which has been suggested to occur in some other system.
B16 variant of murine melanoma cell line, selected "*in vivo*" for enhanced liver metastatic ability, shows a constitutive activation of the proto-oncogene c-met and a more differentiated phenotype in comparison with the parental cell line B16. **Rusciano and co-workers** have tried to identify a possible correlation between differentiation and C-met expression in B16 melanoma cells. C-met expression was strongly induced by melanocyte-stimulating-hormone (alpha-MSH) and this expression was mediated by cAMP elevation and PKA-PKC activation. These results raise the intriguing possibility that autocrine and/or paracrine mechanisms, acting "*in vivo*" in this circuit, might influence the metastatic behaviour of these tumoral cells.
Melanoma cells express in their surface ganglioside antigens (GM2, GD2, GM and GD). Takahashi and co-workers determined levels of IgM anti-ganglioside in sera of melanoma patients, who received melanoma cell vaccine immunotherapy, after surgical removal of regional metastatic melanoma. This evaluation was performed prior to melanoma cell vaccine treatment and 4 weeks after the first melanoma cell vaccine immunisation. All antibody levels increased by week 4 and all increases were significantly associated with survival. These studies suggest that GM2, GD2 and GD3 expressed by melanoma cells, can induce specific IGM antibodies and that high levels of these antibodies might have a beneficial impact on survival.
Transforming growth factor-beta 1 (TGF-beta 1) acts as an autocrine growth inhibitor on normal human melanocytes, while melanoma cells may not respond to this stimulus. The role of its iso-forms, TGF-beta 2 and TGF-beta 3, at presentt is less well characterised. **Krasagakis and co-workers** studied mRNA and protein levels of all three iso-forms on a panel of human melanoma cell lines and in cultures of normal human melanocytes "*in vitro*". The results demonstrated that melanoma cells secrete significantly higher levels of TGF-beta iso-forms as compared to normal melanocytes but are not growth-inhibited by all three TGF-beta iso-forms.
Recruitment of leukocytes from the peripheral blood into the tumor site is mediated predominantly by chemokines and this recruitment can determine beneficial effects. **Mrowietz et al.** have tried to identify peptides released by human melanoma cells with chemotactic properties toward monocytes. Purified fraction from supernatants of melanoma cells shown to react with RANTES-specific antibodies in ELISA and Western Blot analysis and amino acid sequencing of N-terminal fragment confirmed 100% homology to the RANTES protein. High levels of RANTES secretion "*in vitro*" were associated with enhanced tumor formation in nude mice. These data provide evidence that a subset of melanoma cells express and secrete RANTES protein which may be partly responsible of chemotactic effects. Transplantations experiments in nude mice suggest that this chemokine may also favour tumor progression. **Moretti S. and coworkers** have evaluated, by histochemistry, the expression of growth factors and cytokines and the respective receptors in nevi, primary and metastatic melanoma. With respect to nevi, marked up-regulation of several growth factors, cytokines and receptors was observed in thick primary melanoma and in biologically late lesions TGF-beta, GC-CSF and IL1-alfa are highly expressed. Their results suggest that secretion of growth factors and cytokines may be an adjunctive promoting phenomenon in the growth of melanoma cells.
Keratinocytes present in different layers of epidermis are biologically and functionally different and can modulate melanocyte proliferation and function in a different way. In order to investigate this question **Abdel-Naser** analysed normal human epidermal melanocytes incubated with low-Ca++ and high Ca++ keratinocyte-conditioned medium obtained from the same skin source of melanocytes. The results demonstrate that keratinocytes grown at a low Ca++ level release factors that stimulate melanocyte proliferation as well as melanin synthesis, whereas keratinocytes grown at high Ca++ level release factor that only stimulate melanin synthesis. Therefore, it seems that a different chemical environment can modify the response of cells. This may provide a possible explanation of the anatomical position of melanocytes in epidermis and may play a part in the pigmentary changes following injury to epidermal cells.
In order to understand the respective roles of keratinocytes and melanocytes in tanning and photoprotection **Bessou-Touya** at al. studied Chimeric epidermal reconstructs made with Negroid melanocytes and Caucasoid keratinocytes (or vice versa) before and after UVB irradiation. Using this model, they confirmed overall the theory of the epidermal melanin unit and also they show that melanocytes of poorly tanning Caucasoid, which have a comparative higher content of unsaturated fatty acids in their cell membranes are more prone to the peroxidative effects of UV light and that keratinocytes contribute to the photoprotection via phototype-dependent antioxidant enzyme activities.
Bacharach-Buhles and co-workers investigated the mechanism of reduction of melanocytes by apoptosis, that migrate in epidermis after exposition of skin to UVA radiation, to prevent an uncontrolled increase of these cells in the epidermis. The authors observed a dose-dependent shift of apoptotic unaltered melanocytes into the dermis after irradiation and suggested that this mechanism could regulate and control UV-induced proliferation of epidermal melanocytes.
The synthesis of pheomelanin requires the incorporation of thiol-containing compounds and Cysteine and/or Glutatione were proposed as suitable thiols donors. Since melanins are produced only in specialised, membrane-bound organelles the melanosomes, thiols donors must cross membrane barrier from the cytosol to the melanosome interior. In a recent study **Potterf and co-workers** demonstrated that cysteine is transported across membranes of melanosomes in a temperature and

concentration-dependent manner. They demonstrate also that cysteine uptake is a carrier-mediated process. In contrast, the authors were unable to detect any significant uptake of GSH. Their results suggest that cysteine is the physiological thiol source utilised in mammalian melanosomes for pheomelanin synthesis. On the specific question of whether melanin are photoprotective or photosensitizing the group of **S. Pavel and A Schothorst** have presented interesting data showing that following UVA irradiation melanocytes are photosensitized by their own chromophores, most likely pheomelanin and melanin intermediates which results in increasing DNA single strand breaks. In their model skin type VI melanocytes resulted with an higher sensitivity towards UVA radiation than skin type I melanocytes related to the content of melanin/pheomelanin. In fact, increasing the melanin content of skin type I melanocytes resulted in an increase of ssDNA breaks.
In Experimental Dermatology an interesting "controversies" on what the use of generating melanin has been presented with different viewpoint and then some commentary.
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Melanocyte cultures
(Dr N. Smit)

Hedley et al compared normal and vitiligo melanocytes with regard to their expression of MHC class I and II molecules and ICAM-I and how the expression was effected by IFN-gamma and TNF-alpha. Both cells showed similar expression and also the the rates of proliferation of the two types of melanocytes did not differ significantly once the cultures were established.
Iyengar describes in two papers the role of the indoleamines serotonin and melatonin in the melanocyte cell cycle in a whole skin organ culture. Also Johansson et al show the serotonin-like immunoreactivity of melanocytes in the epidermis and in culture.
Potterf et al used two immortalized cell lines, the melan-a and melan-p melanocytes to study cysteine transport to the melanosomes. The two cultures differ significantly in their melanin production with the melan-p cells producing relatively low eumelanin and high pheomelanin. Interestingly also extracellular melanin is measured with a high production of pheomelanin for the melan-p cells in medium with high cysteine content. Just like in the cultured melan-a cells ussed by Potterf a shift towards pheomelanin production is reported by Prota et al for cultured iridial melanocytes as compared to the original pigmentation in iris pigment epithelium. The culture method and sensitive HPLC analysis of melanin made it possible to compare pigmentation in the melanocytes from donors with various degree of iris pigmentation.

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3. MSH, MCH, other hormones, differentiation

(Dr. B. Loir)

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5. Neuromelanins

(Prof. M. d'Ischia)

Some interesting papers dealing more or less peripherically with the subject of neuromelanin have appeared in the end of 1998 and in the first months of 1999, which seem worthy of a comment. The iron-neuromelanin interaction is the main theme of two papers, by Kropf et al. (Biophys. J. 75, 3135, 1998) and by Jellinger (Drugs Aging, 14, 115, 1999). In the first of these papers, the authors employ X-ray absorption fine structure spectroscopy to investigate the iron sites of natural neuromelanin and various synthetic analogues. The results indicate that natural and synthetic pigments have approximately similar environments surrounding the iron centre but display significant differences in the higher coordination shells, which points to a possible inadequacy of synthetic melanins as models for natural pigments. In the second article, Jellinger surveys current pharmacological strategies to treat Parkinson's disease based on the control of iron-induced neurotoxic reactions, including production of reactive oxygen species.
In another study, Offen et al. (Neurosci Lett. 260, 101, 1999) investigated the effects of dopamine melanin, as a model for neuromelanin, on PC12 cells and cerebellar granular cells, and demonstrated the ability of these cells to phagocytise melanin, possibly by an energy-dependent mechanism. This finding may have important implications in relation to the origin of neuromelanin in pigmented neurons and its possible significance as a vulnerability factor in Parkinson's disease, although the validity of conclusions drawn from synthetic melanins still needs to be assessed.
An interesting addition to the current panorama of aminochrome rearranging enzymes is provided by Matsunaga et al. (J. Biol. Chem., 274, 3268, 1999), who showed that macrophage migration inhibitory factor (MIF) a protein widely expressed in nervous tissues, can promote the conversion of catecholamine-derived aminochromes to indolic melanin precursors. If corroborated by further evidence, this finding may open new perspectives in the understanding of the mechanisms underlying the oxidative metabolism of catecholamines in neural cells.
Finally, a new invertebrate model for the study of neuromelanin and its role in oxidative stress is proposed by Fyffe et al. (Cell Tissue Res., 295, 349, 1999) who demonstrated that the brown intraneuronal granules in the dorsal nerve plexus of the earthworm L. terrestris contain material morphologically and histochemically consistent with neuromelanin, which increases upon exposure to high levels of oxygen.

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6. Genetics, molecular biology

(Dr. F. Beermann)

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  Abstract: Experimental strategies have been developed to correct point mutations using chimeric oligonucleotides composed of RNA and DNA. We used these RNA-DNA oligonucleotides to correct a point mutation in mouse tyrosinase, a key enzyme for melanin synthesis and pigmentation. Melanocytes derived from albino mice contain a homozygous point mutation (T (G) under bar T-->T (C) under bar T) in the tyrosinase gene, resulting in an amino acid change from Cys-->Ser. Correction of this point mutation results in the restoration of tyrosinase activity and melanin synthesis, thus changing the pigmentation of the cells. Upon transfection of the RNA-DNA oligonucleotide to albino melanocytes, we detected black-pigmented cells and isolated multiple single clones. All black-pigmented clones exhibited a correction of the point mutation in a single allele of the tyrosinase gene. A full-length tyrosinase was detected by an antityrosinase antibody, and the enzymatic activity was restored in all converted black-pigmented clones. Only degraded fragments were detected in albino cells due to proteolytic cleavage of mutant tyrosinase. The phenotype and genotype of converted black-pigmented clones was stable. These results demonstrate a permanent and stable gene correction by the RNA-DNA oligonucleotide at the level of genomic sequence, protein, and phenotypic change by clonal analysis.

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7. Tyrosinase, TRP1, TRP2 and other enzymes

(Prof. J.C. Garcia-Borron)

Several papers referenced in this issue deal with different aspects of TRP1 biological activity, particularly with its interaction with tyrosinase. Kobayashi et al. (J-Biol-Chem. 1998 Nov 27; 273(48): 31801-5) prove that TRP1 mediates "in vivo" a stabilization of tyrosinase. The intracellular half life of the enzyme, as determined by pulse-chase labeling experiments, is decreased in melan-b melanocytes lacking a functional TRP1, and this decrease can be rescued by infection with the wild type TRP1 gene. Sharma et al. (Pigment Cell Res. 1998 Dec; 11(6): 375-380) further report on the ability of tyrosinase to form high molecular weight complexes. Jimenez-Cervantes et al. (Biochem-Biophys-Res-Commun. 1998 Dec; 253(3): 761-767) have shown by a combination of chromatographic and spectroscopic techniques that purified tyrosinase and TRP1 from mouse melanoma cells associate in solution giving rise to heterodimeric species. Interestingly, TRP1 and tyrosinase seem to prefer a heterodimeric structure rather than homomeric conformations. This specific association provides a molecular basis for the stabilizing effect of TRP1 on tyrosinase. Taken together, all these observations leave little doubt as to the occurrence of strong and specific heterologous interactions between tyrosinase and TRP1. These interactions, at the least, lead to tyrosinase protection against proteolytic degradation, but they may also have still undiscovered functional effects. Certainly, a detailed kinetic study of the heterodimeric species and a vis-a-vis comparison to the kinetic behaviour of purified tyrosinase is needed.

Concerning other possible activities of TRP1, Boissy et al. (Exp-Dermatol. 1998 Aug; 7(4): 198-204) provide evidence that human TRP1 does not function as a DHICA oxidase, as opposed to murine TRP1. This report agrees with unpublished observations in our laboratory, and raises the question of DHICA metabolism in human melanocytes. The answer might be simple in that human tyrosinase appears to be able to use DHICA as a substrate, but further work in this area will be needed in order to distinguish whether tyrosinase accounts for all DHICA oxidation in human melanocytes, or other enzymatic proteins are also involved in the process. Anyhow, the lack of DHICA oxidase activity of human TRP1, if fully confirmed, will raise the question of the function of the human protein. Certainly, it may contribute to tyrosinase stability, and, as pointed out by Kobayashi et al., this role might be more important than in the mouse melanocyte models, since human tyrosinase is far less stable than its mouse counterpart. However, other observations are difficult to account for based exclusively on this stabilizing role. For instance, Del Marmol et al. (FEBS Lett. 1993 327:307-310) described some time ago a close association of TRP1 expression and eumelanogenesis, as opposed to pheomelanogenesis, in human melanocytes. Therefore, TRP1 surely has some hidden aspects that deserve further investigation. Maybe the time is coming to go back to basic enzymology, although this does not seem to be fashionable any more!

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8. Melanoma and other pigmented tumours

Melanoma therapy (Dr. N. Smit)

Many papers dealing with numerous ongoing trials for melanoma treatment. In the Eur. J. Cancer 34, a supplement(3) was devoted to a melanoma meeting in Barcelona. As indicated by Eggermont in the introduction many presentations of this meeting on new immunomodulating strategies using cytokines, vaccines and IFN-a2b are collected in this issue. In his paper Parmiani reviews different types of melanoma associated antigens and their use in *in vitro* and *in vivo* immunotherapy studies. Several other reviews appeared on different therapeutic strategies. In the paper by Punt the current status of IFNalpha in the treatment of cutaneous melanoma is reviewed. In the review by Ollila et al key components of a successful melanoma vaccine are identified, and some of the important clinical trials of active specific immunotherapy for patients with melanoma are summarized. Restifo and Rosenberg review on recent clinical trials using experimental cancer vaccines and recent evidence of objective responses in melanoma patients is included. The paper by Pyrhonen et al reviews major studies that evaluated different treatment options for metastatic uveal melanoma. The use of intra-arterial fotemustine demonstrated a 40% response rate for patients with liver metastases of uveal melanoma. Additionally, chemoimmunotherapy with a four-drug chemotherapy regimen and interferon alfa has provided response rates of approximately 20% and may contribute to prolonged survival. In a review by Johnson et al several recent advances in melanoma therapy are discussed. A special attention is paid to radiolymphatic sentinel node mapping of the lymph nodes. Berd et al describe the use of dinitophenyl (DNP)-modified vaccines. Of 62 patients with clinically evident stage III melanoma who had undergone lymphadenectomy, the 5-year relapse-free survival rate was 45% and the overall survival rate was 58%. The results appear to be better than those obtained with high-dose interferon.

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Melanoma experimental treatment (Dr. N. Smit)

Freuhauf et al describe some important results of melanoma toxicity studies by targeting glutathione in the cells. They show that treatment with BSO is especially toxic towards melanoma when a large number of tumor specimen are treated with this inhibitor of glutathione synthesis in an agarose based thymidine incorporation assay. The active diastereomer L-S-BSO was even more effective. Melanin content in the melanoma tumor specimen was measured by an image analysis technique and a positive correlation between melanin content and inhibition of proliferation after exposure to BSO was found. Also GSH transferase activity was decreased by the BSO treatment. Melanin synthesis and the formation of quinone intermediates and subsequent generation of free radicals may be responsible for the high sensitivity of the melanoma tumours to the BSO treatments since these products are insufficiently removed by GSH especially because also GST activity was inhibited. Jimbow describes the use of cysteaminylphenol and derivatives for inducing a tyrosinase dependent melanoma toxicity. The suggestion was raised to expand the use of these compounds in targeted gene therapy to other non-melanoma tumors transfected with tyrosinase cDNA. Two papers by Fu et al describe the effects of limitations of phenylalanine and tyrosine. In culture medium the restriction of the two amino acids leads to apoptosis in two different melanoma cell lines B16Bl6 and A375. The Tyr-Phe restriction also increased the toxicity of chimeric TGF-alpha toxins in the B16Bl6 melanoma cells. Possibly the limitation of these melanin precursors renders the melanoma tumor cells more sensitive than other tumour types. Interestingly Slominski hypothesizes that inhibition of melanogenesis will make the melanoma tumours less resistant to different types of therapy since melanin protects the cells against these treatments.

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Commentary: Twelve of the 17 patients had a melanotic and 5 had an amelanotic subtype of the tumor. Iodine-123-IBZM accumulation occurred in the metastases of 10 of the 12 patients with melanotic melanoma and in 0 of the 5 patients with the amelanotic tumor type ($p < 0.01$; chi-square test). Furthermore, IBZM accumulation occurred in 0 of the 11 amelanotic metastases but in 20 of the 25 melanotic metastases ($p < 0.001$). Our data suggest that the tracer does not bind to membrane dopamine receptors of the tumor but is built in or closely bound to intracellular melanin.

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Commentary: The purpose of this study was to investigate the effects of retinoid analogues with different retinoid receptor specificity on the growth of human D10 and Cloudman S91 mouse melanoma cells. We compared the growth inhibitory effects with the ability of retinoids to downregulate cell surface expression of the melanocortin receptor (MC1-R). Retinoic acid receptor (RAR)-gamma-selective retinoids exerted the most prominent growth effects, with up to 68% and 69% inhibition in D10 and S91 cells, respectively.

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ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

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

1999 XVIIth International Pigment Cell Conference: Nagoya Congress Center, Japan, October 30 - November 3
Organizer: Prof. S. Ito
E-mail: sito@fujita-hu.ac.jp
Contact: Kazumasa WAKAMATSU, Ph.D.
Secretary-General, IPCC - Nagoya
Fujita Health University School of Health Sciences
J - Toyoake, Aichi 470-1192
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Fax: 81-562-93-4595
Web site: http://www.fujita-hu.ac.jp/IPCCNagoya/
E-mail: kwaka@fujita-hu.ac.jp

2000 IXth Annual Meeting of the PanAmerican Society for Pigment Cell Research College Station, TX, June 25 - 28
Contact: Dr. Lynn Lamoreux
Dept. of Veterinary Pathobiology
The Texas Veterinary Medical Center
Texas A & M University, College Station
TX 77843-4467
Phone: (409)845-6084
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E-mail: llamoreux@cvm.tamu.edu

2000 IXth Annual ESPCR Meeting: Krakow, PL
Contact: Dr T. SARNA
Dept. of Molecular Biology
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Poland - 31 120 Krakow
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Fax: 48-12-336907
E-mail: tsarna@mol.uj.edu.pl

New Members

The ESPCR is delighted to welcome the following colleagues to membership and hope that they will play a full and active part in the Society.

Dr. B. WEHRLE-HALLER
Centre Médical Universitaire
Dept. of Pathology
1 rue Michel-Servet
CH- 1211 Geneva 4

Dr. U. LEITER
University of Ulm
Dept. of Dermatology
40 Oberer Eseslweg
D- 89081 Ulm

Dr. J. HAYCOCK
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!!! NEW !!!

Electronic Application Form for ESPCR membership

Dear Colleague,

A question has been raised by many members concerning the diffusion of information to increase ESPCR membership.

Publishing a blank application form in the Bulletin (here under) is certainly helpful but of limited impact. That's why an electronic application form has been designed and added to the Web site. This facility has numerous advantages, among these a relatively secure way to send the personal information as an electronic mail message. This will help both quickly replying to the sender and an easy transfer of the information to any filing, word-processing programmes ...

So, I would like to invite you to have a look at this new facility (Application for membership section, on the Web page) and eventually send your suggestions. Please, also note that an electronic version of the current bulletin (as well as previous ones) is available in "the members only Web page" at URL: http://www.ulb.ac.be/medecine/loce/espcr.htm

Yours sincerely,

G. Ghanem
ESPCR-Bulletin Editor

MEMBERSHIP OF THE EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH

The ESPCR was founded 12 years ago. The members of the Society share one common denominator - the interest in pigmentation and/or pigment cells. The ESPCR members represent many specific disciplines like chemistry, biochemistry, biophysics, molecular, clinical and developmental biology, genetics, immunology, pathology, oncology, dermatology, pharmacology, epidemiology, etc. This multidisciplinary approach has an important advantage - people can learn from each others' speciality.

The ESPCR organises regular scientific meetings. Regular attendance of the ESPCR Meetings has led to numerous scientific collaborations and friendships. For many of the members the ESPCR has become an indispensible part of scientific life. Together with its sister societies (the Pan-American and the Japanese Society for Pigment Cell Research), the ESPCR forms the International Federation of Pigment Cell Societies (IFPCS), which organises international meetings once in 3 years. The next meeting will be held in September 1999 in Nagoya, Japan as a Joint Meeting with the Japanese Society for Pigment Cell Research and the 9th Annual Meeting of the ESPCR.

The ESPCR membership fee is EUR 48,57 (DM 95.-) and for Ph.D. students and medical residents EUR 24,53 (DM 48.-).

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APPLICATION FORM FOR MEMBERSHIP OF EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH

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