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HAPPY NEW YEAR 2000

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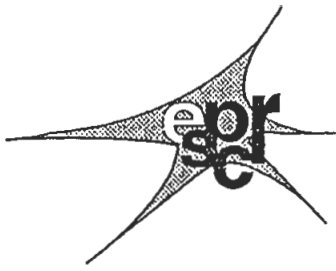

LETTER TO THE EDITOR
DISCUSSION, REVIEW, SHORT COMMUNICATION, ...

INTERNATIONAL FEDERATION OF PIGMENT CELL SOCIETIES

A Letter from the IFPCS President to the ESPCR members

As the chair of the **17th International Pigment Cell Conference (IPCC)** held in Nagoya this fall, I wish to express my sincere thanks to ESPCR and PASPCR members as well as other participants who came a long distance to attend the meeting. We were very pleased to welcome 69 participants from the European continent (13 countries), 71 from the North American continent (2 countries), and 28 from the Asian continent (4 countries), in addition to 139 from Japan. Interest and attendance at the symposia and satellite meetings were great and stimulating discussions were held during and between the formal sessions throughout the meeting. We do believe that most, if not all, of the participants learned new advances in pigment cell research and also enjoyed the social programs.

The outgoing and new members of the **IFPCS** Council held two official meetings during the Nagoya IPCC and elected, as Officers for the next 3 years, Shosuke Ito (JSPCR) *President*, Stan Pavel (ESPCR) *Vice-President* and Richard A. King (PASPCR) *Secretary/Treasurer*. The election was based on the rotation cycle that began 9 years ago when the IFPCS was established at the Kobe IPCC. I am very honored to serve as the President following the successful completion of the terms of the previous 3 Presidents, Drs. Yutaka Mishima, Giuseppe Prota, and Vincent J. Hearing. On behalf of the Officers, I wish to assure you that we will work hard with the Council to continue the growth and interactions initiated during the first 3 IFPCS administrations. We want to congratulate the departing Officers, President Vincent J. Hearing, Vice-President Yoshiaki Hori, and Secretary-Treasurer Patrick A. Riley for their outstanding work and achievements in the last 3 years. Their efforts greatly helped the IFPCS grow and initiate new activities that make the IFPCS more effective in fostering scientific exchange among members. We also would like to welcome new Council members, Drs. Patrick Riley (who served as the Secretary/Treasurer following the untimely death of Dr. Bengt S. Larsson), Richard A. King, and Shigeki Shibahara and express farewell to departing Council members, Drs. Yoshiaki Hori and Yutaka Mishima.

The IFPCS has established the following goals for the Federation (also available on the **IFPCS Web page at http://www.cbc.umn.edu/ifpcs**):
1. To encourage the dissemination of knowledge related to pigment cells by the establishment, sponsorship and support for the publication of books, bulletins, newsletter, journal, reports or other means.
2. To organize a tri-annual international meeting, to honor outstanding contributions in the field by awarding the Myron Gordon award at that meeting, and to select a scientist who has made recent and significant advances in the field to present the Seiji Memorial lecture.
3. To foster and enhance research on pigment cells and pigmentation among the regional Societies and to foster scientific collaboration, cooperation and communication among the regional Societies.

Goal #1 was achieved by establishing an official IFPCS-sponsored journal, ***Pigment Cell Research*** **(http://www.pigment.org)**. Thanks to the efforts of the founding *Editor* Dr. Joseph T. Bagnara (1987-1994) and the subsequent Editors Drs. Takuji Takeuchi (1995) and Jiro Matsumoto (1996-1999), the journal has grown steadily and shown continued improvement to the point that it now has an Impact

Factor score of 1.3. I wish to congratulate Dr. Jiro Matsumoto for his outstanding job in promoting the progress of the journal during his 4-year term as Editor. The journal will now enter a new era under the leadership of Editor Dr. Vincent J. Hearing (2000-2004) who will be supported by a new panel of 15 Associate Editors. To further promote the growth of the journal, it is essential that the numbers of subscribers and submitted papers be increased. I wish to urge all PASPCR members to subscribe to ***Pigment Cell Research,*** as this is the official journal of the Federation. With your help, I am confident that the 5-year editorship of Dr. Hearing will produce even further progress in the rank and value of ***Pigment Cell Research***.

Goal #2 may be the most visible one among the several efforts that the **IFPCS** has been making. Starting with the Nagoya IPCC, the Federation has established the rule that the (International) Scientific Program Committee should include the IFPCS President and 3 Council members, allowing the Local Organizing Committee to work closely with representatives from the Federation in selecting speakers and Symposium topics. As the Chair of the 17th IPCC, I found this system to work very smoothly and effectively. Much of the credit for the scientific success of the Nagoya IPCC should be given to Dr. Hearing and other members of the International Program Committee. As the President of the Federation, I am now looking forward to working closely with the Chair of the 18th IPCC, Dr. Stan Pavel. I am happy to inform you that the venue of the 18th IPCC, to be held in September 2002, is a splendid, five star hotel in Scheveningen on the North Sea coast of the Netherlands.

Goal #3 is being achieved through three related and important initiatives that the IFPCS has taken in the past several years. **Special Interest Groups** have been established and are providing substantial benefits to our scientific community, as shown on our Web page. We now have Special Interest Groups in the subdisciplines of **Biology of Melanoma, Developmental Biology, Genetics of Pigmentation, Hypo/Hyperpigmentation, Ocular/Extracutaneous Pigmentation,** and **Vitiligo.** As the Chair of the 17th IPCC, I was very pleased that three of those groups held Satellite meetings at the IPCC under the themes of 1) Vitiligo: A Manifestation of Apoptosis? 2) Regulation and Genetics of Pigmentary Genes, and 3) Cellular and Molecular Control of Pigment Cell Development. All the satellite meetings were well attended and appreciated. The Federation Council has decided to continue these Interest Groups as a mechanism to promote pigment cell research.

Another initiative to achieve Goal #3 was the establishment of the **IFPCS Visiting Scientist Award.** The grants, established in 1997, are intended to support investigators from one of the regional Societies who wish to visit the laboratory of an investigator in another regional Society to learn specialized techniques and/or to establish collaborations. You will find a full description of the program, the name of generous corporate donors, and the name of awardees on the IFPCS web page. The program was established with funding for 9 visiting scientist awards, with each regional Society being allotted 3 awards for the 3 years' period beginning in 1997. To date 5 individuals have received an award of $3,000 each to cover expenses for their travel and accommodation. The initial 3-year period of the program will end next year, but we hope to continue this program with a renewal of corporate donations.

The third initiative is the establishment of the **InterPig DataBase,** which collects data on research reagents and resources available to the pigment cell community. The database is available to all researchers, especially to members of regional Societies. It includes 115 biochemicals, cell cultures, immunological and molecular biology reagents, and mouse mutants. I hope that ESPCR members will take advantages of the database and will consider adding their new and/or valuable reagents to the database so that these reagents are available to other investigators. This will make the database more useful and will promote pigment cell research collaborations among the scientific community.

Finally, I sincerely hope that we will see healthy and steady progress in our 3 regional Pigment Cell Societies, **ESPCR, JSPCR,** and **PASPCR** as our term on the IFPCS Council extends to the 21st century. I urge each of you to contribute to your Society in any way you can: submitting your abstracts to the regional Society meetings, publishing your papers in the ***Pigment Cell Research,*** collaborating with other members, and recruiting others scientists and clinicians to join us.

Shosuke Ito,
President, IFPCS

2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

A special issue of the Journal of Investigative Dermatology was dedicated to the Proceeding of the Annual Symposium on the Biology of the skin dedicated to the molecular mechanisms of light-induced damage and the effect of light on human skin diseases. Among the paper reported, some reviews are focused on the mechanisms of control of pigmentation and other on the disease of the pigmetary system.. **V. Hearing** summarised the current knowledge on the regulation of mammalian pigmentation at the genetic and biochemical level. Almost half of mammals pigmentation regulatory genes encode proteins that localise, either specifically or non specifically, to melanosomes; mutation in those genes generally lead to phenotypic changes in pigmentation as well as in other pleiotropic changes. The expression and function of these proteins affects both the phenotypic appearance and the melanin properties, especially in their photoprotective characteristics. **I. Suzuki et al** have reported data on the participation of the melanocortin-1 receptor (MC1R) in the mechanism of UV-induced pigmentation trough the stimulation of cAMP formation, which is a principal mechanism for inducing melanogenesis. **B. Gilchrest and M. Eller** reviewed the data on the role of DNA photoproducts in stimulating pigmentation. **R. Setlow**, in considering the UV spectral regions contributing to melanoma, reported the possible role of UVA. **K. Schallreuter and co-workers** reviewed the literature and present new data on oxidative stress in the epidermal compartment during vitiligo. They reported that high H_2O_2 levels can be demonstrated in vivo in the skin of patients with active disease by utilizing Fourier-Transform Raman spectroscopy and that H_2O_2 accumulation was associated with low epidermal catalase levels. The authors conclude that there are several lines of evidence that the entire epidermis of patients with vitiligo is involved in the disease process and that correction of the epidermal redox status is mandatory for repigmentation.

It has recently been shown that cutaneous axon terminals and epidermal melanocytes make contact via chemical synapses in human skin and that calcitonin gene-related peptide (CGRP) induces melanocyte proliferation. To further clarify the effect of neuropeptides on the biology and morphology of melanocytes, especially with respect to melanogenesis and melanocyte dendricity, **Toyoda et al** have exposed organ cultures of normal human skin and cultured melanocytes to various neuropeptides present in intraepidermal nerve endings. Among the neuropeptides examined, CGRP showed to increases melanocyte number, epidermal melanin content, malnosome number and degree of melanization. Their findings suggest that keratinocytes produce and secrete some melanotrophic factors following stimulation with CGRP, which modulate growth, melanin synthesis, and dendricity of melanocytes. For the authors, these data demonstrate intimate interaction between the cutaneous nervous system and melanocytes within the epidermal environment.

Alpha-melanocyte stimulating hormone (alpha-MSH) is produced by several different type including neural cells, entothelial cells, monocytes, and keratinocytes. A biologic role in melanocyte pigmentation is widely recognised, but several recent studies described an its role in modulating inflammatory and immune responses. **J. Haycock et al**, investigate the mechanism by which alpha-MSH was effective in opposing TNFα-stimulated increase in NF-kB DNA binding activity in normal ocular melanocytes; cell cultured from ocular melanoma tumors; and cutaneous melanoma cell lines. The authors showed that alpha-MSH significantly reduced NF-kB DNA binding activity in melanocytes and melanoma cells. Their interesting results suggest that this can be a key pathway by which immunomodulation anti-inflammation may operate.

Funasaka et al have previously reported that advanced melanoma cells generally produce higher amounts of proopiomelanocortin (POMC) peptides than normal melanocytes and that the production correlate with tumor progression. In this study, for elucidate the mechanism of this upregulation, they evaluated the expression of genes encoding corticotropin-releasing hormone (CRH) and its receptor, CRH-R, well as POMC and the MSH receptor (MC1-R), using cultured human melanoma cells, nevus cells , and normal melanocytes. Their results show that all melanocytic cells express CRH, CRH-R, POMC, and MC1-R, with higher intensities in melanoma cells. Furthermore, immunohistochemistry showed that CRH as well as POMC was strongly expressed in advanced melanoma such as vertically growing lesions of acral lentiginous, nodular and metastatic melanomas, in contrast to negative expression in nevus cells. These results indicate tumor progression accentuate CRH, CRH-R, and POMC expression by melanoma cells.

Vitamin A is an intrinsic modulator of proliferation and differentiation in human epidermis, and may be destroyed by ultraviolet radiation (UVR) impinging on the skin. To identify the deleterious effects of a perturbed cellular vitamin A status, **Andersson and co-worker** have investigated the endogenous retinoid concentration and the metabolism of [3H]retinol and all-trans [3H]retinoic acid in cultured human keatinocytes and melanocytes exposed to UVR. Before UVR the retinoid content was similar in keratinocytes and melanocytes, but in both cell types, UVR instantaneously reduced the concentration of retinol and of 3,4-didehyroretinol. The retinoid concentration returned to normal within 1-2 days post-irradiation. However, in both types of irradiated cells, the accumulation of the biologically most active metabolite, all trans [3H]retinoic acid, was higher than in control cells. Furthermore the metabolism of authentically supplied [3H]retinoic acid was reduced, especially in irradiated keratinocytes, which probably contributed to the restoration of retinoid levels after

UV exposure.

K. Schallreuter and J. Wood reported, in cultured melanocytes, a significantly more rapid active transport and autocrine turnover of L-phenylalanine than of L-tyrosine. Moreover, the transport of extracellular L-phenylalanine and its metabolism to L-tyrosine was via phenylalanine hydroxylase and coupled to a calcium-dependent active uptake/efflux, whereas L-tyrosine uptake was calcium independent. According to the authors, these results suggest the importance of an autocrine calcium-dependent active l-phenylalanine uptake/turnover in melanocytes as a major pathway for melanogenesis.

Exposure to phenolic agents contributes to the development occupational vitiligo. Proposed as a causative factor for leukoderma in vivo, the para-substituted phenol 4-tertiary butyl phenol was chosen by **Le Poole and co-worker** to investigate early cellular events responsible for selective disappearance of melanocytes from epidermis of individuals sensitive to such agents. To this end, differential display of melanocyte mRNA isolated from three separate cultures was performed following a 12 h exposure of cells to 4-tertiary butyl phenol or vehicle alone. Alignment analysis revealed that the L30 ribosomial protein was upregulated by the treatment, potentially reflecting altered levels of protein synthesis in response to stress. In addition, a gene sequence upregulated following exposure to 4-tertiary butyl phenol was identified as the A2b receptor (a P1 receptor for adenosine). Flow cytometry confirmed differential expression in melanocytes and fibroblasts. Interestingly, it has been reported that P1 purinoceptor stimulation can induce apoptosis. This is in concordance with results reported demonstrating induction of apoptosis by 4-tertiary butyl phenol in human melanocytes, as well as with morphologic changes observed in this study in cells exposed to 4-tertiary butyl phenol for 72h.

Yang and co-worker have demonstrated that 4-TBP competitively inhibited both tyrosine hydroxylase and dihydrophenylalanine (DOPA) oxidase activities of tyrosinase, i. e., the first two catalytic steps in the biochemical conversion of tyrosinase to melanin in cultured human melanocytes. This inhibition occurred at concentrations that did not influence the viability of melanocytes. Since depigmentation occurred without a cytotoxic response it is unclear whether the interaction between 4-TBP and tyrosinase leads to the destruction of melanocytes in contact/occupational vitiligo.

Sulfur-containing tyrosine analogs such as 4-S-cyteaminylphenol (4-S-CAP) and its N-acetyl derivative, N-acetyl-4-S-CAP, are tyrosinase substrates and can cause selective cytotoxicity and cell death of melanocytes and melanoma cells. It is not clear, however, if the cytotoxicity derives from a cytostatic or cytocidal effect. The latter can also be either apoptotic or necrotic. **Minamitsuji and co-worker** summarize their attempt to clarify the nature of melanocytotoxicity and cell death by using a new derivative of 4-S-CAP, N-propionyl-4-S-CAP , (NPr-CAP). The tyrosinase-mediated cytotoxicity of NPr-CAP was further confirmed by the decreased viability of COS 7 monkey-kidney cells, transfected with human tyrosinase cDNA. NPr-CAP, however, also transiently inhibited the proliferation of melan c cells, a control tyrosinase-negative albino melanocyte line, and vector-transfected COS 7 cells. Thus, the major process of NPr-CAP-mediated melanocytotoxicity involves cytocidal apoptosis associated with active tyrosinase. In addition, there was a transient, non-tyrosinase-mediated cytotoxicity.

Microphtalmia (mi) is a transcription factor that plays a major role in the regulation of growth and function in mast cells and melanocytes. Association of mi with other proteins is a critical step of mi-mediated transcriptional activation. **Razin et al** have found protein kinase C-interacting protein (PKCI) specifically associated with mi in yeast two-hybrid screening. PKCI by itself, although localized in the cytosol and nucleus of the cells, has no know physiological function and did not demonstrate transcriptional activity. Its ability to suppress mi transcriptional activity in the transient transfected fibroblast system suggests that it can function in vivo as a negative regulator of mi-induced transcriptional activation.

In order to study whether mechanical stretching plays a role for human melanocytes, **Kippenberger et al** have established a culture technique to mimic the physical stretching. Primary cultures of human melanocytes were plated on silicon supports which undergo a stretching of about 10% of the initial length. After application of repeated stretching and relaxation for 4 days, cell count was significantly enhanced. In addition, they found 2-fold increase in heat shock protein (HSP) 90, both at the protein and mRNA level. The stretch-mediated up-regulation of HSP90 expression in melanocytes appeared to be independent of stretch-mediated growth stimulation. For the authors, these findings have strong implications for the in vitro cultivation of melanocytes for transplantation purposes.

In mice a molecular motor of the myosin V class (designated myosin Va) is known to be the product of dilute locus, where a mutation prevents melanosome transport in melanocytes. There is conflicting evidence about whether it has a role in dendrite outgrowth. **Edgar and Bennet** have demonstrated its role by transiently transfecting antisense oligonucleotides to inhibit its expression in a melanocyte cell line. Myosin Va protein levels were similar in 3 melanocyte cell lines with differing amounts of pigmentation, indicating that expression of myosin Va is not tightly coupled to expression of melanin. Immunocytochemistry showed 2 types of myosin Va localization. A puntate pattern of staining concentrated in the perinuclear region was indicative of organelle association, and the observation of occasional puntate staining aligned with F-actin bundles supported the idea that myosin Va has a role in trensporting melanosomes along actin filaments. Staining was also intense at tips of dendrites and at sites of dendrite-cell contact, consistent with a possible role in dendrite growth. The authors conclude that in addition to its involvement in melanosome transport, myosin Va has a role in the extension of new dendrites by melanocytes but not in maintenance of pre-existing dendrites.

Rogers et al demonstrate that myosin V is the actin-based motor responsible for melanosome transport. Mobility of melanosomes treated with mitotic extract was found to decrease dramatically, as compared with untreated or interphase

extract treated melanosomes. This mitotic inhibition of mobility correlated with the dissociation of myosin V from melanosomes, whereas the activity of soluble motor remained unaffected. The authors conclude that organelle transport by myosin V is controlled by a cell cycle-regulated association of this motor to organelles, and that this binding is likely regulated by phosphorylation of myosin V during mitotis.

Melanocytes (Mc) and their progenitors melanoblasts (Mb) are derived from the neural crest and migrate along the dorsolateral pathway to colonize the dermis, the epidermis, and finally the hair matrix. To examine the involvement of cadherins in the migration of Mc lineage cells, **Nishimura et al** combined flow cytometric analysis of dissociated live cells with immunohistochemical staining of tissue section to quantify the level of cadherin expression on the surface of Mb/Mc. While most of the epidermal Mb/Mc disappear after the neonatal stage in normal mice, forced expression of steel factor in the epidermis of transgenic mice promotes survival of epidermal Mb/Mc, maintaining epidermal-type cadherin expression pattern (E-cad(high)P-cad(low)) throughout the podtnatal life. These finding indicate the involvement of extrinsic cues in coordinating the cadherin expression pattern and suggest a role for E- and P-cadherins in guiding Mc progenitors to their final destinations.

Slominski et al reported that cultured melanoma cells can synthesize steroids such as corticosterone from progesterone or deoxycorticosterone. Corticosterone production was strongly responsive to deoxycorticosterone substrate addition (12-fold increase), but unresponsive to the adrenal stimulating factors ACTH and angiotensin II.

Francia and co-worker described the occurence of 4 transcripts differentially displayed between syngenic murine B16F10 (metastatic melanoma) and melan-a (immortalised melanocytes) cell line. They report that one such transcript, which is B16F10-specific, represent a protein phosphatase-2A B' regulatory subunit. In situ hybridisation studies on human clinical samples detected high expression of this gene in a number of malignant melanomas. For the authors these results imply strongly that this protein phosphatase-2A regulatory subunit may have a role in melanoma tumor progression.

Ciotti et al have investigated the expression of intercellular adhesion molecule-1 (ICAM-1) and granulocyte-macrophage colony stimulating factor (GM-CSF) in melanoma patients by evaluating fresh biopsy specimens by in situ hybridization and immunochemistry. Most of their metastatic melanoma samples and a few of the primary melanoma lesions showed ICAM-1 expression. The expression of ICAM-1 was significantly ($P<0.01$) higher in metastatic lesions than in primary tumours. GM-CSF mRNA and protein ewre higher detected in 10 of the 18 metastatic samples and in two of the 15 primary lesions. These finding, if confirmed by a wider number of patients, could suggest the prognostic value of the simultaneous, and probably co-ordinated, expression of ICAM-1 and GM-CSF. They also highlight the importance preventive molecular and biochemical characterisation of neoplastic cell cytokine receptors, specifically focusing on the particular cytokine to be used as anticancer therapy and/or as adjunct to chemotherapy.

Growth hormone (GH) exerts its regulatory functions in controlling metabolism, balanced growth and differentiated cell expression by acting on specific receptors, which trigger a phosphorylation cascade resulting in the modulation of numerous signalling pathways, and dictate gene expression. **Lincoln and co-worker** use immunohistochemical techniques to demonstrate the presence of GH receptors in 126 formalin-fixed, paraffin-embedded melanocytic tumours comprising melanocytic naevi, superficial spreading melanoma, nodular melanoma, lentigo melanoma and metastatic melanomas. The relative proportion of positive cells and intensity of staining was higher in neoplastic cells, compared to normal cutaneous cells. In the primary lesions, dermal tumour cells tended to be more immunoreactive relative to those seen in dermal region. Metastatic lesions in various organs also expressed GH receptors in secondary tumour cells and all of the metastatic cases were positive. The expression of GH-receptors in human melanoma cells means that these cells are directly responsive to GH action and that GH may stimulate local production of IGF-I, which then acts in an autocrine mechanism.

Graeven et al assess the expression patterns of vascular endothelial growth factor (VEGF) and its two receptors, flt-1 and KDR, in normal human melanocytes, transformed melanocytes expressing the simian virus 40 Tgene (SV40T), and melanoma cells derived from primary and metastatic lesions. Constitutive expression of VEGF, flt-1 and KDR mRNA and proteins was observed in the majority of primary and metastatic melanoma cell lines, and in SV40T-transformed melanocytes. In summary, co-expression of VEGF and its receptors seems to be a tumor-associated phenomenon in melanoma development. However VEGF production did not support autocrine proliferation of the melanoma cell lines studied.

Dipeptidyl peptidase IV (DPPIV) is a cell surface peptidase expressed by normal melanocytes, epithelial cells, and other cells. Malignant cells, including melanomas and carcinomas, frequently lose or alter DPPIV cell surface expression. Loss of DPPIV expression occuring during melanoma progression at a stage where they transformed melanocytes become independent of exogenous growth factors for survival. **Wesley and co-worker** suggest that rescue of fibroblast activation protein alpha, which can form a heterodimer with DPPIV, may play a role in regulation growth of melanocytic cells. These results support the view that down-regulation of DPPIV is an important early event in the pathogenesis of melanoma.

Macrophage migration inhibitory factor (MIF) is known to function as a cytokine, hormone, and glucocorticoid-induced immunoregulator. **Shimizu et al** have reported for the first time that human melanocytes and melanoma cells express MIF mRNA and produce MIF proteins. To assess the role of MIF overexpression in melanoma cells, G361 cells, a widely available human melanoma cell line, were transfected with an antisense human MIF plasmid. Their results demonstrate that the cell growth rate of the transfected cells was markedly suppressed, suggesting that MIF participates in the

mechanism of proliferation of melanoma cells. Further more, the administration of anti-MIF antibody significantly suppressed tumor-induced angiogenesis. The authors suggest that MIF may function as a novel growth factor that stimulate incessant growth and invasion of melanoma concomitant with neovascularization.

Ephrin-A1, a new melanoma growth factor, is angiogenic and chemoattractant for endothelial cells. EPH-A2, or ECK (a receptor for ephrin-A1), is ectopically expressed in most melanoma cell lines; the possible role of the receptor in tumor progression are unknown. **Easty et al** have studied the expression of this ligand and receptor in biopsies of benign and malignant melanocytic lesions. Their findings are consistent with 2 possible roles for ephrin-A1 in melanoma development: it may promote melanocyte growth or survival and induce vascularization in advanced melanomas. Both effects may be potentiated by inflammatory responses.

Activation of the endothelial receptor B (ETRB) in cultured melanocyte precursors promotes cell proliferation while inhibiting differentiation, two hallmarks of malignant transformation. **Lahav et al** have tested whether ETRB has a similar role in malignant transformation of melanoma. When tested in culture, they find that the selective ETRB antagonist BQ788 can inhibit the growth of seven human melanoma cell lines. Extending these study in vivo, they find that administration of BQ788 significantly slows human melanoma tumor growth in nude mice. Thus, the authors suggest that ETRB inhibitors may be beneficial for the treatment of melanoma.

Sauder reports the expression of p53, p16, and Bcl in malignant melanoma arriving in benign nevi. P53 immunoreactivity was found only in the malignant component, with no expression being seen in the benign components of the lesions. In opinion of the author, this suggests that this tumour suppressor gene is involved in the pathogenesis of melanoma

Pavey et al have demonstrated the existence of a UV-induced response pathway involving up-regulated p16 expression may provide a mechanism linking the loss of p16 and UV exposure with the development of melanoma.

The Cdc2L locus encoding the PITSLRE protein kinases maps to chromosome band 1p36 and consists of two duplicated and tandemly linked genes. **Ariza and co-worker** have determined whether diminution of PITSLRE kinases leads to deregulation of apoptosis. The authors suggest that alterations in PITSLRE gene expression and protein localization may result in the loss of apoptotic signaling.

It is not known if immune response to T cell-defined human histocompatibility leukocyte antigen (HLA) class I-restricted melanoma antigens leads to an expanded peripheral pool of T cells in all patients, affects cytotoxic T lymphocyte (CTL) generation, and correlates with anti-tumor response in metastatic lesions. To this end, **Anichini and co-worker** were developed a limiting dilution analysis technique that allowed to evaluate the same frequency of peptide-specific T cells as by staining T cells with HLA-peptide tetrameric complexes. They demonstrate that expansion of peripheral immune repertoire to melan-A/Mart-1 takes place in some metastatic patients and leads to enhanced CTL induction after antigen-presenting cell-mediated selection, but, in most metastatic lesions, it does not overcome tumor escape from immune surveillance.

Picardo et al have tested the existence of a correlation between antioxidant and phototypes in melanocytes cultures. Their results suggest that the different pattern of antioxidants and of membrane fatty acids of melanocytes is correlated with their physiologic response to UV light. The decreased antioxidant enzyme activities and the increased percentage of polyunsaturated fatty acids in the membranes can be considered as adjunctive risk factors for people with low photoype when exposed to a level of UV light higher than in their original region.

Blasi et al investigate the antioxidant status of cultured uveal melanocytes from patients with uvela melanoma and uveal melanoma cells to characterize some of the biochemical properties of these cells in respect to the normal cutaneous melanocytes. Their results show a different pattern of antioxidants in uveal melanocytes with respect cutaneous ones, possibly related to their anatomic distribution. However two subgroups were identified on the basis of the antioxidant pattern that could be the expression of a constitutional increased susceptibility to oxidative stress in some subjects. Moreover, an imbalance of the antioxidants was observed in melanoma cells, possibly related to the disease status and progression.

The melanocytes in the mammalian eye have been thought to produce melanin only during fetal development and in the very young individual; recent discovery that latanoprost a protaglandin analogue used in the treatment of glaucoma, causes increased pigmentation of the iris in monkeys and humans indicates that the iridial melanocytes can produce melanin in adult individuals. **Lindquist et al** have observed that in the iris of the treated eye the only difference from the untreated eye was an increased amount of melanin in these cells. Thus it seem likely that treatment with latanoprost in some individuals cause an increase of the low normal melanin synthesis in iridial melanocytes.

Grossniklaus et al have demonstrate that a subset of melanocytic proliferations of the conjunctiva exists that cannot be reproducibly classified by pathologists as benign, malignant, or indeterminate.

Ichimiya et al report that a sex steroid-thyroid hormone (Metharmon-F; MF) was a potent drug for treatment of vitiligo. The efficacy of MF treatment of vitiligo was proven to be due to the stimulatory effect of melanocyte proliferation and melanin production via alpha-MSH.

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Calcitonin gene-related peptide upregulates melanogenesis and enhances melanocyte dendricity via induction of keratinocytes-derived melanotrophic factors. J Invest Dermatol Symp Proc, 4:116-25, 1999.

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A role for dipeptidyl peptidase IV in suppressing the malignant phenotype of melanocytic cells. J Exp Med, 190: 311-22, 1999.

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Effects of 4-tertiary butylphenol on the tyrosinase activity in human melanocytes. Pigment Cell Res, 12: 237-45, 1999.

3. MSH, MCH, other hormones, differentiation

(Dr. B. Loir)

- Dennis JW., Granovsky M., Warren CE.
Glycoprotein glycosylation and cancer progression. Biochim. Biophys. Acta 1473(1):21-34, 1999.
Shortened abstract: The authors review evidence that beta1,6GlcNAc-branching of N-glycans contributes directly to cancer progression, and they consider possible functions for the glycans. "Mgat5 encodes N-acetylglucosaminyltransferase V (GlcNAc-TV), the Golgi enzyme required in the biosynthesis of beta1,6GlcNAc-branched N-glycans. Mgat5 expression is regulated by RAS-RAF-MAPK, a signaling pathway commonly activated in tumor cells. Ectopic expression of GlcNAc-TIII, an enzyme that competes with GlcNAc-TV for acceptor, suppresses metastasis in B16 melanoma cells".

- Funasaka Y., Sato H., Chakraborty AK., Ohashi A., Chrousos GP., Ichihashi M.
Expression of proopiomelanocortin, corticotropin-releasing hormone (CRH), and CRH receptor in melanoma cells, nevus cells, and normal human melanocytes. J. Investig. Dermatol. Symp. Proc. 4(2):105-9, 1999.
Shortened abstract: The authors have "previously showed that normal human melanocytes produce and secrete alpha-MSH and ACTH, and furthermore, that advanced melanoma cells generally produce higher amounts of POMC peptides that correlate with tumor progression". Using reverse transcriptase-polymerase chain reaction, they show in this paper that "all melanocytic cells (cultured human melanoma cells, nevus cells, and normal melanocytes) express CRH, CRH-R, POMC, and MSH receptor (MC1-R), with highest intensities in melanoma cells. Furthermore, immunohistochemistry shows that CRH as well as POMC is strongly expressed in advanced melanomas, such as vertically growing lesions of acral lentiginous, nodular and metastatic melanomas, in contrast to negative expression in nevus cells. These results indicate that tumor progression accentuates CRH, CRH-R, and POMC expression by melanoma cells".

- Graeven U., Fiedler W., Karpinski S., Ergun S., Kilic N., Rodeck U., Schmiegel W., Hossfeld DK.
Melanoma-associated expression of vascular endothelial growth factor and its receptors FLT-1 and KDR. J. Cancer Res. Clin. Oncol. 125(11):621-9, 1999.
Summary: "Coexpression of VEGF and its receptors is a tumor-associated phenomenon in melanoma development. However VEGF production does not support autocrine proliferation of the melanoma cell lines tested".

- Haycock JW., Wagner M., Morandini R., Ghanem G., Rennie IG., Mac Neil S.
Alpha-melanocyte-stimulating hormone inhibits NF-kappaB activation in human melanocytes and melanoma cells. J. Invest. Dermatol. 113(4):560-6, 1999.
Commentary: The authors have showed that "α-MSH has a pronounced effect on NF-kappaB activity in melanocytes and melanoma cells, identifying a specific dimeric complex, and suggest this to be a key pathway by which immunomodulation/anti-inflammation may operate".

A complementary paper about the central action of α-MSH to inhibit peripheral NF-kappaB activation was published on the same month (october 1999), in the following reference:
Ichiyama T., Sakai T., Catania A., Barsh GS., Furukawa S., Lipton JM.

Inhibition of peripheral NF-kappaB activation by central action of alpha-melanocyte-stimulating hormone. J. Neuroimmunol. 99(2):211-7, 1999.

- Ichimiya M.
  Immunohistochemical study of ACTH and alpha-MSH in vitiligo patients successfully treated with a sex steroid-thyroid hormone mixture. J. Dermatol. 26(8):502-6, 1999.
  Summary: This immunohistochemical analysis was performed on samples from five patients with generalized vitiligo successfully treated with oral administration of Metharmon-F (MF). Immunoreactivity to alpha-MSH in melanocytes became much stronger after the treatment, than before the treatment. However, the immunoreactivity to ACTH in melanocytes (both before and after the treatment) was minimal and there was little significant difference in these immunoreactivities in keratinocytes between the depigmented lesions before treatment and the repigmented lesion after treatment. The efficacy of MF in treatment of vitiligo was proven to be due to the stimulatory effect of melanocyte proliferation and melanin production via alpha-MSH.

- Jansen B., Schlagbauer-Wadl H., Kahr H., Heere-Ress E., Mayer BX., Eichler H., Pehamberger H., Gana-Weisz M., Ben-David E., Kloog Y., Wolff K.
  Novel Ras antagonist blocks human melanoma growth. Proc. Natl. Acad. Sci. USA 96(24):14019-14024, 1999.

- Rachkovsky M., Pawelek J.
  Acquired melanocyte stimulating hormone-inducible chemotaxis following macrophage fusion with Cloudman S91 melanoma cells. Cell Growth Differ. 10(7):517-24, 1999.
  Shortened abstract: "Fusion of Cloudman S91 melanoma cells with macrophages results in hybrids with increased metastatic potential". The authors report that such hybrids acquire new pathways for motility, including an acquired melanocyte-stimulating hormone-inducible motility, perhaps reflecting the FN fragment chemotaxis of macrophages. Their "results support a long-standing hypothesis that metastasis is initiated following hybridization between tumor-invading phagocytes and cells of the primary tumor".

- Shimizu T., Abe R., Nakamura H., Ohkawara A., Suzuki M., Nishihira J.
  High expression of macrophage migration inhibitory factor in human melanoma cells and its role in tumor cell growth and angiogenesis. Biochem. Biophys. Res. Commun. 264(3):751-8, 1999.

4. Photobiology

(Dr. E. Wenczl)

- Bacharach-Buhles M, Lubowietzki M, Altmeyer P.
  Dose -dependent shift of apoptotic and unaltered melanocytes into the dermis after irradiation with UVA 1. Dermatology. 198:5-10, 1999.

- Bessou-Touya S, Morichon F, Surleve-Bazeille JE, Bioulac-Sage P, Pain C, Taieb A.
  An ex vivo study of congenital pigmented nevi in epidermal reconstructs. Pigment Cell Res. 12:164-174, 1999.
  Summary: The authors have demonstrated that it was possible to maintain ex vivo nevus cells from congenital pigmented nevi and to produce their 3-dimentional in vivo organization. In the described model UVB exposure induced an upwards migration of nevus cells in the suprabasal layers of the epidermis.

- Eves P, Smith-Thomas L, Hedley S, Wagner M, Balafa C, Mac Neil S.
  A comparative study of the effect of pigment on drug toxicity in human choroidal melanocytes and retinal pigment epithelial cells. Pigment Cell Res. 12:22-35, 1999.
  Summary: Three drugs known to induce toxicity in the eye, tamoxifen, chloroquine and thioridazine, were used to assess the sensitivity of cells to xenobiotic drugs. UVB irradiation was used to achieve an increase in the pigment content of the cells. Retinal pigment epithelial cell were shown to be much more resistant to the effects of all three drugs than the choroidal melanocytes and this resistance appeared to be largely independent of the presence of pigment. Further the results illustrated that it is inadvisible to generalise about drug binding to melanin.

- Gellardon FM, Moll I, Meyer M, Michaelidid TM.
  Alterations in cell death and cell progression in the UV-irradiated epidermis of bcl-2-deficient mice. Cell Death and differentiation. 6:55-60, 1999.
  Summary: The findings of this in vivo and in vitro study suggest that effects of UVB irradiation on epidermal cell death and cell cycle progression are influenced by survival-promoting Bcl-2.

- Gilchrest BA, Eller MS, Geller AC, Yaar M.
  The pathogenesis of melanoma induced by ultraviolet radiation. N Engl J Med. 340:1341-1348, 1999.
  Summary: It is an interesting review that hypothesize the photoprotective role of melanocytes in the UV-induced pathomechanism of melanoma.

- Haapajarvi T, Pitkanen K, Laiho M.
Human melanoma cell line UV responses show independency of p53 function. Cell Growth and Differentiation. 10:163-71, 1999.
Summary: UV radiation-induced mutation of the p53 gene is suggested as a causative event in skin cancer, including melanoma. The results of this study performed on different melanoma cell lines suggest that in melanoma several p53 regulatory steps are dislodged; its basal expression is high, its activation in response to UVC damage is diminished and the regulation of its target genes p21Cip1/Waf1 and GADD45 are dissociated from p53 regulation.

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The human melanocyte as a particular target for UVA radiation and an endpoint for photoprotection assessment. Photochem Photobiol. 69:686-693, 1999.
Summary: The induction of DNA breaks by UVA in the nucleus of normal cultured human melanocytes was investigated using the comet assay. Endogenous pigment and/or melanin-related molecules were found to enhance DNA breakage. After UVA doses where strong comets were observed, neither cytotoxicity nor stimulation of tyrosinase activity were detected. However, the accumulation of p53 protein suggested that cells reacted to genotoxic stress under these genotoxic conditions. The same approach was used to compare two sunscreens with indentical sun protection factors but different UVA protection factors.

- Welsh BM, Mason RS, Halliday GM.
Topical all-trans retinoic acid augments ultraviolet radiation-induced increases in activated melanocyte numbers in mice. J Invest Dermatol. 112:271-278, 1999.
Summary: The authors have shown retinoic acid (RA) to augment both constitutive and UVR induced pigmentation in the lightly pigmented HRA:Skh-2 murine model. The mode of action of RA in this system is unknown, but RA and UVR needed to be applied on the same site and UVB was effective whereas UVA was not. It is probable that complex synergistic interactions are occuring between UVR, RA and keratinocyte-derived paracrine factors to enhance tyrosinase activity and cell division in these quiescent melanocytes.

5. Neuromelanins

(Prof. M. d'Ischia)

The binding of metal ions to neuromelanin as a possible underlying cause of neurotoxicity and neuronal degeneration continues to be an active focus of research during the last months of 1999. The role of iron and its pathophysiological relevance to Parkinson's disease was addressed by Bridelli et al. who reinvestigated the structure of human neuromelanin and synthetic analogues and succeded in demonstrating by IR techniques that iron binding involves phenolic OH groups in human neuromelanin and both phenolic OH and NH indolic groups in synthetic melanin. The presence of an aliphatic component specific to neuromelanin was also confirmed, in accord with the hetereogeneous nature of the pigment. Along the same line of thought, Kienzl et al. used energy dispersive X-ray electron microscopy analysis to confirm an abnormal iron accumulation in the melanized neurons of the substantia nigra pars compacta in patients with Parkinson's disease. A mechanisms of iron/neuromelanin-dependent cytotoxicity via continuous production of reactive oxygen species was proposed. In another paper, Meglio et al.
reported a melanin-dependent enhancement of lipid peroxidation in liposomes and caudate putamen homogenates induced by Al3+ ions. The enhancing effect was shown to depend on interaction with superoxide ions produced by autoxidation of melanin, and is therefore consistent with a prooxidant mechanism basically different from that of Fe and other redox active metals.
Two related studies from the same group on the role of catecholamine metabolism in major depression (Zhu et al., Ordway et al.) indicated that tyrosine hydroxylase, but not monoamine oxidase, was overexpressed in the locus coeruleus of depressive patients. The distribution of tyrosine hydroxylase-like immunoreactivity in the locus coeruleus was uneven and was paralleled by a similar distribution of neuromelanin-containing cells in normal and depressive subjects. It was suggested that in major depressive patients there is either an overactivation of locus coeruleus neurons or a deficiency in norepinephrine levels.
Using in situ hybridization histochemistry Miller et al. demonstrated, inter alia, that there is a significant increase in the percentage of neuromelanin-pigmented cells that coexpress galanin, an inhibitory modulator of cholinergic and noradrenergic neurotransmission, in patients with Alzheimer's disease. The role of galanin in Alzheimer's disease is discussed.

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The structure of neuromelanin and its iron binding site studied by infrared spectroscopy. FEBS Lett. 20;457(1):18-22, 1999.

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Iron as catalyst for oxidative stress in the pathogenesis of Parkinson's disease? Life Sci. 65(18-19):1973-6, 1999.

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Aluminum enhances melanin-induced lipid peroxidation. Neurochem Res. 24(8):1001-8, 1999.

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Preservation of noradrenergic neurons in the locus ceruleus that coexpress galanin mRNA in Alzheimer's disease. J Neurochem. 73(5):2028-36, 1999.

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Quantitative distribution of monoamine oxidase A in brainstem monoamine nuclei is normal in major depression. Brain Res. 13;847(1):71-79, 1999.

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6. Genetics, molecular biology

(Dr. F. Beermann)

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Induction of melanoma in TPras transgenic mice. Carcinogenesis 20(9):1747-1753, 1999.

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Inhibition of dendrite formation in mouse melanocytes transiently transfected with antisense DNA to myosin Va. Journal of Anatomy 1999.

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An endothelin receptor B antagonist inhibits growth and induces cell death in human melanoma cells in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America 96(20):11496-11500, 1999.

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An agouti mutation lacking the basic domain induces yellow pigmentation but not obesity in transgenic mice. Proceedings of the National Academy of Sciences of the United States of America 96(15):8579-8584, 1999.

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Characterization of the promoter region of the human melanocortin-I receptor(MC1R) gene. Biochemical & Biophysical Research Communications 262(2):452-460, 1999.

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The mahogany protein is a receptor involved in suppression of obesity. Nature 398(6723):148-152, 1999.

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Expression of the microphthalmia-associated basic helix-loop-helix leucine zipper transcription factor mi in avian neuroretina cells induces a pigmented phenotype. Cell Growth & Differentiation 10(7):525-536, 1999.

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7. Tyrosinase, TRP1, TRP2 and other enzymes

(Prof. J.C. Garcia-Borron)

The number of papers dealing with the posttranslational events associated to the maturation, transport and intracellular degradation of the melanogenic enzymes is increasing steadily. Since the experimental techniques to approach these issues are progressively more refined and powerful, the relevance of the results is also raising.
Branza-Nichita et al (Biochem-Biophys-Res-Commun. 11; 261(3): 720-5) have investigated the folding of tyrosinase as related to calnexin. The correct interaction with the chaperone is reported to be necessary for proper folding and copper binding. Thus, any situation interfering with this interaction might be expected to decrease the amount of active enzyme. Calvo et al. (J-Biol-Chem. 30; 274(18): 12780-9) and Simmen et al. (J-Cell-Sci. 112 (Pt 1): 45-53) have analyzed the sorting signals responsible for delivery of tyrosinase to the melanosomes/lysosomes. Both studies emphasize the crucial role of a dileucine motif in correct sorting. Interestingly, the paper by Calvo and coworkers raises the possibility of a specific melanosomal pathway in melanocytes. Further studies in this direction are needed to fully understand the differences between melanosomal and lysosomal sorting. Finally, Ando et al. (J-Lipid-Res. 40(7): 1312-6) describe the regulation of tyrosinase intracellular stability by fatty acids. Linoleic acid increases the rate of degradation of tyrosinase, while palmitic acid stabilizes the enzyme in the B16 model. This effect seems specific in that it is not observed for TRP1 and TRP2.
All these observations underscore the importance of posttranslational regulatory events on melanogenesis. It is becoming evident that the regulation of the levels and activity of the melanogenic enzymes cannot be explained exclusively in terms of translational control.

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8. Melanosomes (New Topic)

(Dr. J. Borovansky)

This newly introduced section will be devoted to melanosomes. Melanosome is a term commonly used in geology (where it refers to the darker-coloured portions of migmatite rocks - see e.g. Mehnert K.R.: Migmatites and the origin of granitic rocks, Elsevier, Amsterdam 1968 or Berger A ,Kalt A. J.Petrology 40(11): 1699-1719, 1999) and in life sciences. Our attention wil be, of course, focused on melanosomes in the latter sense. These subcellular particles have an interesting biogenesis, fate so far unexplained in terms of molecular events, typical ultrastructure and specific chemical composition. They represent the site of melanogenesis including production of potentially cytotoxic species. Melanosomes play multiple physiological and pathological roles (absorption and conversion of energy, involvement in redox and free radical reactions, ion exchange capacity, affinity to various compounds) and they display unique antigens. All these properties can be exploited in practice, particularly in the diagnosis and treatment of malignant melanoma. The demonstration of melanosomes presence is also important in differential diagnostic meditations in human and veterinary pathology.

- Bhatnagar V, Ramaiah A.
  Characterization of Mg^{2+}-ATPase activity in isolated B16 murine melanoma melanosomes. Mol Cell Biochem. 189(1-2):99-106, 1998.
  Comments: ATPase activity may be related to melanosomal proton pump.

- Borovanský J, Hach P, Smetana K, Elleder M, Matouš-Malbohan I.
  Attempts to induce melanosome degradation in vivo. Folia Biologica 45(2):47-52, 1999.
  Comments: Melanosomes devoid of their limiting membranes are not prone to extensive disintegration in vivo. Hydrolytic reactions are not involved in the destruction of their pigment moiety.

- Kendereski A, Micic D, Sumarac M, Zoric S, Macut D, Colic M, Skaro-Milic A, Bogdanovic Z.
  White Addison's disease: What is the possible cause? J Endocrinol Invest 22(5):395-400, 1999.
  Comments: Absence of hyperpigmentation is explained by high degree melanosome degradation in secondary lysosomes.

- Kim IT, Choi JB.
  Melanosomes of retinal pigment epithelium - distribution, shape, and acid phosphatase activity. Korean J Ophthalmol 12(2):85-91, 1998.
  Comments: Evidence of melanosome synthesis in adult eyes and of their association with acid phosphatase.

- Lindquist NG, Larsson BS, Stjernschantz J, Sjoquist B.
Age-related melanogenesis in the eye of mice, studied by microautoradiography or H-3-methimazole, a specific marker of melanin synthesis. Experimental Eye Res 67(3):254-264, 1998
Comments: Evidence that new melanosomes are formed in iridial pigment cells in adult mice.

- Matsumoto Y, Horiba K, Usuki J, Chu SC, Ferrans VJ, Moss J.
Markers of cell proliferation and expression of melanosomal antigen in lymphangioleiomyomatosis. Am J Resp Cell Molec Biol 21(3):327-336, 1999.
Comments: In 3 human melanoma cell lines HMB45 reacted with melanosomes, whereas in pulmonary lymphangioleiomyomatosis cells the binding sites for HMB45 were located in cytoplasmic granules resembling immature melanosomes.

- Orlow SJ, Brilliant MH.
The pink-eyed dilution locus controls the biogenesis of melanosomes and levels of melanosomal proteins in the eye. Experimental Eye Res 68(2):147-154, 1999.
Comments: Mutations at the p locus affect the size, number, shape and contents of melanosomes.

- Osthold W, Beck J.
Black Hair Follicular Dysplasia in a mixed-bree dog. Kleintierpraxis 44(9):685, 1999.
Comments: Case report of a disease characterized by clumping of macromelanosomes.

- Park HY, Perez JM, Laursen R, Hara M, Gilchrest BA.
Protein kinase C-beta activates tyrosinase by phosphorylating serine residues in it cytoplasmic domain. J Biol Chem 274(23):16470-16478, 1999.
Comments: PKC-beta is closely associated with tyrosinase on the outer surface of melanosomes.

- Potterf SB, Virador V, Wakamatsu K, Furumura M, Santis C, Ito S, Hearing VJ.
Cysteine transport in melanosomes from murine melanocytes. Pigment Cell Res 12(1):4-12, 1999.
Comments: Demonstration of cysteine transport by a carrier-mediated process into melanosomal fraction.

- Rege JD, Shet D, Sawant HV, Naik LP.
Cytologic diagnosis of a melanotic neuroectodermal tumor of infancy occurring in the cranial bones. Diagnostic Cytopathology 21(4):280-283, 1999.
Comments: Tumor consists of neuroblast-like cells admixed with large epitheloid cells containing melanosomes.

- Schepis C, Siragusa M, Gagliardi ME, Torre V, Cicciarello R, Albiero F, Cavallari V.
Primary macular amyloidosis: An ultrastructural approach to diagnosis. Ultrastructural Pathology 23(5):279-284, 1999.
Comments: Melanosome aggregates were consistently observed in macrophages and in Schwann cells.

- Spritz RA.
Multi-organellar disorders of pigmentation; tied up in traffic. Clin Genetics 55(5):309-317, 1999.
Comments: Review summarizing clinical manifestations of syndromes associated with defects of melanosomes, lysosomes and cytoplassmic granular elements with an effort to explain the molecular basis of these disorders and with emphasis to the AP3-mediated pathway of organelle-specific protein biogenesis and trafficking.

- Stout PR, Ruth JA.
Deposition of [H-3]cocaine, [H-3]nicotine, and [H-3]flunitra-zepam in mouse hair melanosomes after systemic administration. Drug Metabolism and Disposition 27(6):731-736, 1999.
Comments: Microautoradiographic study exploiting melanosomal affinity and aiming at a better basis for using hair for analyses of drug and environmental toxin exposure.

- Vancoillie G, Lambert J, Naeyert JM.
Melanocyte biology and its implications for the clinician. Europ J Dermatol. 9(3):241-251, 1999.
Comments: Concise but highly didactic review in an excellent graphic arrangement covering all the aspects of melanosomes.

- Wu XF, Bowers B, Rao K, Wei Q, Hammer JA.
Visualization of melanosome dynamics within wild-type and dilute melanocytes suggests a paradigm for myosin V function in vivo. J Cell Biol 143(7):1899-1918, 1998.
Comments: Bidirectional, microtubule-dependent melanosome movement coupled with actomyosin Va dependent capture of melanosomes in the periphery is the predominant mechanism responsible for the centrifugal transport and peripheral accumulation of melanosomes in melanocytes.

9. Melanoma experimental, Cell culture

(Dr. N. Smit)

A: Drug toxicity

The paper by Glass-Marmor et al shows the effects of the Ca^{2+} ionophore A23187 and the importance of the levels of free Ca^{2+} in melanoma cells. Accumulation of intracellular-free Ca^{2+} was considered to cause a reduction in the energy-producing systems of the cell, leading to melanoma cell death. This could be an indication that a proper regulation of the energy production may be of great importance for the viability of melanoma cells but also for melanocytes in general.
Plant indole alkaloids have been tested by Keawpradub et al for their toxicity towards different cancer cell lines and fibroblasts. No discernible cell-type specificity among the cancer cell lines (including StMI1 1a melanoma) was found.
Davol et al have used the FGF receptor on melanoma cells for targeting bFGF-saporin and used this in combination with suramin resulting in 68% tumour growth inhibition compared to controls in a model of beige mice bearing SK-Mel-5 human melanoma xenografts. In the study by Lahav et al the presence of the endothelin receptor B (ETRB) on melanoma cell lines was utilized and the ETRB antagonist BQ788 was found to inhibit growth of seven melanoma lines.
Minamitsuji et al describe that the cytotoxic effects of tyrosine analogs such as 4-S-cysteaminylphenol (4-S-CAP) and its derivative N-propionyl-4-S-CAP (NPr-CAP) are tyrosinase mediated. Differences between the toxicities towards the (tyrosinase +) melan-a2 and (tyrosinase-) melan-c melanocyte cultures and tyrosinase transfected COS cells and the controls are shown. Hasegawa et al have shown already in 1997 (Bioch. Pharmacol. 53, 1435-44) that the dihydro-1,4-benzothiazine-1,6-dione (BQ) is the ultimate toxic product of 4-S-CAP. The importance of GSH adduct formation and free radical mediated toxicity of these compounds is indicated in both the papers. The present paper by Minamitsuji now shows that this may even trigger apoptosis in these cells. In the paper by Pervais et al it is described that the apoptosis induced in e.g. M14 melanoma cells by two different merocyanine 540 photoproducts is mediated by caspase 8. Different mechanisms for the release of mitochondrial cytochrome C by the two products were found.
Wang et al show that the enzyme DT-diaphorase may be an interesting target enzyme in melanoma and other tumours since it has been shown to be elevated in different tumour tissues. The bioactivation of anti-tumour agents such as mitomycin-C by this enzyme and possibilities to further increase its activity may be utilized for specific induction of cytotoxicity in various tumour cells.

- Breathnach AS.
  Azelaic acid: potential as a general antitumoural agent. Med. Hypotheses. 52(3):221-6, 1999.

- Cree IA, Neale MH, Myatt NE, de Takats PG, Hall P, Grant J, Kurbacher CM, Reinhold U, Neuber K, MacKie RM, et al.
  Heterogeneity of chemosensitivity of metastatic cutaneous melanoma. Anticancer Drugs. 10(5):437-44, 1999.
  Comments: We have examined the heterogeneity of chemosensitivity in metastatic cutaneous melanoma specimens using an ex vivo ATP-based chemosensitivity assay (ATP-TCA). The degree of heterogeneity observed suggests that the ATP-TCA could be used to select patients who might benefit from specific chemotherapeutic agents alone or in combination.

- Davol PA, Garza S, Frackelton ARJ.
  Combining suramin and a chimeric toxin directed to basic fibroblast growth factor receptors increases therapeutic efficacy against human melanoma in an animal model. Cancer. 86(9):1733-41, 1999.

- Glass-Marmor L, Penso J, Beitner R.
  Ca2+-induced changes in energy metabolism and viability of melanoma cells. Br. J. Cancer. 81(2):219-24, 1999.

- Hamanaka H, Mizutani H, Asahig K, Shimizu M.
  Melanocyte melanin augments sparfloxacin-induced phototoxicity. J. Dermatol. Sci. 21(1):27-33, 1999.

- Jackson TL, Lubkin SR, Siemers NO, Kerr DE, Senter PD, Murray JD.
  Mathematical and experimental analysis of localization of anti-tumour antibody-enzyme conjugates. Br. J. Cancer. 80(11):1747-53, 1999.

- Jackson TL, Lubkin SR, Murray JD.
  Theoretical analysis of conjugate localization in two-step cancer chemotherapy [In Process Citation]. J. Math. Biol. 39(4):353-76, 1999.
  Comments: A promising two-step approach that is designed to minimize systemic drug toxicity while maximizing activity in tumours employs monoclonal antibody (mAb)-enzyme conjugates for the activation of anticancer prodrugs.

- Keawpradub N, Eno-Amooquaye E, Burke PJ, Houghton PJ.
  Cytotoxic activity of indole alkaloids from Alstonia macrophylla. Planta Med. 65(4):311-5, 1999.

- Klein JL, Roberts JD, George MD, Kurtzberg J, Breton P, Chermann JC, Olden K.
  Swainsonine protects both murine and human haematopoietic systems from chemotherapeutic toxicity. Br. J. Cancer. 80(1-2):87-95, 1999.

Comments: We demonstrate that swainsonine protects C57BL/6 mice bearing melanoma-derived tumours from cyclophosphamide-induced toxicity without interfering with the drug's ability to inhibit tumour growth.

- Lahav R, Heffner G, Patterson PH.
An endothelin receptor B antagonist inhibits growth and induces cell death in human melanoma cells in vitro and in vivo. Proc. Natl. Acad. Sci. U.S.A. 96(20): 11496-500, 1999.

- Li D, Yee JA, Thompson LU, Yan L.
Dietary supplementation with secoisolariciresinol diglycoside (SDG) reduces experimental metastasis of melanoma cells in mice. Cancer Lett. 142(1):91-6, 1999.
Comments: The effect of dietary supplementation with secoisolariciresinol diglycoside (SDG), a lignan precursor isolated from flaxseed, on experimental metastasis of B16BL6 murine melanoma cells in C57BL/6 mice was investigated.

- McDevitt TC, Nelson KE, Stayton PS.
Constrained cell recognition peptides engineered into streptavidin. Biotechnol. Prog. 15(3):391-6Other, 1999.

- Minamitsuji Y, Toyofuku K, Sugiyama S, Yamada K, Jimbow K.
Sulfur containing tyrosine analogs can cause selective melanocytotoxicity involving tyrosinase-mediated apoptosis. J. Investig. Dermatol. Symp. Proc. 4(2):130-6, 1999.

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Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA). Int. J. Cancer. 81(6):918-22, 1999.

- Pervaiz S, Seyed MA, Hirpara JL, Clement MV, Loh KW.
Purified photoproducts of merocyanine 540 trigger cytochrome C release and caspase 8-dependent apoptosis in human leukemia and melanoma cells. Blood 93(12):4096-108, 1999.

- Qiu L, Kelso MJ, Hansen C, West ML, Fairlie DP, Parsons PG.
Anti-tumour activity in vitro and in vivo of selective differentiating agents containing hydroxamate. Br. J. Cancer. 80(8):1252-8, 1999.
Comments: A series of hydroxamates, which are not metalloprotease inhibitors, have been found to be selectively toxic to a range of transformed and human tumour cells without killing normal cells (fibroblasts, melanocytes) at the same concentrations. Two hydroxamates inhibited growth of xenografts of human melanoma cells in nude mice; resistance did not develop in vivo or in vitro.

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Comparative cytotoxicity and pharmacokinetics of antimelanoma immunotoxins containing either natural or recombinant gelonin. Cancer Chemother. Pharmacol. 44(4):343-8, 1999.

- Serafino A, Sinibaldi-Vallebona P, Pierimarchi P, Bernard P, Gaudiano G, Massa C, Rasi G, Ranagnan G.
Induction of apoptosis in neoplastic cells by anthracycline antitumor drugs: nuclear and cytoplasmic triggering? Anticancer Res. 19(3A):1909-18, 1999.

- Teronen O, Heikkila P, Konttinen YT, Laitinen M, Salo T, Hanemaaijer R, Teronen A, Maisi P, Sorsa T.
MMP inhibition and downregulation by bisphosphonates. Ann. N.Y. Acad. Sci. 878:453-65:453-65, 1999.

- Wang X, Doherty GP, Leith MK, Curphey TJ, Begleiter A.
Enhanced cytotoxicity of mitomycin C in human tumour cells with inducers of DT-diaphorase. Br. J. Cancer. 80(8):1223-30, 1999.

- Yang F, Boissy RE.
Effects of 4-tertiary butylphenol on the tyrosinase activity in human melanocytes. Pigment Cell Res. 12(4):237-45, 1999.

- Zagozdzon R, Golab J, Mucha K, Foroncewicz B, Jakobisiak M.
Potentiation of antitumor effects of IL-12 in combination with paclitaxel in murine melanoma model in vivo. Int. J. Mol. Med. 4(6):645-8, 1999.

B: Gene and immunotherapy

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Interleukin-2-induced, melanoma-specific T cells recognize CAMEL, an unexpected translation product of LAGE-

1. Int. J. Cancer. 82(3):442-8, 1999.
Comments: In summary, CTL induction with IL-2- transfected melanoma cells has revealed a new tumor antigen that may serve as a target for immunotherapy.

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Polycations and cationic lipids enhance adenovirus transduction and transgene expression in tumor cells. Cancer Gene Ther. 6(5):437-46, 1999.
Comments: We demonstrate increased transgene expression after mixing adenovirus preparations with polycations, cationic lipids, and CaCl2 prior to transduction in vitro. Further studies will determine whether polycations can improve intratumoral gene transfer.

- de Ines C, Cochlovius B, Schmidt S, Kipriyanov S, Rode HJ, Little M.
Apoptosis of a human melanoma cell line specifically induced by membrane-bound single-chain antibodies. J. Immunol. 163(7):3948-56, 1999.

- Gillespie AM, Coleman RE.
The potential of melanoma antigen expression in cancer therapy. Cancer Treat. Rev. 25(4):219-27, 1999.

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Sensitization of tumor necrosis factor alpha-resistant human melanoma by tumor-specific in vivo transfer of the gene encoding endothelial monocyte-activating polypeptide II using recombinant vaccinia virus. Cancer Res. 59(18):4668-74, 1999.

- Gu DL, Gonzalez AM, Printz MA, Doukas J, Ying W, D'Andrea M, Hoganson DK, Curiel DT, Douglas JT, Sosnowski BA, et al.
Fibroblast growth factor 2 retargeted adenovirus has redirected cellular tropism: evidence for reduced toxicity and enhanced antitumor activity in mice. Cancer Res. 59(11):2608-14, 1999.

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Antitumor effects of interferon-omega: in vivo therapy of human tumor xenografts in nude mice. Cancer Res. 59(16):4064-8, 1999.

- Huang S, Ullrich SE, Bar-Eli M.
Regulation of tumor growth and metastasis by interleukin-10: the melanoma experience. J. Interferon. Cytokine. Res. 19(7):697-703, 1999.
Comments: We transfected human A375P melanoma cells that do not express IL-10 with the murine IL-10 gene and subsequently analyzed for changes in tumor growth and metastasis in nude mice. Surprisingly, IL- 10 gene transfer resulted in a loss of metastasis and significant inhibition of tumor growth.

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Induction of complement attack on human cells by Gal(alpha1,3)Gal xenoantigen expression as a gene therapy approach to cancer. Gene Ther. 6(6):1073-83, 1999.

- Mateo L, Gardner J, Chen Q, Schmidt C, Down M, Elliott SL, Pye SJ, Firat H, Lemonnier FA, Cebon J, et al.
An HLA-A2 polyepitope vaccine for melanoma immunotherapy. J. Immunol. 163(7):4058-63, 1999.

- Rongcun Y, Salazar-Onfray F, Charo J, Malmberg KJ, Evrin K, Maes H, Kono K, Hising C, Petersson M, Larsson O, et al.
Identification of new HER2/neu-derived peptide epitopes that can elicit specific CTL against autologous and allogeneic carcinomas and melanomas. J. Immunol. 163(2):1037-44Other, 1999.

- Skipper JC, Gulden PH, Hendrickson RC, Harthun N, Caldwell JA, Shabanowitz J, Engelhard VH, Hunt DF, Slingluff CLJ.
Mass-spectrometric evaluation of HLA-A*0201-associated peptides identifies dominant naturally processed forms of CTL epitopes from MART- 1 and gp100. Int. J. Cancer. 82(5):669-77Other, 1999.

C: Melanocyte culture

The paper by Edgar and Bennett describes the effects of transient transfection of antisense phosphorothioate oligodeoxynucleotides targeted against myosin Va mRNA. In Melan-a mouse melanocytes this resulted in a reduced expression of the myosin Va protein. Replating the cells after trypsinization showed that the capacity to regain the dendritic morphology was reduced in the cells containing the specific antisense oligonucleotides but unaffected by the control oligonucleotide. This work shows the importance of myosin Va in new dendrite formation in cultured melanocytes in addition to its involvement in melanosome transport. Katagata et al describe the presence of keratin subunits in neonatal epidermal melanocytes from a commercially available cell kit. As the authors mention it was not expected to find keratin

expression in melanocytes. The subunits do not form any keratin filaments and the question about their possible role in melanocyte (development)remains to be answered. In the papers by Kaufmann et al differences in the half-life of neurofibromin is studied in melanocytes of NF1 patients. Melanocyte growth factors bFGF and TPA increased the half life of neurofibromin which seems to be dependent on lysosomal (or melanosomal) degradation.
The paper by Schallreuter and Wood nicely demonstrates that phenylalanine may be an important precursor for melanin formation in cultured melanocytes since it can be converted to L-tyrosine by phenylalanine hydroxylase. Since the phenylalanine uptake seems to be an active transport which is calcium dependent the levels of phenylalanine and calcium in culture medium may be important for the final melanin production in the melanocyte cultures.
Suzuki et al describe the use of melanocyte cultures of skin type V or VI that responded to MSH at an effective dose of 0.1 nM. One culture of skin type I or II was much less responsive. Genetic analysis of the MC1-receptor variants are being used to study these responses to MSH as has also been presented by Abdel-Malek at the XVIIth IPCC meeting in Nagoya (Pigment Cell Res suppl 7, p.44). These studies may be of major importance for our understanding of different responsiveness of melanocyte cultures to stimulation of melanogenesis.

- Alanko T, Rosenberg M, Saksela O.
FGF expression allows nevus cells to survive in three-dimensional collagen gel under conditions that induce apoptosis in normal human melanocytes. J. Invest. Dermatol. 113(1):111-6, 1999.
Partial abstract: Here we report growth characteristics of in vitro cultured normal human melanocytes and dermal nevus-derived melanocytes. As previously reported, nevus cells have a moderate to high FGF-2 expression level. Here we demonstrate that dermal nevus cells are able to survive in three-dimensional type 1 collagen culture, while normal human melanocytes rapidly undergo apoptosis.

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An ex vivo study of congenital pigmented nevi in epidermal reconstructs. Pigment Cell Res. 12(3):164-74, 1999.
Partial abstract: Typical nesting of nevus cells was observed in the dermal- epidermal junction or in the superficial dermis. UVB exposure induced an upward migration of nevus cells in the suprabasal layers of the epidermis. This tissue model can be considered as an excellent system for the ex vivo reproduction of pigmented nevi and as an assay of the sensitivity of nevus cells towards UVB irradiation.

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Inhibition of dendrite formation in mouse melanocytes transiently transfected with antisense DNA to myosin Va. J. Anat. 195(Pt 2):173-84, 1999.

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Melanoma-associated expression of vascular endothelial growth factor and its receptors FLT-1 and KDR. J. Cancer Res. Clin. Oncol. 125(11):621-9, 1999.
Partial abstract: Neonatal melanocytes did not express VEGF or VEGF receptors and VEGF expression could not be induced by exogenous growth factors.

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Genetic and epigenetic control of the proliferation and differentiation of mouse epidermal melanocytes in culture. Pigment Cell Res. 12(3):147-63, 1999.

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Keratin subunit expression in human cultured melanocytes and mouse neural crest cells without formation of filamentous structures. J. Investig. Dermatol. Symp. Proc. 4(2):110-5, 1999.

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Posttranslational regulation of neurofibromin content in melanocytes of neurofibromatosis type 1 patients. Arch. Dermatol. Res. 291(6):312-7, 1999.

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On the lysosomal degradation of neurofibromin and its phosphorylation in cultured. Biol. Chem. 380(9):1071-8, 1999.

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Cyclic stretch up-regulates proliferation and heat shock protein 90 expression in human melanocytes. Pigment Cell Res. 12(4):246-51, 1999.

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Human melanocytes grown in epidermal equivalents transfer their melanin to follicular outer root sheath keratinocytes. Arch. Dermatol. Res. 291(6):325-32, 1999.

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Sulphorhodamine B assay for measuring proliferation of a pigmented melanocyte cell line and its application to the evaluation of crude drugs used in the treatment of vitiligo. J. Ethnopharmacol. 66(2):141-50, 1999.

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Diversity of desmosomal proteins in regenerating epidermis: immunohistochemical study using a human skin organ culture model. Arch. Dermatol. Res. 291(7-8):437-46, 1999.

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Pigmented human skin equivalent as a model of the mechanisms of control of cell-cell and cell-matrix interactions. Med. Biol. Eng. Comput. 36(6):813-20, 1998.

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Endothelin signalling in the development of neural crest-derived melanocytes. Biochem. Cell Biol. 76(6):1093-9, 1998.

- Pellegrini G, Bondanza S, Guerra L, De Luca M.
Cultivation of human keratinocyte stem cells: current and future clinical applications. Med. Biol. Eng. Comput. 36(6):778-90, 1998.

- Pianigiani E, Andreassi A, Taddeucci P, Alessandrini C, Fimiani M, Andreassi L.
A new model for studying differentiation and growth of epidermal cultures on hyaluronan-based carrier. Biomaterials. 20(18):1689-94, 1999.
Partial abstract: Hyaluronic acid is, in fact, considered to be an optimal biomaterial allowing proliferation of both keratinocytes and melanocytes, and it is already used for clinical aims.

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Correlation between antioxidants and phototypes in melanocytes cultures. A possible link of physiologic and pathologic relevance [letter]. J. Invest. Dermatol. 113(3):424-5, 1999.

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Reconstructed human epidermis composed of keratinocytes, melanocytes and Langerhans cells. Med. Biol. Eng. Comput. 36(6):821-4, 1998.

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The importance of L-phenylalanine transport and its autocrine turnover to L-tyrosine for melanogenesis in human epidermal melanocytes. Biochem. Biophys. Res. Commun. 262(2):423-8, 1999.

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Growth factor synergism and antagonism in early neural crest development. Biochem. Cell Biol. 76(6):1039-50, 1998.

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Participation of the melanocortin-1 receptor in the UV control of pigmentation. J. Investig. Dermatol. Symp. Proc. 4(1):29-34, 1999.

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Calcitonin gene-related peptide upregulates melanogenesis and enhances melanocyte dendricity via induction of keratinocyte-derived melanotrophic factors. J. Investig. Dermatol. Symp. Proc. 4(2):116-25, 1999.
Partial abstract: To clarify the effect of neuropeptides on the biology and morphology of melanocytes, organ cultures of normal human skin and cultured melanocytes were exposed to various neuropeptides. The findings suggest that keratinocytes produce and secrete some melanotrophic factors following stimulation with calcitonin gene-related peptide (CGRP), which modulate growth, melanin synthesis, and dendricity of melanocytes.

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Elasmobranch color change: A short review and novel data on hormone regulation. J. Exp. Zool. 284(5):485-91, 1999.

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

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2000 **Melanoma: Basic Biology and Immunological Approaches to Therapy**
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Website: http: //www.aacr.org

2000 **IXth Annual Meeting of the Pan American Society for Pigment Cell Research (PASPCR)**
June 25-28 **College Station, TX**
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
Fax: (409)845-9972
E-mail: llamoreux@cvm.tamu.edu

2000 **9th ESPCR Meeting: Ulm, Germany**
Sept 28 -Oct 1 **Contact**: Prof. PETER R.U.
University of Ulm (BWK)
Dept of Dermatology
Oberer Eselsberg 40
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Tel: 49-731 502-3770
Fax: 49-731 502-3772
E-mail: ralf.peter@medizin.uni-ulm.de

2001 **5th World Conference on Melanoma: Venice**
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SAD NEWS OF THE DEATH OF A COLLEAGUE

With much sadness, we must tell the membership of the death in September 1999 of Giovanna Prota (wife of Professor Giuseppe Prota). Giovanna was well known within the Society and much admired as a gentle, lovely lady. She will be sadly missed by all who knew her. We offer our condolences to Giuseppe and their family.

Sheila Mac Neil and Stan Pavel