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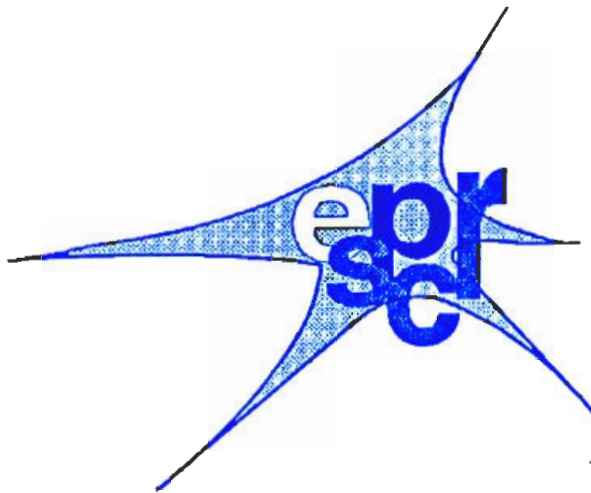
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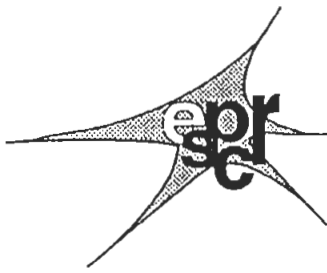
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LETTER TO THE EDITOR DISCUSSION, REVIEW, SHORT COMMUNICATION, ...

MEETING REPORT XVIIth IPCC and Satellite Meetings

Satellite Meeting No. 1

Vitiligo: A Manifestation of Apoptosis?

Report by James J. Nordlund

Symposium Chairmen: James J. Nordlund (University of Cincinnati) and Seung-Kyung Hann (Korean Institute for Study of Vitiligo and Yonsei University)

Goal of Symposium: This symposium on vitiligo emphasized one mechanism, i.e., apoptosis. The goal was to stimulate new ideas and approaches to the problem of vitiligo and its treatment.

Major Problems for Investigators: (James J. Nordlund, M.D., University of Cincinnati, USA) The most important goal for investigators is to find a safe, effective medication that will stop the spread of vitiligo when it first begins or that retains pigmentation after successful treatments. The second most important goal is to find better ways to transplant autologous melanocytes in those whose depigmentation affects glabrous (hairless) skin.

Mechanisms of Cell Death:

Ultrastructure of apoptosis: (Raymond Boissy, Ph.D., Caroline LePoole, Ph.D. and Ying Boissy, University of Cincinnati) Apoptosis is a programmed induced destruction of cells by which unneeded or senescent cells are eliminated from the body without induction of an immune process. Necrosis is another form of cell death by which the cells are disrupted and release cellular antigens that might induce an immune response.

Apoptosis has morphological features including 1) alterations in membrane fluidity; 2) blebbing of the plasma membrane; 3) cell fragmentation in which apoptotic bodies are released into the environment; 4) vacuolization of the cytoplasm; 5) cellular shrinkage; 6) condensation of the chromatin along the nuclear membrane; 7) activation of calcium or potassium sensitive endonucleases that fragment DNA.

Apoptosis has been demonstrated as a mechanism of melanocyte death in a model system in which human melanocytes in culture are exposed to chemical toxins such as 4-tertiary butyl phenol (4-TBP). Melanocytes obtained from patients with vitiligo that are grown in culture also exhibit features of apoptosis especially if the cells are not maintained in very special conditions.

Apoptosis has not been clearly observed in the skin of patients with advancing vitiligo. However some melanocytes in the spreading lesion exhibit features that suggest cellular shrinkage and condensation of heterochromatin, features consistent with apoptosis.

Molecular Mechanisms of Apoptosis: (Yoshihide Tsujimoto, Ph.D., Osaka University, Japan) Apoptosis is a method of destruction of cells that must be selectively eliminated as part of tissue homeostasis, for morphogenesis or removal of other harmful cells such as cancers. The process is driven by a family of cysteine proteases called caspases. Caspases cleave a set of special proteins that initiate apoptosis. The Bcl-2, Bcl-x and Bcl-w are three of the better characterized anti-apoptotic proteins that inhibit apoptosis and protect a cell from destruction. Pro-apoptotic molecules include molecules such as Bid, Bik and Bim. The anti and pro apoptotic proteins form heterodimers inactivating each other.

Mitochondria play an essential role in apoptosis. The electrical potential across a cell destined for destruction by apoptosis is lost and cytochrome c and other factors are released. Bcl-2 prevents cytochrome c release, whereas Bax, Bak and Bid accelerate these processes.

Cytochrome c activates caspase 9 and ultimately caspase 3 releasing cytochrome c through a permeability transition pore. The pro and anti apoptotic factors enhance or close pore permeability respectively. A newly discovered factor, Acinus, induces chromatin condensation after cleavage by caspase-3.

Apoptosis and necrosis share some common steps. The concentration of ATP within a cell is one factor that determines whether a cell undergoes necrosis or apoptosis.

Immune Mechanisms of Apoptosis: (Tatsuya Horikawa, M.D. and David Norris, M.D., University of Colorado) Cells exposed to toxins can die by either apoptosis or necrosis. Severe damage induces necrosis, intermediate damage initiates apoptosis. Many of the immune activated processes induce apoptosis such as TNF- α or TNF- β . Cytotoxic lymphocytes induce apoptosis through three distinct mechanisms. 1) coordinate release of perforin and granzymes adjacent to the target cell membrane; 2) cytokine release (i.e., IFN- γ , TNF- α , IL-1); 3) triggering of the Fas receptor.

Cells maintain complex anti-apoptotic defenses such as Bcl-2. Interruption of these defenses by immune mechanisms facilitate apoptosis in susceptible cellular targets. When activated cytotoxic lymphocytes encounter cells expressing high levels of ICAM-1, they bind to and engage Fas on the target surface. Those cells susceptible to apoptosis undergo programmed cell death. Others are destroyed by necrosis.

Apoptosis is a key mechanism controlling the balance among the highly changing interactive cell populations in the immune response. Activated lymphocytes expressing Fas ligand on their cell surface induce apoptosis of lymphocyte and macrophage targets bearing Fas. Apoptosis is a complex process involving multiple mechanisms of induction. But Fas-dependent lysis of cytotoxic T lymphocytes is the most efficient and highly developed form of immune cytotoxicity.

Mechanisms of repigmentation:

Melanocyte reservoir: (James J. Nordlund, University of Cincinnati) Data show almost conclusively that depigmentation of vitiligo is caused by loss of melanocytes. Histological data confirm absence of cells by routine, histochemical, and ultrastructural techniques. Attempts to culture cells from depigmented skin have been unsuccessful.

Glabrous skin (hairless skin) or depigmented skin with white hair do not respond to medical therapies for repigmentation. These areas do repigmented by surgical techniques. These data indicate that the hair follicle is the normal reservoir for repigmentation and that surgical transplants are a method to replace the follicular reservoir.

Mechanisms of melanocyte proliferation and survival: (Zalfa Abdel Malek, Ph.D., University of Cincinnati) Melanocytes can be induced to proliferate in response to stimuli such as ultraviolet light. Many growth factors have been identified that stimulate growth of melanocytes. Some are synthesized in the epidermis, some in the dermis. Factors such as b-FGF, endothelin-1, α -MSH and ACTH are synthesized by keratinocytes and melanocytes.

Proliferation of melanocytes requires the activation of multiple signaling pathways. Together cAMP and activation of protein kinase C induce melanocyte mitosis. Endothelin-1 has a complex action including activation of protein kinase C, calcium mobilization and inositol triphosphate formation. These factors subsequently activate map kinase erk 1/erk 2 and the transcription factor CREB (calcium/camp response element binding protein) which are necessary for proliferation.

Endothelin 1 also enhances melanocyte motility and reduces adhesion to fibronectin. Mutations in receptors for endothelins such as endothelin-3 are responsible for a syndrome characterized by absence of both melanocytes and enteric neurons. Mutations in the c-kit gene are responsible for piebaldism.

Challenges for the future: (Seung-Kyung Hann, Vitiligo Research Center and Yonsei University). The causes and cures of vitiligo have remained elusive. It should be a disease of great importance to pigment biologists because the disorder provides clues to the normal function of the pigment cell with the other epidermal cells, the keratinocyte and Langerhans cells to form the KLM complex.

In this symposium we have suggested that apoptosis might be a mechanism by which the melanocytes are destroyed and we have reviewed the factors that can cause the cells to return to the depigmented skin. It is hoped that these presentations will be the stimulus for more discussion and conversations among pigment biologists to find new ways to solve the problem of vitiligo and its cure. One idea worth presenting is that there are weak and strong melanocytes in the skin of a given person. By identifying why some cells are stronger and resist vitiligo, we will find ways to prevent or halt the spread of vitiligo. When the human genome is mapped, it is hoped that the genes involved in vitiligo will be identified and that rapid progress on the cause and cure of this disease will be made. _

PS1 - Biochemistry of Melanogenesis, Melanosomes, and Melanocytes

Report by John Pawelek

Dr. K. Jimbow presented the opening lecture "Signals and Molecules Involved in Melanosome Biogenesis and Melanocyte Differentiation". Melanosomes share many biological properties with lysosomes. Early, stage I melanosomes appear to be linked to endosomal vacuoles. Melanosomal proteins are glycosylated and are assembled through interaction with calnexin in the ER, eventually exiting through the trans Golgi network to the final melanosome compartments. Adapter protein-3 is important in intracellular transport of tyrosinase gene family proteins. Also, small GTP-binding proteins, e.g. Rab 5 and 7, as well

as phosphatidyl inositol 3-kinase regulate traffic. Thus, the cascade of melanosome biogenesis is regulated by several factors: 1) glycosylation of melanosomal proteins and correct folding and accumulation within the ER and Golgi, and 2) specific signals and molecules involved in intracellular transport and vesicular traffic of these glycoproteins from the Golgi to melanosomes.

Using HPLC and X-ray microanalyses, Smit et al. studied the relative proportions of pheo- and eumelanins in melanocytes from dark- and light-skinned individuals. Light-skinned individuals have a higher level of pheomelanins and lower level of eumelanins than those with darker skin. These differences can be accentuated if the cells are cultured with excess L-tyrosine. Cultured nevus cells from patients with dysplastic nevus syndrome tend to produce predominantly pheomelanins, and again this phenomenon is enhanced if the nevus cells are cultured in high levels of tyrosine.

Solano et al. presented "New Insights on the Murine Silver Locus: On the Expression of the Transcript in Wild-Type and Silver Mutation and Function of the Protein". Wild type mouse cells express a gp87 silver locus protein that, though smaller, is similar to human gp100, whereas silver mutant cells express a truncated version of gp87, due to a premature stop codon at nucleotide 1808. Studies on the oxidation of DHICA by extracts of mutant and wild-type cells suggested that DHICA oxidation is catalyzed by TRP-1, and apparently not gp87.

Kobayashi and Hearing continued their studies on the stabilization of tyrosinase through binding interactions with TRP-1, and here demonstrated direct interaction of the two proteins *in vivo*. Employing a chemical cross-linker to stabilize the association of tyrosinase with other cellular proteins, they showed by immunoblotting that TRP-1 was indeed associated with tyrosinase in amounts much greater than could be accounted for by nonspecific cross-linking. Together, the data indicated that TRP-1 interacts directly with tyrosinase, stabilizing it *in vivo*.

Toyofuku et al. studied aberrant folding and transport of mutant tyrosinases in type I oculocutaneous albinism (OCA1). Computer models suggest that some amino acid substitutions in OCA1 may alter folding of tyrosinase, but it is not yet clear why this eliminates tyrosinase activity. Accordingly, wild-type and OCA1 mutant tyrosinases were transfected into COS7 cells. Some mutant tyrosinases were retained in the ER and not transported to lysosomes, the final destination of tyrosinase in cells other than melanocytes. Tyrosinases with temperature-sensitive mutations were transported appropriately to lysosomes, but not expressed at physiological temperatures. It was concluded that some aa substitutions altering the 3-D structure of tyrosinase result in temperature-sensitivity of the enzyme, while others result in trapping of mutant enzymes in the ER, preventing transport to melanosomes.

Peters et al. reported the presence of POMC-related peptides in melanosomes of cultured human melanocytes. Probing with specific antibodies they reported cross-reactivity with alpha MSH, proconvertases 1 and 2 (PC 1 and 2) and the PC2 regulatory protein, 7B2. Thus they identified elements of the POMC system in melanosomes, providing supportive evidence for their model of complex formation between MSH and co-factor 6BH4, which activates tyrosinase by relieving 6BH4 inhibition of the enzyme. Such a model suggests that MSH action does not necessarily involve MSH receptors.

CS2 - Chemistry and Biophysics of Melanin & Melanogenesis

Report by Patrick Riley

This session began with a Keynote Lecture given by the Chairman of the Conference, Professor Shosuke Ito. His talk, entitled "Chemical Analysis of Melanins and its Application to the study of Melanogenesis Regulation" began with a detailed review of a range of studies using the assay introduced in 1983 as a means of estimating the relative amounts of phaeomelanin and eumelanin. This assay is based on the conversion of DHICA moieties in eumelanin to the derivative pyrrole tricarboxylic acid (PTCA) by permanganate oxidation in approximately 2.8% yield, and benzothiazine moieties (in phaeomelanin) to 4-amino-3-hydroxyphenylalanine (AHP) by hydriodic acid hydrolysis in 20% yield under defined conditions of treatment of melanin samples. Professor Ito suggested that the proportional generation of phaeomelanin, assessed by these criteria, is inversely related to the level of tyrosinase activity. Application of this model to murine coat colour mutants was considered to be consistent with this general proposal. Professor Ito further suggested that the inner region of polymeric melanins consists of phaeomelanin with an outer layer composed predominantly of eumelanin. In response to questions Professor Ito suggested that at very low levels of tyrosinase activity any dopaquinone generated is rapidly converted to cysteinyl-dopa and eliminated from cells resulting in the absence of detectable melanin. With regard to the structural model of melanin proposed it was at the molecular level and would not be easily discernible by techniques such as electron microscopy.

Professor Patrick Riley then presented a talk entitled "The Source & Significance of Dopa in Phase I Melanogenesis" which outlined the overwhelming accumulation of evidence favouring the indirect generation of dopa by reduction of dopachrome in the initial steps of *in vitro* melanogenesis. He argued that

this mechanism, coupled with the reduction of active site copper atoms, was able to account for the observed 'lag phase' kinetics of tyrosinase. In response to a question from Professor Frank Meyskens he agreed that UV-induced changes in tyrosinase synthesis may be significant but expressed doubts concerning the activation of tyrosinase by superoxide anion generated by UV exposure. In his view the intracellular proton concentration was more likely to be a significant regulator of tyrosinase recruitment.

The next talk was given by Professor Tadeusz Sarna in which he outlined the functions of the retinal pigment epithelium (RPE) in providing metabolic support for photoreceptor cells. He drew attention to the decrease in RPE melanization as a function of age. Experiments on melanosomes obtained from differentially melanised bovine eye RPE demonstrate that photo-oxidation is more pronounced in the absence of melanin. Thus, decreasing retinal melanization may have a bearing on pathological conditions such as age-related macular degeneration.

Dr. Jun Matsunaga then presented evidence from clonogenic assays that a short (30-minute) exposure of B16 or Neuro2A cultures to selected melanogenic intermediates exhibited differential cytotoxicity. Of the agents tested the most toxic were dopachrome and dopaminachrome. This finding was interpreted to imply an important cytoprotective role for dopachrome tautomerase activity as demonstrated for migration inhibitory factor (MIF).

Dr. Malgorzata Rozanowska presented data from pulse-radiolysis and laser flash-photolysis experiments showing that carotenoid cation radicals are quenched by synthetic melanin. In other experiments it was shown that light- or chemically-induced lipid peroxidation was abrogated by the addition of a series of carotenoids and that their efficiency was increased in the presence of melanin.

Finally, Dr. Jeffrey Tosk gave an ebullient account of studies of Rayleigh scattering by suspensions of dopa melanin. Using a polarised light scattering technique the effects of microenvironmental alterations, such as pH and redox state, on the aggregation of the pigment could be demonstrated. These effects were considered to be relevant to the association between pigmentation and neuronal damage observed in Parkinson's disease. In response to a question from Professor Raimondo Crippa he agreed that the aggregation state may regulate the extent of the reactive surface of melanin.

PS2 - Basic Biology of Melanoma

Report by Frank Meyskens

The sessions on "Basic Biology of Melanoma" was represented by six platform and eleven poster presentations and covered a wide range of topics including keratinocyte / melanocyte/melanoma cross-talk and its importance in invasion, immunogenic properties of melanosomal proteins, angiogenesis, control of reactive oxygen species, and molecular genetic alterations.

Herlyn (USA) gave a superb overview of the topic of keratinocyte/melanocyte and biochemical interactions and regulation and its importance in maintaining tissue integrity. Substantial evidence was offered that the replacement of E-cadherin by N-cadherin during melanocyte transformation is a critical event that permits invasion to occur. Using a reconstructed model system MacNiel (England) offers evidence that erosion of the basement membrane was necessary for invasion by melanoma cells and that this process was reduced by 17β -estradiol and estrogen. This observation is, of course, of great interest since longstanding clinical data clearly shows that young women with melanoma have a much better prognosis, even after adjusted for Breslow level. Related to this general topic were two papers on angiogenesis: Both calponin (Koganchiua, Japan) and $\alpha\nu\beta_3$ integrin (Kageshita, Japan) respectively were found to be altered expression in the blood vessels and melanoma cells. As is being documented by many investigators the role of angiogenesis is crucial in the pathophysiology of carcinogenesis and many new therapies are being developed based on such fundamental observations.

Papers were presented by four different groups (Kawasaki, Japan); Bartido, USA; Cochran, and Pawelick, all USA, New Haven) demonstrating the complexity of response of immune responses in melanocyte biology. Melanosomal proteins, dendrite cells, and T-lymphocytes all play a role and in a manner too complex to review here. Perhaps, the most intriguing of the observations is the necessary evidence being amassed by Pawelek & his group that metastatic melanoma cells may be a hybrid of host (macrophage) and melanoma cells. If this hypothesis can be proven, the theoretical and practical consequences would be enormous for tumor biology.

A group of papers also dealt with various aspects of biochemical controls including the inter-relationships between tyrosinase, trp-1, and δ -glutamyltranspeptidase (Chasuble, India), various oncogenes products

(Ueda, Japan) and the role of α -PKC in phospholipase alterations. Two complex papers (Takara, Japan; Andrs Germany) on the genetics of melanoma were presented as well.

Two posters were presented on the general topic of reactive oxygen species. Haycock (England) nicely demonstrated that α -MSH blocked the acute TNF or peroxide stress response in melanoma cells which

has implications for the study of early inflammatory changes in melanocyte carcinogenesis. Our own group (Spillane, U.S.A) has presented the unique observation that apoptosis of melanoma cells is promoted by heavy ion chelators, inhibited by classical antioxidants, and that ferrous (anti-apoptotic) and ferric (pro-apoptotic) ions change the intracellular redox state in an opposing manner and affect apoptosis accordingly.

CS3 - Melanoma: Ultraviolet Light, Diagnosis and Treatment.

Report by Alistair J Cochran

CHAIRS: Alistair J Cochran, Bertil Kagedal, Juichiro Nakayama

Papers presented:

Understanding Regulation of NF κ B as Basis for Melanocyte Transformation and Melanoma Cell Therapeutic Resistance, Frank L. Meyskens Jr., Susan McNulty, Niloufar Tohidian, and Julie Buckmeier, Chao

Effective Early Detection of Malignant Melanoma on the Sole, Toshiaki Saida,

Does PCR Detect Melanomas in Sentinel Nodes (SNs) that are Not Detected by Immunohistochemistry?

J. Guo, J. Messina, D.-R. Wen, R.R. Huang, D. Reintgen, and A.J. Cochran,

Primary Cutaneous and Vulvar Melanomas are Dissimilar in Clinical and Histopathological Features, but Similar in TP53 Mutations, Boel Ragnarsson-Olding, Anton Platz, Sofia Karsberg, Lena Kanter-Lewensohn, and Ulrick Ringborg,

This session brought together a series of interesting if somewhat diverse papers.

Dr Meyskens reported that melanoma cell lines have elevated levels of reactive oxygen species relative to normal melanocytes. He and his colleagues studied nuclear factor kappa B (NF κ B), a transcriptional regulatory complex that responds to reactive oxygen species and ultraviolet B, as a potential factor in melanoma progression and chemoresistance. UVB increases NF κ B binding by normal melanocytes, but not by melanoma cell lines. Oxidative stress induces increased NF κ B binding in both melanocytes and melanoma cell lines. The NF κ B response of melanoma cells to UVB is apparently independent of oxidative stress and is mediated at the transcriptional level.

Dr Saida reported melanocytic nevi, visible as brownish black macules, on the soles of 8% of Japanese. These must be separated from early melanomas which may appear quite similar. With the exception of congenital nevi, most nevi on the sole are less than 7mm in diameter, leading to a recommendation that lesions larger than 7mm be excised. In contrast most melanomas are larger than 9mm in diameter. Epiluminescence microscopy (ELM) may provide further assistance in making this separation. Patterns seen on ELM that may be used to separate melanomas and nevi were reported. An algorithm for the management of these lesions has been developed.

The paper by Guo et al. described a study of the tumor status of sentinel lymph nodes (SN), comparing standard histology (SH), immunoperoxidase (IPX), RT-PCR (PCR), using a primer for m-tyrosinase and RT in situ PCR (ISPCR) (same primer). A series of 15 SN was studied. Five were positive for tumor by SH, IPX and PCR, 5 were negative for tumor by these three techniques and 5 were negative by SH and IPX, but positive by PCR. Additional sections were cut from the blocks of these 15 nodes and studied by ISPCR, using a primer for m-tyrosinase. In the 5 nodes that were negative by SH, IPX and PCR, ISPCR detected no tumor, but found neural tissue (Schwann cells containing m-tyrosinase) in 4 of 5 and a capsular nevus in one. In the 5 nodes positive by SH, IPX and PCR, ISPCR confirmed the presence of melanoma, but additionally found neural tissues in three. In the 5 nodes that were negative by SH and IPX, but positive by PCR, ISPCR demonstrated no melanoma, but all five contained neural tissue and two had a capsular nevus. It was concluded that sources of PCR signal for m-tyrosinase other than melanoma cells are present in lymph nodes. This must be considered in interpreting results from PCR studies of complex tissues such as lymph nodes.

Dr Ragnarsson-Olding reported a comparison of malignant melanomas (MM) arising on sun-exposed and non sun-exposed (vulvar) body sites and, within the vulva, on hair-bearing and glabrous skin. From a study of 219 patients the density of MM on the vulva is 2.5 times that on other areas of the body. On the vulva MM are often amelanotic. Mucosal lentiginous MM was most common, followed by nodular and superficial spreading melanoma, the reverse of the frequency on non-vulvar sites. In a study of TP53 mutations in 35 MM, 27% of those from sun-exposed and 34% from non-exposed sites displayed the C-T mutations at dipyrimidine sites that are considered fingerprints of UV radiation-induced DNA damage. It was concluded that the similarity in frequency of the TP53 mutations in MM from exposed and non-exposed sites points to a complex genesis for these alterations. The clinical and histologic differences between melanomas of the two sites are striking but inexplicable at this time.

Papers by Cascinelli and by Lee et al. were withdrawn.

PS4 - Inter/Intracellular Signaling Pathways in Melanocytes

Report by Glynis Scott

The session on inter/intracellular signaling pathways in melanocytes was opened by Dr. Nishikawa who spoke about the microenvironment control of melanocyte migration and localization to the hair follicle. Their studies on the role of stem cell factor (SCF), c-kit and endothelin-1 show that SCF and ET-1 are critical growth factors for the localization of melanocytes to the skin. Their studies show that E-cadherin levels increase in melanocytes prior to entry of the melanocytes to the epidermis, and that this increase in expression is highly synchronized. They also show that overexpression of SCF through the use of transgenic mice results in population of melanocytes in the interfollicular areas. Dr. Nishikawa and co-workers show that the soluble and the membrane bound forms of SCF have differing functions in hematopoietic and future work in their laboratory will focus on defining the effects of the soluble Vs membrane bound SCF on melanocyte migration and localization to the skin.

Dr. Thody and co-workers presented data on the regulation of melanocyte function by α -MSH. Dr. Thody reviewed findings confirming the importance of the MC-1 receptor in mediating the diverse effects of α -MSH on skin pigmentation. He then reviewed data showing that other POMC peptides, such as ACTH, are available ligands for the MC-1 receptor in the skin and may indeed have differing effects on melanocyte function compared with α -MSH. He shows that ACTH peptide 1-17 coupling to cAMP is greater than that seen with α -MSH and that certain ACTH peptides activate IP3 production. Another point of potential regulation is the levels and activity of proconvertase enzymes, which convert PMC to ACTH. Future studies will involve defining the dose response curve for POMC peptide effects on melanocyte function including melanogenesis, dendricity and adhesion, and the signaling intermediates involved. Dr. Medrano presented data on the effect of pigmentation associated genes on cell cycle and terminal differentiation in human melanocytes. Dr. Medranos' laboratory has made the observation that activation of cAMP results in senescence of human melanocytes and that senescence is accompanied by other markers of differentiation such as increased pigmentation. Their data indicates that changes in the levels of E2F/p130 repressor complexes is associated with terminal differentiation and increased pigmentation, suggesting a possible mechanism for increased melanoma susceptibility in light skinned individuals who cannot tan. Dr. Medrano also shows that cyclin E is markedly decreased in senescent cells, whereas it is increased in primary melanoma. Future work will be focused on defining the potential regulatory sites at which pigmentation associated genes affect cell cycle.

Dr. Tuma presented data on the role of the microtubule motor protein kinesin II and the actin binding protein myosin V on melanophore movement in *Xenopus*. By using dominant negative mutants of these two proteins fused to green fluorescent protein they show that cells expressing both mutants fail to exhibit any melanophore movement whatsoever. In cells expressing only the dominant negative kinesin mutant, melanophores failed to demonstrate the usual quick saltations towards the periphery, but did show slow, random movements. Cells expressing the dominant negative myosin V mutant showed some movement of melanophores to the periphery, but melanophores fail to remain at the periphery. These results demonstrate the importance of these two proteins in melanophore movement and future studies will be directed to understanding the regulation of these motor proteins in melanophore movement.

Dr. Tada presented data on the signal transduction intermediates involved in ET-1, α -MSH and bFGF signaling in human melanocytes. A main point of their presentation is that CREB activation may occur through two distinct signaling pathways: one that is mitogen activated and ERK2 dependent and one that is stress induced and p-38 dependent. They show that ET-1 and bFGF, but not α -MSH, induce CREB phosphorylation, and that ERK2 activation is necessary for ET-1 induced CREB phosphorylation. Ultraviolet light also resulted in CREB activation, but through phosphorylation of p38 rather than ERK2. These results highlight the importance of CREB phosphorylation as a signaling intermediate in mitogen induced proliferation/differentiation and in response to stressors such as UV light.

Mr. Wagner presented the final talk in the session from Dr. MacNeils laboratory. In the studies presented the investigators demonstrated the effect of α -MSH on Ca²⁺ mobilization. Using the human melanoma cell line HBL as a model system, the investigators show that when cells were cultured on fibronectin, and when the α -MSH induced cAMP response was inhibited by an adenosine agonist, α -MSH induced increased intracellular Ca²⁺ levels in the majority of cells studied. This dual response of the MC-1 receptor to α -MSH is similar to that observed with the MC-3 receptor found in the brain. Future studies will be directed at determining the biological significance of these observations.

CS5 - Melanocyte Photobiology/Chemistry and UV protection

Report by Zalfa Abdel-Malek

Chairpersons: Zalfa Abdel-Malek, Masamitsu Ichihashi, Mauro Picardo

The melanocyte plays a central role in the cutaneous photobiological response to sun exposure. This

response is determined to a large extent by constitutive pigmentation and subsequently by the tanning ability that confers photoprotection against further sun-induced DNA damage.

Gilchrest and Eller described the melanogenic response of S91 melanoma cells to the introduction of small DNA nucleotides into these cells. These DNA fragments enhanced DNA repair and resulted in activation of the p53 pathway, which includes induction of p21, PCNA, XPA, XPC, GADD45, and tyrosinase.

Hill and Hill emphasized the differences in the responses of melanoma cells synthesizing different types of melanin (eu- versus pheomelanin) to different UV spectra. Their presentation also emphasized the importance of comparing survival curves, as well as mutagenicity following exposure to different UV spectra. The conclusion was that melanin may be a sensitizer or a photoprotector depending on the UV wavelength, form of melanin, and the assays used to determine the function of melanin.

The correlation between skin phototype and antioxidant levels are evaluated following sun exposure by Picardo et al. Although SOD/catalase ratio correlated with the MED, it was found that a specific allelic form of catalase is expressed at a high frequency in low skin phototypes.

Moro et al. reported that the differential sensitivity of melanocytes, keratinocytes, and fibroblasts to ultraviolet radiation (keratinocytes = fibroblasts > melanocytes) might explain the higher incidents of basal and squamous cell carcinoma than melanoma, and the photoaging response to sun exposure. Keratinocytes are also more sensitive than melanocytes to the cytotoxic effect of sodium arsenite, which results in basal and squamous cell carcinoma, hyperpigmentation, but not melanoma.

Smit et al. showed that in cultured human melanocytes, formation of cyclobutane dimers and 6,4-photoproducts in response to UVB light correlated directly with melanin content. However, no significant differences were found in the rates of DNA repair or in survival among melanocytes with different melanin contents.

CS6 - Ocular/Extracutaneous Melanin and Melanogenesis

Report by Dan-Ning Hu

A Symposium on Ocular/Extracutaneous Melanin and Melanogenesis was chaired by Dan-Ning Hu and Shosuke Ito. Dan-Ning Hu (USA) delivered the keynote lecture. The methods for cultivation and studying uveal melanocytes have been established in the past 10 years. Human uveal melanocytes grow well and produce melanin *in vitro*. The growth and melanogenesis of cultured uveal melanocytes are regulated by various biologic substances. The stimulators consist of some growth factors (etc. bFGF, HGF), Endothelin, adrenergic agonists and various prostaglandins. The inhibitors consist of TGF β , cholinergic agonists and IL-6. The uveal melanocytes are similar to epidermal melanocytes except in their response to α -MSH and ACTH. Both hormones stimulate the growth and melanogenesis of epidermal but not uveal melanocytes in cAMP-elevating agents deleted medium.

Giuseppe Prota (Italy) and Hu's work on types of ocular melanin was presented, they found that the iris pigment epithelium contains mainly eumelanin. Iridal melanocytes contain both eumelanin and pheomelanin, the ratio being variable depending on iris color. The pheomelanin/eumelanin ratio is higher in growing cultured uveal melanocytes than those in senescent cells. Kazumasa Wakamatsu (Japan) used an improved alkaline peroxide oxidation method to study neuromelanin. They found that neuromelanin consists mostly of dopamine with 10-20% incorporation of cysteine and that its structure is rather complex as compared to synthetic melanin.

Raymond Boissy (USA) reported that α -MSH does not stimulate the growth of uveal melanocytes *in vitro* and they could not detect receptors of α -MSH in uveal melanocytes by northern blot analysis and immunocytochemical studies. Khaled Abul-Hassen (UK) reported the transfer of gene to retinal pigment epithelium by a liposome-based method and to uveal melanocytes by adenoviral vector. After gene transfer, tyrosinase activity of melanocytes responds to various factors, but pigment epithelium lacks a factor for post transcriptional and/or translational modification of tyrosinase. Joan Roberts (USA) reported that cultured uveal melanocytes eliminate exogenous nitric oxide, an important regulator in ocular, neural and vascular tissues. Clearance of nitric oxide is directly related to melanin content. In the discussion, S. MacNeil (UK) mentioned that although α -MSH does not simulate growth and melanogenesis of cultured uveal melanocytes, intracellular calcium is elevated by α -MSH when the cell is cultured on fibronectin and adenosine agonist is added into the medium.

Hot Topics Symposium

Report by Vince Hearing

This Symposium was an innovation of the XVIIth IPCC and attempted to give a special forum to the most interesting and exciting abstracts submitted. As you know, all abstracts were judged blindly by the cochairs of each Symposium to select the best 3 to be presented orally in that Symposium. The highest

scoring abstract from each topic was then considered for the 'Hot Topics Symposium' and the top 5 were selected for this special forum (in other words, it was quite an honor to present here not to mention that fact speakers got an extra 5 minutes).

Robert Hoffman (a PASPCR member) presented the first paper and reported his group's attempts towards regulating pigmentation using a *tyrosinase* gene encapsulated within liposomes for delivery to follicular melanocytes. The *tyrosinase* gene used was from *Streptomyces* and was linked with an internal ribosome entry site to allow expression (the bacterial enzyme was used rather than the mammalian enzyme because of its significantly smaller size). Albino mouse skins in organ culture were used as targets and some hair repigmentation was observed following this gene therapy approach. Since hair and skin melanocytes are relatively accessible to such targeting, the potential use of such gene therapy is a tantalizing goal for correcting pigmentary defects. A similar approach using antisense strategy would potentially down-regulate pigmentation, and their future research is aimed at increasing the efficiency and application of this approach.

Bernhard Wehrle-Haller next reported his group's work on stem cell factor (SCF), an important paracrine signaling factor for melanoblast survival. Various segments of the cytoplasmic tail of SCF were deleted in order to characterize the role(s) of various motifs on intracellular processing and secretion by keratinocytes. As an approach to identify where the mutant SCFs went, he incorporated green fluorescent protein (GFP) into the extracellular part and used fluorescent microscopy to track their localization. Wild-type GFP-SCF was transported to the cell surface where c-kit (the receptor) positive melanocytes would bind to it. Deletion of various segments of the cytoplasmic tail identified a short sequence required for this specific processing and distribution. That sequence is completely conserved in avian and mammalian SCF and Dr. Wehrle-Haller postulates that this motif is required for SCF function and correct melanoblast localization during development. In co-culture experiments, melanocytes and melanoblasts actively associated with wild-type SCF expressing fibroblasts, but not with mutant SCF expressing fibroblasts, leading to the hypothesis that SCF is not only important for localization of melanoblasts towards SCF-positive cells, but also that it might play other roles in cross-talk between these types of cells, perhaps even facilitating melanosome transfer.

Masashi Kato next described their further studies with the *ret* transgenic system in which *ret*-overexpressing mice have high rates of spontaneous melanoma generation. He now reported that the increases in *ret* expression were accompanied by increases in MAP kinases and c-Jun, as well as in matrix metalloproteinases. Their group proposes that increased expression of *ret* disrupts normal intracellular signaling which may play a role in malignant transformation of melanocytes. He used this melanoma system as an appropriate model to examine the efficacy of agents targeted against melanoma, and he reports that an herbal medicine (active component = ???) delayed the onset of tumors, and significantly decreased metastasis of melanomas to various organs.

Miri Seiberg (another PASPCR member) reported interesting studies that are attempting to sort out factors responsible for melanosome transfer to keratinocytes. She reports that they have recently found that the protease activated receptor-2 (PAR2) is important to this exchange, and that agents which inhibit or stimulate the function of PAR2 have dramatic effects on melanosome transfer in vitro, and also in vivo in a Sinclair swine model. PAR2 is expressed on the surface of keratinocytes (not melanocytes) and seems to play an important role in regulating melanocyte-keratinocyte interactions. Her results further suggest that increased transfer has a feedback mechanism that stimulates the melanocytes to produce more melanin, and vice-versa. Physical contact between melanocytes and keratinocytes is essential for these effects and co-culture of those cells separated by a permeable membrane prevented those effects. This study is an important first step in understanding mechanisms involved in the physical transfer of pigment granules from melanocytes to keratinocytes, a process that heretofore has essentially been poorly understood.

Shigeru Sato presented the final paper in this session, which dealt with the characterization of the mutation in *Mitf* found in the *Black-Eyed White* mouse. As we know, *Mitf* not only plays an important role in the regulation of melanoblast development, but also plays an important role in regulating differentiation of functional melanocytes. Null mutants of *Mitf* have a complete loss of pigment cells in all normally pigmented tissues, but the *Black-Eyed White* mutation is an interesting one wherein mutant mice have a normally pigmented retinal pigment epithelium, but lack melanocytes in the skin and inner ear. This mutation was shown to result from an insertion into intron 3 of *Mitf*, which affected the distribution of *Mitf* isoforms. *Mitf* is now known to be transcribed in 3 active forms, *Mitf*-A (predominantly expressed in the RPE), *Mitf*-H (predominantly expressed in the heart) and *Mitf*-M (predominantly expressed in non-RPE melanocytes). The *Black-Eyed White* mutation affects the processing of these isoform mRNAs, leading to only slight decreases in *Mitf*-A and *Mitf*-H, but to complete down-regulation of *Mitf*-M, which explains its dramatic effects on melanocytes in the skin and inner ear, and lack of effect on RPE. This study demonstrates the critical nature of the *Mitf*-M isoform for normal melanocyte development.

In sum, this Hot Topics Symposium was a novel venue to highlight the most topical and important

abstracts submitted to the IPCC. It will be interesting to see if this becomes a traditional feature of future IPCC.

CS8 - Control of Hyper/Hypopigmentation

Report by Ray Boissy

The development of facultative cutaneous pigmentation is under the regulation of extramelanocytic factors generally produced by the keratinocytes of the epidermis, that can in turn be modulated by the environment, predominantly by ultraviolet irradiation. In order to begin to assess the facultative development of skin hyper/hypopigmentation analyses using co-cultures of melanocytes and keratinocytes is beginning to be exploited. Presentations in this seminar focused on such model systems. "Keratinocyte-melanocyte co-cultures and pigmented reconstructed epidermis as models to study skin pigmentation and its modulation" presented by R. Schmidt began the session. He reviewed techniques developed at L'Oreal to screen for inhibitors of melanization. He specifically discussed the following. [a] Cultured melanocytes are routinely developed using a medium reported by Dr. Olsson of Sweden designed for the culturing and expansion of vitiligo derived melanocytes. [b] Total cellular melanin was recovered from sonicated cells using a DEAE cellulose filter. [c] Keratinocyte-melanocyte co-cultures were established using a 10:1 ratio in keratinocyte specific medium resulting in successful melanosome transfer. [d] A technique using C¹⁴-thiouracil to assess rate of melanin synthesis was developed. [e] A solar simulator was used to irradiate melanocyte cultures and co-cultures in which the threshold for increased melanogenesis was lower in the latter condition demonstrating that keratinocytes are a major mediator of UV induced cutaneous hyperpigmentation. [f] A reconstructed skin equivalent model was presented which was utilized to investigate the effects of sunscreens and UVR, Kojic acid, IBMX, and melanocytes from various ethnic donors on pigmentation of the model system. M. Kim presented "Modulation of melanin neosynthesis by plant extracts in human keratinocyte-melanocyte co-culture system" in which a Transwell® system was used to test for diffusible molecules mediating the keratinocyte regulation of melanocytes. They demonstrated that the hypopigmentary effect of two specific plant extracts occurred only in the co-culture system and not in pure melanocyte cultures. The keratinocyte effect appeared to be modulated by IL-1 α , IL-5 and IL-6. A. Hachiya presented "A paracrine role of stem cell factor/c-kit linkage in UVB-induced pigmentation" which investigated the expression of SCF and c-kit in co-cultures. They demonstrated that transcript and protein expression of SCF (the membrane bound form only) and c-kit were upregulated by UVB in a dose dependent manner. This upregulation was confirmed immunocytochemically *in vivo*. In addition, using a guinea pig skin system, UVB-induced hyperpigmentation could be inhibited by subepidermal injection of anti-c-kit antibody.

Melanocyte stimulating hormone and the agouti protein are also extramelanocytic regulators of cutaneous pigmentation via the receptor, MC1R. Z. Abdel-Malek presented "The melanocortin-1 receptor is a key regulator of human cutaneous pigmentation" in which she reviewed data demonstrating that MSH and ASP could increase and decrease respectively the activity of cultured human melanocytes in response to ultraviolet irradiation. Significant data was presented demonstrating that cultured human melanocytes homozygous for an Arg160Trp mutation in the MC1R failed to respond to the effects of both MSH and ASP. ASP is a ten fold larger molecule than MSH. V. Virador presented "Bioactive domains and expression patterns of the agouti signal protein" in which the ASP was molecularly dissected to identify its bioactive domain. Synthetic 15mer segments of the ASP were assessed for their ability to reduce melanization of cultured melanocytes. A region containing KKVARPR, just outside of the basic domain of ASP, was most effective. A model was presented suggesting how ASP binds to the MC1R to alter the pocket used for MSH binding. In addition, a synthetic peptide (α PEP16) was developed which demonstrated reactivity in hair follicles consistent with ASP being secreted by keratinocytes.

Successful cutaneous pigmentation is the result of the transfer of melanosomes from the dendrite tip of the melanocytes to the neighboring keratinocytes. This process has remained molecularly elusive. G. Scott presented "Dendrite formation and melanosome transfer - beyond the electron microscope" which clearly demonstrated in transfection studies that a member of the Rho family of molecules associated with actin assembly (i.e., Rac 1) is involved as a downstream regulator of dendrite extension. UVR and MSH can also influence rac-1 function. Rac 1 function appears to be regulated by TIAM-1. Analysis of sucrose density purification of melanosomes demonstrated expression for specific SNARE attachment protein receptors for vesicle fusion (i.e., SNAP 23, SNAP 25, syntaxin 4, and VAMP-2) along with a SNARE associated docking protein (Rab 3a). These molecules putative mediate the fusion of the melanosome with the plasma membrane of the melanocyte and its transfer through the plasma membrane of the recipient keratinocyte. Regulation of these molecules may ultimately underlies the development of hyper/hypopigmentation of the skin and hair.

Satellite Meeting 4

Sentinel Node mapping and dissection in the Staging and Treatment of Melanoma.

Report by Alistair J Cochran

Chairs: Alistair J Cochran, M.D., FRCP, FRCPath, and Toshiaki Saida, M.D., Ph.D.

Meeting Program:

Lymphatic Mapping and Sentinel Node Surgery, Alistair J. Cochran, M.D., FRCP, FRCPath, Department of Pathology and Laboratory Medicine, 13-145 CHS, UCLA School of Medicine, Los Angeles, California, 90095.

Sentinel Node Dissection for Melanoma, Richard Essner, M.D., FACS, Assistant Director of Surgical Oncology, John Wayne Cancer Institute, Santa Monica, California.

The role of Lymphoscintigraphy and Other Radionuclide Techniques in the Localization of Sentinel Lymph Nodes, E.C. Glass, R. Essner, A.J. Cochran, Haigh, D. Morton, John Wayne Cancer Institute, Santa Monica, California.

Occult melanoma micrometastases in the regional lymph nodes: Their detection, distribution and clinical implication, Minoru Takata and Naohito Hatta, Department of Dermatology, Kanazawa University School of Medicine, Kanazawa, Japan.

Clinical Experience with the Sentinel Node Technique in Japanese Patients with Melanoma, Naoya Yamazaki National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-Ku, Tokyo 104-0045, Japan.

Clinical Experience with the Sentinel Node Technique in Non-melanoma Skin Cancers, Yoshio Kiyohara, Masahito Taguchi, Tadashi Suzuki, Tetsuya Tsuchida, Department of Dermatology, Saitama Medical School, Saitama, Japan

Dr Cochran presented the background and significance of techniques that were the subject of the meeting. The management of high-risk (deep, thick) primary melanoma, clinically localized to the primary site, has been unsatisfactory. Treatment options were elective nodal dissection, an operation with substantial morbidity, that is probably unnecessary for 80% of patients or a policy of "wait and see" that delays definitive treatment for the 20% of patients who eventually recurred. Lymphatic mapping and the selective removal and close evaluation of the sentinel node (SN) provides a more rational approach. The symposium brought together experts who were active in originating and developing the techniques and clinicians involved in their application. Areas covered included localization of the SN, evaluation of the SN for tumor by histology, immunohistology and molecular techniques and the treatment and prospects of patients with tumor-positive SN. Current clinical experience in the US and Japan was presented.

Dr Essner described the surgical aspects of the approach noting that success requires close cooperation by skilled surgical, nuclear medicine and pathology personnel. There are three separate components: mapping of the direct lymphatic route from the primary site to the regional lymph node basin, identification of the first lymph node in that basin that receives lymph via the direct lymphatic (the sentinel node) and selective excision of the SN, leaving the non-sentinel nodes in place. Complete lymph node dissection is reserved for individuals with tumor in the SN, those most likely to benefit from node dissection. Identification of the SN originally depended on visualization of the afferent lymphatics and target node based on their blue color after injection of a blue dye (isosulfan blue). The technique is more accurate and easier to master when the blue dye is combined with preoperative identification of the lymphatics and SN by early read (dynamic) lymphoscintigraphy and intraoperative detection of the SN by a hand-held gamma probe. It was stressed, however, that the technique requires an initial learning period during which the performance of surgeon, pathologist and nuclear medicine personnel will improve quite rapidly. Since its development by Morton et al (1992) the effectiveness of the approach has been widely validated by other workers. The technique has also been applied to many other tumors, such as breast cancer, vulvar cancer and colon cancer. Lymphatic mapping and sentinel lymph node evaluation is at least as efficient as elective node dissection as a staging technique and may be superior as it permits the pathologist to focus on a limited number of nodes and apply special approaches, such as immunohistology and molecular pathology to this limited target. Two major studies are in progress. The Multicenter Selective Lymphadenectomy Trial randomizes patients to receive either wide local excision or wide local excision and lymphatic mapping and selective lymphadenectomy. If the sentinel node contains tumor the patient receives a complete lymphadenectomy. The Sunbelt Melanoma Trial is examining the role of SN evaluation in determining patients likely to benefit from treatment with interferon-alpha. From the results of these trials the therapeutic role of selective lymph node dissection will become clear.

Dr Glass provided an overview of the role of the nuclear medicine physician. Preoperative lymphoscintigraphy (LS) is essential to avoid the errors implicit in clinical assessment of likely lymphatic drainage patterns, to chart the route of the afferent lymphatic and to identify the SN within the relevant

nodal basin. The site of the SN can then be marked on the skin to facilitate placement of the surgical incision. Preoperative LS requires the intradermal injection of radiopharmaceuticals such as Tc99m antimony trisulfide colloid, Tc99m albumin nanocolloid or Tc99m sulfur colloid. Optimal agents feature rapid migration times, good retention in the SN, minimal spillover into non-sentinel nodes and low radiation dose to the patient. After intradermal injection imaging with a scintillation camera should begin immediately to capture images of the afferent lymphatics and SN. Body outlines and the position of the SN are to be clearly marked to assist the surgeon in localizing his approach. The optimal arrangement is to undertake surgery within two hours of LS, obviating the need for a second perioperative injection of isotope. Unusual drainage paths are not uncommon. Special attention is to be paid to cervical, facial, epitrochlear, popliteal, umbilical and scapular nodes.

Problems encountered by new users are most often due to relatively simple factors. These include the use of excessive tracer (> 18MBq), too long a period between tracer injection and imaging, poor positioning of the patient, failure to mark the outline of the body and failure to accurately mark the position of the SN on the skin. With attention to detail and moderate experience satisfactory results may be expected.

Dr Cochran outlined the role of the pathologist who, once the surgeon and nuclear medicine physician have identified and removed the SN, has the critical responsibility of determining whether the SN contains tumor. A single SN will be provided from 67% of patients, two SN from 25% and three or more from the remaining 12%. Because of the relative difficulty of interpreting frozen section material and the likelihood of losing diagnostic material because of distortion during freezing and thawing and tissue removal to obtain "full face" sections the use of frozen sections is not recommended. Sentinel nodes are carefully bisected through their longest circumference and ten serial full-face sections removed from each cut face. Sections 1, 3, 5 and 10 are stained with hematoxylin and eosin, section 2 with S100 and section 4 with HMB-45. Tumor cells are to be separated from capsular and trabecular nevus cells, paracortical dendritic cells, macrophages and intranodal nerves. It is essential to use immunohistochemistry to avoid missing up to 24% of positive SN. In numerous studies between 18-20% of SN have been found to contain tumor. The risk to personnel from technetium 99 at the doses used is considered slight, but standard radiation precautions should be utilized. Studies with molecular biology approaches are of great interest, but have not yet become standard. In providing material for experimental studies pathologists should divide the tissues in such a manner that the diagnostic process is not compromised.

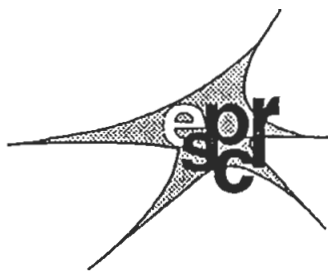
Drs Takata and Hatta reported a study of 436 nodes from 32 patients. HMB-45 detected tumor in the nodes of 15/24 patients who were negative by HE. In 23 nodes nested PCR for tyrosinase was more sensitive than a combination of single round PCR for the melanoma associated markers tyrosinase, MART-1, Pmel-17, TRP-1 and TRP-2. Tyrosinase mRNA was detected in 6 nodes that were negative by HE and HMB-45.

Using HMB-45 and nested RT-PCR for m-tyrosinase, maps of distribution of microscopic and "submicroscopic" metastases were generated for 13 inguinal basins. The authors reported considerable variation in the distribution patterns of occult metastases in the node basins studied. The authors conclude that the main role of molecular biology is to identify the subgroup of patients whose nodes are negative by histology and molecular biology and who require no additional treatment.

Dr Yamazaki reported on clinical experience at the National Cancer Center Hospital, Tokyo. In a study of 14 patients with acral lentiginous melanoma, using the dye patent blue V alone, the SN was identified in 13/14 cases. Sensitivity and accuracy were similar to those reported previously and the authors found the approach "feasible and successful."

Dr Kiyohara and his colleagues reported their experience with the techniques in the management of 24 patients with non-melanoma skin cancer (squamous cancer, porocarcinoma, invasive Bowen's disease and invasive Paget's disease) at Saitama Medical School. In 11 cases the SN contained tumor and a complete lymph node dissection was undertaken. In no case was a non-sentinel node found positive in the presence of a negative sentinel node. A single sentinel node was found in 76% of patients and two in 24%. The authors conclude that the approach is "reasonable and reliable."

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2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

Tsatmali M et al have investigated the effect of the treatment of melanocytes with α -MSH on the nitric oxide production in response to ultraviolet and lipopolysaccharide. In B16 mouse melanoma cells and in human melanocytes, the pre-incubation with the α -MSH induce a bipolar effect: at concentration from 10^{-11} to 10^{-9} the treatment produced an enhancement of stimulation, indeed at concentration in excess to 10^{-9} induced a decrease on nitric oxide production. α -MSH produced a contrasting effects also on the induction of nitric oxide synthetase with an inhibition of lipopolysaccharide-mediated induction and an enhanced effect those UV-determined. These evidences suggest that α -MSH regulate nitric oxide production in melanocytes by modulating the stimulation of inducible nitric oxide synthetase. Peters EM et al from the group of K Schallreuter, have studied the mechanism with the melanosomes produce pro-opiomelanocortin-related peptides in their acidic environment which could be essential for the melanogenic effect of α -MSH. Recently, the same group have reported that α -MSH could directly activate tyrosinase by removing the allosteric regulator 6(R)-L-erythro 5,6,7,8, tetrahydrobiopterin resulting in a stable α -MSH/6(R)-L-erythro 5,6,7,8, tetrahydrobiopterin complex. The authors report the presence of the entire system for pro-opiomelanocortin processing in melanosomes, with the pH of these organelles optimal for the activity of prohormone convertases 1 and 2 (PC1 and PC2) that seems to be the key for the entire process. Therefore, these results are in agreement with an autocrine production and recycling of the cofactor 6(R)-L-erythro 5,6,7,8, tetrahydrobiopterin in melanocytes.

In contrast to the large number of studies on the stimulatory effects of a number of hormones on pigmentation, very few studies have reported on signalling molecules that lower tyrosinase activity. Fuller BB et al have examined the effect of various alpha and beta adrenergic agonists and antagonists on tyrosinase activity in human melanocyte cultures. Because the alpha-2 adrenergic receptor antagonist yohimbine acts to block the lowering of cAMP by alpha-2 adrenoceptor agonists, they carried out studies to determine whether yohimbine might increase tyrosinase activity in human melanocytes. The treatment with yohimbine induced a marked down-regulation of tyrosinase activity. The inhibition was dose dependent, reversible and occurred in human melanocytes derived from either black or white skin types, and also in mouse melanoma cells. The experimental evidences suggest that the yohimbine has not effect on cell proliferation, cellular translation, DNA synthesis or intracellular levels of cAMP; moreover, an intact cell was required for yohimbine action and the drug did not act as a direct inhibitor of the enzyme. Therefore, the authors concluded that yohimbine can act through an yet unidentified signalling pathway to reduce the catalytic activity of pre-existing tyrosinase molecules present in melanosomes.

Several researchers have reported increase in the melanin synthesis of cultured melanoma cells and melanocytes on the addition of histamine in order to evaluate a possible role of this mediators in post-inflammatory high pigmentation, Yoshida M et al, attempted to evaluate the effect of histamine on the melanogenesis of human cultured melanocytes. Treatment of human melanocytes with histamine induced an increase in tyrosinase activity and a subsequent increment in melanin synthesis. These stimulatory effects of histamine were completely inhibited by famotidine, an H2 antagonist. Moreover, an intracellular cAMP accumulation and a subsequent protein kinase A (PKA) activation in histamine-treated melanocytes were found. Therefore, the authors suggest that the melanogenic activity of histamine is specifically mediated by protein kinase A activation via H2 receptors.

It is well-known that the pigmentation is the result of the interaction between melanocytes, that produce the melanin, and keratinocytes, that are the pigment recipients. Seiberg M et al have studied some aspects of the molecular mechanism of melanosome transfer. They have observed that the protease-activated receptor 2 (PAR-2) expressed on keratinocytes, but not in melanocytes, is involved in melanosome transfer and therefore may regulate pigmentation. The pigmentation PAR-2-induced seems to need an intimate cell-cell contact. Also the depigmenting effect, determined by the inhibition of PAR-2 activation, required a keratinocyte-melanocyte contact. These experiments showed a novel mechanism for the regulation of the pigmentation, mediated by the activation or inhibition of the keratinocyte receptor PAR-2.

On the possible mechanism of disappearance of melanocytes in vitiligo Yang F et al from the group of Raymond Boissy investigate the mechanism of the 4-TBP induced cytotoxicity. 4- tertiary butylphenol (4-TBP) appears to be the most potent phenolic derivative causing occupational vitiligo that is morphologically indistinguishable from idiopathic vitiligo. For this reason the melanocyte treatment with 4-TBP was considered a valid experimental model for the vitiligo study. Melanocyte cultures derived from African-American and Caucasian donors, with a 3 fold difference in tyrosinase activity and 14-fold difference in melanin content, demonstrated a comparable concentration-dependent sensitivity to 4-TBP indicating that tyrosinase does not mediate this cytotoxicity. Moreover, the authors have demonstrated by three different assays, that the death of the melanocytes follow an apoptotic process.

All melanomas produced, both in vitro than in vivo, the basic fibroblast growth factor (bFGF), whereas normal melanocytes require exogenous bFGF for growth. Nesbit M et al have transduced normal human melanocytes to overexpress two forms of bFGF: (bFGF-Long and B-FGF-Short) using replication-deficient 5 vectors. Like primary melanoma cells, transduced normal melanocytes grew anchorage independently in soft agar. These results showed that bFGF upregulation is a critical component in melanoma progression.

To understand how the melanoma cells escape senescence, it is essential to define what genes control senescence in the normal cell. Haddad MM et al have exposed human melanocytes to high levels of cAMP and have observed an accumulation of melanin and terminal differentiation. In particular, they present evidence that activation of a cAMP pathway correlates with multiples changes in these cells, as increased melanogenesis; increased association of the cyclin-dependent kinase inhibitors (CDK-Is) p27 (KIP1) and p16 (INK4) with CDK2 and CDK4, respectively; loss of E2F DNA-binding activity; and phenotypic changes characteristics of senescent cells. They postulated that the disruption of cAMP-mediated and melanogenesis-induced senescence may cause immortalization of human melanocytes, an early step in the development of melanomas.

A special issue of Cellular Molecular Biology collect the principal studies presented in the last International Pigment Cell Conference held in Nagoya. Among the paper reported there is those of Loir B et al on a semiquantitative RT-PCR assay for melanocortin 1 receptor (MC1-R) in mammalian melanocytes, the paper of Bowers RR et al on the role of the antioxidants in the survival of normal and vitiliginous avian melanocytes; the presentation of Martinez-Esparza M et al on the tyrosinase regulation by α -MSH in the presence of the hypopigmenting cytokines, such as TGF beta 1 and TNF alpha; the paper of Benathan M et al on the role of the balance of CysH and GSH in regulating the levels of 5-S-CD and DOPA and the melanogenic activity of pigment cells and the presentation of the group of Alain Taieb on the use of reconstructed epidermis to study pigmentation and photoprotection.

The new Editorial management of Pigment Cell Research has renewed and improved the Journal. In the last issue is reported an important review on the regulation of melanocyte proliferation from Ruth Halaban. Thorneby-Andersson et al reported the chemical characterization of the oxidation products of 4-ter-butylphenol and 4-ter butylcatechol by tyrosinase. The authors, using a cell free system, have demonstrated the generation of quinones following exposure of the TBP and TBC to tyrosinase capable of reacting with cysteine or GSH suggesting that these events could be implicated in the depigmentary activity of these compounds. The group of Giovanni Orecchia has described higher plasma level of catecholamine and of their metabolites in the early phase of non segmental vitiligo indicating an increased activity of monoamionergic systems possibly due to stressful events.

The authors suggest that the increase could represent an epiphenomenon rather than an important cause of the depigmentation.

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Remark : Many interesting papers in these fields, selected from the last ESPCR Conference, were published in the november 99 issue of *Cell. Mol. Biol*. 45(7).

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5. Neuromelanins

(Prof. M. d'Ischia)

The structure of neuromelanin and its interaction with iron under normal and pathological conditions, i.e. Parkinson's disease, are the subject of two interesting papers which appeared in the beginning of 2000. Zecca et al. isolated neuromelanin from human substantia nigra using different procedures, and found a peptide component covalently bound to the melanic structure corresponding to approximately 15% of the neuromelanin weight.

Moreover, they found that neuromelanin can absorb specifically lipid molecules, approximately 20% of its weight, including cholesterol. On this basis the authors postulate novel possible functional roles of neuromelanin depending on its ability to bind lipidic cellular components. Using electron paramagnetic spectroscopy (EPR) Lopiano et al. examined the organization of iron in NM and showed it to be in the form of polynuclear superparamagnetic/antiferromagnetic aggregates. The lack of one or more signals in the EPR spectra of a pigment preparation from a Parkinsonian patient suggested a decreased ability of the pigment to bind iron in pathological substantia nigra. Minor structural differences in the iron binding sites of neuromelanin in controls and in Parkinson's disease were also determined by monitoring the Mn (II) signal in the Q-band spectra.

Drukarch and van Muiswinkel proposed a neuroprotective treatment strategy for Parkinson's disease based on stimulation of pathways implicated in the detoxication of DA-derived quinones produced during autoxidative processes in the brain. In particular the authors suggest use of dithiolethiones, phenolic anti-oxidants, and isothiocyanates to upregulate enzymes such as NAD(P)H:quinone oxidoreductase (NQO) and glutathione transferase(s), both of which are expressed in the human substantia nigra.

Finally, Matsunaga et al. reported evidence showing that macrophage migration inhibitory factor (MIF) is able to catalyze the conversion of dopaminechrome and norepinephrinechrome, oxidation products of the neurotransmitters dopamine and norepinephrine, respectively, to indole derivatives that may serve as precursors to neuromelanin. This and other results are taken to suggest possible roles of MIF in detoxification of catecholamine toxic products and neuromelanin formation.

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(Prof. J.C. Garcia-Borrón)

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Inhibition of tyrosinase by flavonoids, stilbenes and related 4-substituted resorcinols: structure-activity investigations. *Planta Med*. 66(1):11-5, 2000.
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Characterization of a new phenoloxidase inhibitor from the cuticle of *Manduca sexta*. *Biochem Biophys Res Commun*. 268(2):379-83, 2000.
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Tyrosinase-mediated formation of a reactive quinone from the depigmenting agents, 4-tert-butylphenol and 4-tert-butylcatechol. *Pigment Cell Res*. 13(1):33-8, 2000.
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Expression of genes for microphthalmia isoforms, Pax3 and MSG1, in human melanomas. *Cell Mol Biol (Noisy-le-grand)*. 45(7):1075-82, 1999.
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Tyrosinase catalysed biphenyl construction from flavan-3-ol substrates. *Phytochemistry*. 53(2):285-92, 2000.
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New EIA technique for tyrosinase in human melanocytes and its application. *Life Sci*. 66(1):PL1-6, 2000.
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Cloning and overexpression of a tyrosinase gene mel from *Pseudomonas maltophilia*. *FEMS Microbiol Lett*. 185(1):23-27, 2000.
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Albinism: its implications for refractive development. *Invest Ophthalmol Vis Sci*. 41(1):1-7, 2000.
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The cytotoxicity and apoptosis induced by 4-tertiary butylphenol in human melanocytes are independent of tyrosinase activity. *J Invest Dermatol*. 114(1):157-64, 2000.
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Histamine induces melanogenesis and morphologic changes by protein kinase A activation via H2 receptors in human normal melanocytes. *J Invest Dermatol*. 114(2):334-42, 2000.

8. Melanosomes

(Dr. J. Borovansky)

Two reviews concerning the role of microtubule- and actin-associated motor proteins and their role in melanosome transport (Lambert et al., Tuma and Gelfand) and two reviews summarizing the genetic control of melanosomal organization (Aldaihari et al., Hearing) have appeared recently. Original papers added to our knowledge of proteins and enzymes associated with melanosomes (Oh et al., Samaraweera et al., Borovansky and Hach) and brought new data on the relationship of melanosomes to lysosomes (Schraermeyer et al., Thumann et al). Retinal pigment epithelium melanosomes were shown to stimulate the photooxidation of unsaturated fatty acids (Dontsov et al.).

- Aldaihari Z, Olivry T, Ortonne JP.
Melanocytogenesis and melanogenesis: genetic regulation and comparative clinical diseases. *Veterinary Dermatol* 10: 3-16, 1999.
Comments: Review devoted to melanocytogenesis and melanogenesis with emphasis to hereditary hypomelanoses (colour illustrations) and to genes influencing pigmentation both at the cellular and subcellular level.
- Borovansky J, Hach P.
Disparate behaviour of two melanosomal enzymes (α -mannosidase and γ -glutamyltransferase). *Cell mol Biol* 45(7): 1047-1052, 1999.
Comments: α -mannosidase (acid, lysosomal type) and γ -glutamyltransferase (GGT) activities were detected and studied in melanosomes and premelanosomes isolated from B16 melanoma. α -mannosidase was firmly immobilized in the melanosome matrix whereas the membrane bound GGT was easily released with detergents. Unlike the α -mannosidase, the GGT serum levels were increasing in relation to the melanoma growth.
- Carlson JA, Healy K, Slominski A, Mihm Jr MC.
Melanocytic matricoma: A report of two cases of a new entity. *Am J Dermatopathol* 21(4): 344-349, 1999.
Comments: Presence of premelanosomes is common in the neoplastic cells of tumours of neural crest origin (cf. Kameyama et al) or in tumours containing benign passage melanocytes. Two cases of a unique neoplasm composed of matrical cells and melanocytes recapitulating epithelial-melanocyte interaction (typically occurring in the follicular anagen bulb) are described.
- Dontsov AE, Glickman RD, Ostrovsky MA.
Retinal pigment epithelium pigment granules stimulate the photo-oxidation of unsaturated fatty acids. *Free Rad Biol Med* 26(11-12): 1436-1446, 1999.
Comments: Melanosomes, melanolipofuscin and lipofuscin granules isolated from human and bovine RPE stimulated fatty acid peroxidation when irradiated with visible light, with the melanosomes exhibiting the greatest light-induced activity. An attempt to calculate the concentration of melanosomes, melanolipofuscin and lipofuscin granules in the human RPE.
- Duchod J.
Tradition of pigment cell research at Charles University in Prague. *Cell mol Biol* 45(7): 883-894, 1999.
Comments: Historical review covering various aspects of pigment cell research including melanosomes. The first piece of evidence that melanosomes consist of several proteins came from Prague.
- Fetisov SO, Barcza MA, Megud MM, Oler A.
Hypophysial and meningeal melanocytes in the Zucker rat. *Pigment Cell Res* 12(5): 323-330, 1999.
Comments: Zucker rats possess functionally active melanocytes producing lamellar stage II-IV melanosomes in the leptomeninges and hypophysis. Numerous pigment granules were observed in the extracellular matrix of the pia matter around melanocytes and within the cytoplasm of pial, glial and neural cells of the brain.
- Hearing VJ.
Biochemical control of melanogenesis and melanosomal organization. *J Invest Dermatol Symp Proc* 4(1): 24-28, 1999.
Comments: Review summarizing current knowledge on the regulation of mammalian pigmentation at the genetic and biochemical level with a special attention to melanosomal proteins (and their potential use in immunologic targeting of malignant melanoma) and to the genes encoding them.
- Kameyama M, Ishikawa Y, Shibahara T, Kadota K.
Melanotic neurofibroma in a steer. *J Vet Med Sci* 62(1): 125-128, 2000.
Comments: Melanotic neurofibroma is a tumour of neural crest origin showing schwannian and perineurial differentiation with ectopic production of granular melanosomes (stage II-IV) often with uneven deposition of melanin.
- Lambert J, Vancoillie G, Naeyaert JM.
Molecular motors and their role in pigmentation. *Cell mol Biol* 45(7): 905-918, 1999.

Comments: Detailed review dealing with microtubule- and actin-based organelle transport, microtubule-associated and actin-based motor proteins and with their roles in melanosome transport.

- Nobukawa B, Fujii H, Hirai S, Kumasaka T, Shimizu H, Matsumoto T, Suda K, Futagawa S.
Breast carcinoma diverging to aberrant melanocytic differentiation – A case report with histopathologic and loss of heterozygosity analyses. *Am J Surg Pathol* 23(10): 1280-1287, 1999.
Comments: Histopathologic description of a case of primary breast carcinoma showing differentiation to malignant melanoma. EM analysis revealed the presence of typical lamellar premelanosomes and melanosomes in the melanoma component.
- Oh J, Liu ZX, Feng GH, Raposo G, Spritz RA.
The Hermansky-Pudlak syndrome (HPS) protein is part of a high molecular weight complex involved in biogenesis of early melanosomes. *Human Mol Genetics* 9(3): 375-385, 2000.
Comments: Hermansky-Pudlak protein consisting of 700 amino acids is neither N-glycosylated nor an integral membrane protein. In pigment cells the protein is divided between a cytosolic 200kDa complex and a >500kDa membrane-associated complex localized in association with tubulovesicular and non-coated vesicular structures in the region of the Golgi apparatus and with the peripheral membrane of nascent and early stage melanosomes.
- Samaraweera P, Donatien PD, Qazi S, Kobayashi T, Hearing VJ, Panthier JJ, Orlow SJ.
Identification and characterization of a melanocyte-specific novel 65-kDa peripheral membrane protein. *Eur J Biochem* 266(3): 924-934, 1999.
Comments: Using an antiserum raised against the Triton X100 insoluble fraction of melanosomes from a murine melanoma cell line a novel 65kDa protein was identified. Combined biochemical and histochemical approach suggested that the protein was preferentially associated with the cytoplasmic face both of stage I,II melanosomes and vesicles.
- Schraermeyer U, Peters S, Thumann G, Kociok N, Heimann K.
Melanin granules of retinal pigment epithelium are connected with the lysosomal degradation pathway. *Exp Eye Res* 68(2): 237-245, 1999.
Comments: Gold granules were detected inside early stage and mature melanosomes after subretinal injection of the gold-labelled rod outer segments in rats. Latex beads fused with melanosomes in cultured RPE cells. Evidence for the existence of proton pump in melanosomes was derived from the experiments with chloroquine.
- Thumann G, Bartz-Schmidt KU, Kociok N, Heimann K, Schraermeyer U.
Ultimate fate of rod outer segments in the retinal pigment epithelium. *Pigment Cell Res* 12(5): 311-315, 1999.
Comments: Gold-labelled rod outer segments (ROS) were injected into the subretinal space of rabbits and the gold granules were later localized inside melanosomes. The results suggested that melanosome formation is a pathway within RPE cells to degrade ROS and to handle non-degradable substances.
- Tuma MC, Gelfand VI.
Molecular mechanisms of pigment transport in melanophores. *Pigment Cell Res* 12(5): 283-294, 1999.
Comments: Review describing cooperation of microtubules with actin in the transport of melanosomes and kinesin II, myosin V and dynein as three motors involved in pigment granule movement. Putative mechanisms of regulation of pigment transport are discussed.
- Wang SQ, Bartido S, Yang G, Qin J, Moroi Y, Panageas KS, Lewis JJ, Houghton AN.
A role for a melanosome transport signal in accessing the MHC class II presentation pathway and in eliciting CD4(+) T cell responses. *J. Immunol* 163(11): 5820-5826, 1999.
Comments: Melanoma cell membranes have been known to share antigens with melanosomes. To explain mechanism(s) of this phenomenon a fusion product containing melanosomal transport signal from gp75/TRP-1 and ovalbumin as a reporter antigen was expressed as intracellular membrane protein, which was sorted to the endocytic pathway, processed and presented by class II MHC molecules.

9. Melanoma experimental, Cell culture

(Dr. N. Smit)

A. Melanoma cytotoxicity, experimental

In the paper by Gili et al phenolic thioether amines were tested for their cytotoxicity towards melanoma and neuroblastoma cell cultures. The toxicity of the compounds was correlated to the tyrosinase activity in the melanoma cells and tyrosine hydroxylase activity in the neuroblastoma cells. The results indicate a non-tyrosinase-mediated mechanism for the compounds in addition to their tyrosinase-mediated toxicity. Another report (Yang et al) also describes that the cytotoxicity of a phenolic compound (4-tertiary butylphenol) is not mediated by tyrosinase. On the other hand in the paper by Thomas et al the selective action towards melanoma cells of sulfur containing phenol

derivatives is considered to be the result of oxidative stress induced by tyrosinase mediated oxidation of the drugs. In our paper (Smit et al) we describe some aspects of DT-diaphorase, a two-electron quinone reductase in relation to its function in melanogenesis. Inhibition of this enzyme in melanoma cells induces cytotoxicity. In the paper by Schulz and Skibo structural variants of pyrrolo [1, 2-a] benzimidazole are described which were designed to inhibit topoisomerase II. The active quinone forms can be deactivated by DT-diaphorase. Prevention of the inactivation of the compounds by DT-diaphorase was aimed at increasing the cytotoxicity of these new variants. One of the quinones showed toxicity selectively against melanoma cell cultures. In the paper by Xing et al N-unsubstituted indoles and cyclopent[b]indoles were tested for their cytotoxicity making use of DT-diaphorase as the activating enzyme. A selectivity for melanoma and other cancers was found depending on the substituents present.

- Bandak S, Goren D, Horowitz A, Tzemach D, Gabizon A.
Pharmacological studies of cisplatin encapsulated in long-circulating liposomes in mouse tumor models. *Anticancer Drugs* 10(10):911-20, 1999.
- Campbell WE, Nair JJ, Gammon DW, Codina C, Bastida J, Viladomat F, Smith PJ, Albrecht CF.
Bioactive alkaloids from *Brunsvigia radulosa*. *Phytochemistry* 53(5):587-91, 2000.
- Crosasso P, Ceruti M, Brusa P, Arpicco S, Dosio F, Cattel L.
Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. *J. Controlled Release* 63(1-2):19-30, 2000.
- Dassonneville L, Wattez N, Mahieu C, Colson P, Houssier C, Frederich M, Tits M, Angenot L, Bailly C.
The plant alkaloid usambarensine intercalates into DNA and induces apoptosis in human HL60 leukemia cells. *Anticancer Res.* 19 (6B):5245-50, 1999.
- Futami J, Seno M, Ueda M, Tada H, Yamada H.
Inhibition of cell growth by a fused protein of human ribonuclease 1 and human basic fibroblast growth factor. *Protein Eng.* 12(11):1013-9, 1999.
- Gili A, Thomas PD, Ota M, Jimbow K.
Comparison of in vitro cytotoxicity of N-acetyl and N-propionyl derivatives of phenolic thioether amines in melanoma and neuroblastoma cells and the relationship to tyrosinase and tyrosine hydroxylase enzyme activity. *Melanoma Res.* 10(1):9-15, 2000.
- Hasegawa H, Lee KS, Nagaoka T, Tezuka Y, Uchiyama M, Kadota S, Saiki I.
Pharmacokinetics of ginsenoside deglycosylated by intestinal bacteria and its transformation to biologically active fatty acid esters. *Biol. Pharm. Bull.* 23(3):298-304, 2000.
- Hirpara JL, Seyed MA, Loh KW, Dong H, Kini RM, Pervaiz S.
Induction of mitochondrial permeability transition and cytochrome C release in the absence of caspase activation is insufficient for effective apoptosis in human leukemia cells. *Blood* 95(5):1773-80, 2000.
- Hoogerhout P, Stittelaar KJ, Brugghe HF, Timmermans JA, ten Hove GJ, Jiskoot W, Hoekman JH, Roholl PJ.
Solid-phase synthesis and application of double-fluorescent-labeled lipopeptides, containing a CTL-epitope from the measles fusion protein. *J. Pept. Res.* 54(5):436-43, 1999.
- Jordan AM, Khan TH, Osborn HM, Photiou A, Riley PA.
Melanocyte-directed enzyme prodrug therapy (MDEPT): development of a targeted treatment for malignant melanoma. *Bioorg. Med. Chem.* 7(9):1775-80, 1999.
- Juranic Z, Anastasova F, Juranic I, Stanojkovic T, Radulovic S, Vuletic N.
Antiproliferative action of isatine-beta-thiocarbohydrazone and N-ethylisatine-beta-thiocarbohydrazone on human PBMC and on two neoplastic cell lines. *J. Exp. Clin. Cancer Res.* 18(3):317-24, 1999.
- MacEwen EG, Kurzman ID, Vail DM, Dubielzig RR, Everlith K, Madewell BR, Rodriguez COJ, Phillips B, Zwahlen CH, Obradovich J, et al.
Adjuvant therapy for melanoma in dogs: results of randomized clinical trials using surgery, liposome-encapsulated muramyl tripeptide, and granulocyte macrophage colony-stimulating factor. *Clin. Cancer Res.* 5(12):4249-58, 1999.
- McClay EF, Jones JA.
Characterization of a human melanoma cell line with non-oestrogen receptor dependent tamoxifen resistance. *Melanoma Res.* 9(6):531-7, 1999.
- Ryu CK, Kang HY, Yi YJ, Lee CO.
Cytotoxic activities of 6-arylamino-7-halo-5,8-quinolinediones against human tumor cell lines. *Arch. Pharm.*

Res. 23(1):42-5, 2000.

- Schulz WG, Skibo EB.
Inhibitors of topoisomerase II based on the benzodiazole and dipyrroloimidazobenzimidazole ring systems: controlling DT-diaphorase reductive inactivation with steric bulk. *J. Med. Chem.* 43(4):629-38, 2000.
- Smit NP, Hoogduijn MJ, Riley PA, Pavel S.
Study of DT-diaphorase in pigment-producing cells. *Cell Mol. Biol. (Noisy-Le-Grand)* 45(7):1041-6, 1999.
- Suh ME, Park HK, Yoo HW, Lee CO.
Cytotoxic effects of pyridino[2,3-f]indole-4,9-diones on human tumor cell lines. *Biol. Pharm. Bull.* 23(3):354-5, 2000.
- Thomas PD, Kishi H, Cao H, Ota M, Yamashita T, Singh S, Jimbow K.
Selective incorporation and specific cytotoxic effect as the cellular basis for the antimelanoma action of sulphur containing tyrosine analogs. *J. Invest. Dermatol.* 113(6):928-34, 1999.
- Ueno T, Ohtawa K, Kimoto Y, Sakurai K, Kidera Y, Hiroto M, Matsushima A, Nishimura H, Inada Y.
Polyethylene glycol-modified concanavalin A as an effective agent to stimulate anti-tumor cytotoxicity. *Cancer Detect. Prev.* 24(1):100-6, 2000.
- Wang H, Ng TB, Ooi VE, Liu WK.
Effects of lectins with different carbohydrate-binding specificities on hepatoma, choriocarcinoma, melanoma and osteosarcoma cell lines. *Int. J. Biochem. Cell Biol.* 32(3):365-72, 2000.
- Xing C, Wu P, Skibo EB, Dorr RT.
Design of cancer-specific antitumor agents based on aziridinylcyclopent[b] indoloquinones. *J. Med. Chem.* 43(3):457-66, 2000.
- Yang F, Sarangarajan R, Le P, I, Medrano EE, Boissy RE.
The cytotoxicity and apoptosis induced by 4-tertiary butylphenol in human melanocytes are independent of tyrosinase activity. *J. Invest. Dermatol.* 114(1):157-64, 2000.
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Roxithromycin and clarithromycin, 14-membered ring macrolides, potentiate the antitumor activity of cytotoxic agents against mouse B16 melanoma cells. *Cancer Lett.* 147(1-2):17-24, 1999.

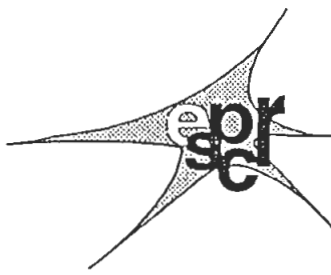
B. Culture systems to study melanocytes

A special issue of *Cell. Mol. Biol.* was devoted to papers presented at the 8th ESPCR meeting in Prague, 1998. Two papers (Cario-Andre et al and Regnier et al) describe the use of reconstructed epidermis to study melanogenesis in a system that most closely resembles that of melanocytes in skin. Different skin type melanocytes can be investigated in this system and also the effects of stimulators and inhibitors of melanogenesis (Regnier et al). Also pathological conditions, like vitiligo and nevi may be studied in this system (Cario-Andre et al). An example of such an approach is described by Eves et al who studied the invasion of melanocytes and one melanoma cell line in a reconstructed human skin model. The influence of keratinocytes and fibroblast and the role of the basement membrane on invasive behaviour of the cells was studied in this system. A fourth paper where the reconstructed skin system is used also studies invasive behaviour of different types of melanoma (Meier et al). In this case changes in invasive behaviour of radial growth phase melanoma cells were found when the cells were transduced with the bFGF gene. The effect of bFGF overexpression in normal melanocytes is described by Nesbit et al and the changes in melanocyte growth behaviour suggest a critical role of bFGF in melanoma progression.

Both two and three dimensional cocultures of keratinocytes and melanocytes were used to study melanosome transfer by Seiberg et al. A role for the protease-activated receptor 2 in melanosome transport is proposed in the systems with intimate cell-cell contacts. Another paper also describes the importance of keratinocyte and melanocyte interactions. In a coculture model Hsu et al examined gap-junctional communication of keratinocytes and melanocytic cells from various stages of tumor progression. Two models are used that allow the study of the development of embryonic stem (ES) cells. Beauvais-Jouneau et al describe a new cellular system in which the grafting of mouse ES cells into chicken embryos were used for both in vitro and in vivo studies of the molecular mechanisms controlling dorsolateral migration. Yamane et al have used cocultures of ES and a bone marrow-derived stromal cell line for generation of melanocytes. This process was enhanced by dexamethasone and strictly depended on the presence of steel factor.

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A novel model to study the dorsolateral migration of melanoblasts. *Mech. Dev.* 89(1-2):3-14, 1999.
- Bowers RR, Nguyen B, Buckner S, Gonzalez Y, Ruiz F.

- Role of antioxidants in the survival of normal and vitiliginous avian melanocytes. *Cell Mol. Biol. (Noisy-le-grand)* 45(7):1065-74, 1999.
- Cario-Andre M, Bessou S, Gontier E, Maresca V, Picardo M, Taieb A.
The reconstructed epidermis with melanocytes: a new tool to study pigmentation and photoprotection. *Cell Mol. Biol. (Noisy-le-grand)* 45(7):931-42, 1999.
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Characterization of an in vitro model of human melanoma invasion based on reconstructed human skin. *Br. J. Dermatol.* 142(2):210-22, 2000.
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Activation of a cAMP pathway and induction of melanogenesis correlate with association of p16(INK4) and p27(KIP1) to CDKs, loss of E2F-binding activity, and premature senescence of human melanocytes. *Exp. Cell Res.* 253(2):561-72, 1999.
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The regulation of normal melanocyte proliferation. *Pigment Cell Res.* 13(1): 4-14, 2000.
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Cadherin repertoire determines partner-specific gap junctional communication during melanoma progression. *J. Cell Sci.* 113(Pt.9):1535-1542, 2000.
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Effect of prostaglandins A(2), E(1), F(2 alpha) and latanoprost on cultured human iridal melanocytes. *Exp. Eye Res.* 70(1):113-20, 2000.
 - Lin Z, Hoult JR, Bennett DC, Raman A.
Stimulation of mouse melanocyte proliferation by Piper nigrum fruit extract and its main alkaloid, piperine. *Planta Med.* 65(7):600-3, 1999.
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Human melanoma progression in skin reconstructs : biological significance of bFGF. *Am. J. Pathol.* 156(1):193-200, 2000.
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Attachment and chemotaxis of melanocytes after ultraviolet irradiation in vitro. *Br. J. Dermatol.* 141(5):794-801, 1999.
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Basic fibroblast growth factor induces a transformed phenotype in normal human melanocytes. *Oncogene* 18(47):6469-76, 1999.
 - Regnier M, Duval C, Galey JB, Philippe M, Lagrange A, Tuloup R, Schmidt R.
Keratinocyte-melanocyte co-cultures and pigmented reconstructed human epidermis: models to study modulation of melanogenesis. *Cell Mol. Biol. (Noisy-le-grand)* 45(7):969-80, 1999.
 - Roberts JE, Wiechmann AF, Hu DN.
Melatonin receptors in human uveal melanocytes and melanoma cells . *J. Pineal Res.* 28(3):165-71, 2000.
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The protease-activated receptor 2 regulates pigmentation via keratinocyte-melanocyte interactions. *Exp. Cell Res.* 254(1):25-32, 2000.
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The treatment of hypopigmented lesions with cultured epithelial autograft. *J. Burn. Care Rehabil.* 21(1 Pt 1):50-4, 2000.
 - Yamane T, Hayashi SI, Mizoguchi M, Yamazaki H, Kunisada T.
Derivation of melanocytes from embryonic stem cells in culture. *Dev. Dyn.* 216(4/5):450-8, 1999.
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Histamine induces melanogenesis and morphologic changes by protein kinase A activation via H2 receptors in human normal melanocytes. *J. Invest. Dermatol.* 114(2):334-42, 2000.



ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

Also available from address: <http://www.ulb.ac.be/medecine/loce/escpr.htm>

2000 Melanoma: Basic Biology and Immunological Approaches to Therapy
May 3-7 **The Woodlands Resort, The Woodlands (near Houston), TX**

Contact: American Association for Cancer Research
Public Ledger Building, Suite 826
150 S. Independence Mall West
Philadelphia, PA 19106-3483
Phone: 215-440-9300
Fax: 215-351-9165
E-Mail: meetings@aacr.org
Website: <http://www.aacr.org>

2000 Melanoma: Milan, Italy

May 11-12 Contact: ESO - European School of Oncology
Viale Beatrice d'Este, 37 - 20122 Milan, Italy
Teaching Division: E. Ferrerio, V. Di Renzo
Tel: 39 0258317850
Fax: 39 0258321266
E-Mail: esoteaching@tin.it

2000 Perspectives in Melanoma IV: Pittsburgh, Pennsylvania

June 1-2 Contact: Imedex™ USA, Inc.
"Experts in Medical Meetings"
70 Technology Drive
Alpharetta, Georgia
30005-3969 USA
Tel: 770 751 7332
Fax: 770 751 7334
E-Mail: meetings@imedex.com

2000 The Research View Point: II Melanoma Research Meeting for Specialists
June 23-24 **in Dermatology, Surgery and Medical Oncology: Milan, Italy**

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2000 IXth Annual Meeting of the PASPCR
June 25-28 College Station, TX
Contact: Dr. Lynn LAMOREUX
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2000 9th ESPCR Meeting: Ulm, Germany
Sept 28 Contact: Prof. PETER R.U.
- Oct 1 University of Ulm (BWK)
Dept of Dermatology
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D - 89081 ULM
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TRAVEL AWARDS

Dear ESPCR members,

A notice about the ESPCR meeting (Ulm, Germany, September 2000).
Because the venue and organizers were changed for this meeting and program details are rather late in emerging, the awards committee headed by Friedrich Beermann has decided to defer the deadline for application for travel awards to attend the meeting.

The new deadline will be July 1st 2000

This should be updated on the web pages shortly. Please ignore any previous information about the deadline. Further details of the awards are available at:

<http://www.ulb.ac.be/medecine/loce/espccr/awards.htm>

or from F. Beermann at Friedrich.Beermann@isrec.unil.ch

These awards are intended for younger scientists, who are members of the ESPCR. (There is still time to join before applying for an award - and the subscription is very reasonable). The number of applications is currently very low, compared to the funds available, so it is well worth a shot. Please pass this on to any younger scientists you know who may be interested in attending this meeting.

Many thanks
The Awards Committee

2001 5th World Conference on Melanoma: Venice
Feb 28- Contact: Dr. Mario SANTINAMI
Mar 3 Secretary General
5th World Conference on Melanoma
Casa di Cura S. Pio X
Via F. Nava 31
I- 20159 Milano
Phone/Fax: 39-02-69516449
E-Mail: info@melanoma2001.org
Website: www.melanoma2001.org

2001 Xth Annual Meeting of the PASPCR, Minneapolis, MN

Contact: Dr. Richard KING

E-Mail: king@mail.ahc.umn.edu

2002 2002 XVIIIth International Pigment Cell Conference: The Hague, NL

Contact: Dr. Stan PAVEL

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Dear Colleagues,

Following a suggestion by Dr. W. Westerhof and the advise of the International Editors of the Bulletin, we thought that it was a good idea to publish summaries of PhD theses presented by ESPCR members or done in an ESPCR members' laboratory.

The aim is to inform ESPCR members on: research activities that can remain unpublished, the expertise field of the author,... and also to acknowledge the work of ESPCR members.

This should trigger even more scientific exchange and encourage collaborations that are also the goals of our Society.

Should you have such a material or any other that you think it deserves to be reported, please do not hesitate to send it to the Editorial office.

The first is reported below.

In advance, thank you.

The Bulletin Editor

TITLE OF THE THESIS:
TREATMENT OF VITILIGO

By Dr. M.D NJOO

University of Amsterdam, 29/3/2000

PROMOTERS: Prof. dr. J.D. Bos
Prof. P.M.M. Bossuyt
Dr. W. Westerhof

SUMMARY AND CONCLUSIONS:

Treatments for vitiligo are stated to be ineffective. Rather than prescribing active therapy, most dermatologists prefer just to explain to patients the harmless nature of the disease and to give them advice regarding the use of sunscreens and camouflage products. Clinical practise is usually based on personal and institutional experience that is supported by limited scientific evidence and may therefore be based on biased and imprecise information. Physicians are not expected to be aware of the results of every study that is

performed on the treatment of vitiligo. Moreover, many articles are not easily identifiable as they are published in a wide variety of professional journals around the world. During past years, many articles have been published about vitiligo therapies. The studies described in Chapters 3.2 and 3.3 attempted to summarize all the available evidence by systematically reviewing the literature and to provide recommendations concerning the most effective and safest vitiligo therapies. It is important to note that selection bias and publication bias may have interfered with our data. Also, the way of scoring repigmentation grade as measure for treatment effect may be different between the studies. Furthermore, most studies included in the analysis were nonrandomized, noncontrolled trials so that only indirect comparisons of treatment effects were possible. Nevertheless, the results allowed us to conclude that vitiligo is a treatable skin condition although complete (100 %) repigmentation is only rarely reported. More than 75 % repigmentation is regarded as a cosmetically acceptable grade of repigmentation. The effects of treatment seem to vary with certain patient and disease characteristics. Studies have identified that age, skin type, duration of disease, presence of leukotrichosis, localization of the lesions, extend of the depigmentation and disease activity may all influence treatment outcome. The choice of the most effective and safest therapy should therefore take into account all these factors.

The development of evidence-based clinical guidelines described in Chapter 3.4 can be regarded as an important step in reducing inappropriate care and improving treatment outcome in the treatment of patients with vitiligo. The guidelines were adjusted for local circumstances and preferences. Such guidelines are not static and should be regularly updated with data from clinical and experimental studies. In this respect, the studies of Chapters 3.5, 3.6 and 3.7 contain data that could be of value, if confirmed at other centers with other patient groups.

The literature studies have also identified some shortcomings in current vitiligo research. So far, only a few RCTs have been performed for patients with localized as well as generalized forms of vitiligo. RCTs are regarded as the "best available scientific evidence" (JAMA 1995; 274: 1630-1632). The inclusion of the results of such trials into practise guidelines can increase the strength and validity of treatment recommendations. Physicians would also feel more confident with guidelines that contain the best available evidence.

Future clinical trials in vitiligo should also evaluate treatment outcome in relation to quality of life. Quality of life assessment enables the investigator to collect information from the patient's perspective about the impact of vitiligo on daily life and provides a systematic and scientific basis for evaluating the benefits of treatment in terms of what patients value. Treatment effects that are measured solely on the basis of physical symptoms do not necessarily correlate with quality of life measures. The results of Chapter 3.6 showed that although some patients showed only partial repigmentation, their CDLQI scores had improved, because the vitiligo lesions had become less obvious. Quality of life scores may therefore provide an additional view of the overall effectiveness of therapy.

There are also political and financial reasons to include quality of life scores for future clinical trials in vitiligo. Because vitiligo is not regarded as a serious skin disease, more evidence is needed to convince the medical profession and also governmental institutions of the possible disabling effects of this disorder. Quality of life scores can be helpful to compare the impact of vitiligo with other cutaneous and noncutaneous diseases, provided that the appropriate instruments are used. The results of such studies may help solve current problems in fund raising for vitiligo research and reimbursement of treatment costs by health insurance companies.

Furthermore, follow-up studies are needed to assess the long-term benefits and long-term side effects of novel forms of phototherapy. The results that are reported with narrowband UV-B are promising but the full potential of this therapy is not yet established.

Presently, a maximum treatment duration of 2 years is recommended, but the effectiveness of prolonged therapy should also be investigated. There are also no data available regarding the permanence of the observed repigmentations after cessation of therapy.

Since UV radiation is known to have carcinogenic properties, future studies should also focus on the determination of skin cancer risks for patients with vitiligo who receive prolonged photo(chemo) therapy.

BOOKS

A special Proceedings Supplement to Pigment Cell Research

is now in press (200 pages). It contains 25 manuscripts from the Keynote Lectures presented at the IPCC, as well as Summaries of the various Symposia, Satellite Meetings and Evening Sessions held at the IPCC in Nagoya last November.

Authors: Z.A. Abdel-Malek, G. Barsh, D. Fisher, S. Frost-Mason, Y. Funasaka, B.A. Gilchrest, V.J. Hearing, M. Herlyn, H. Z. Hill, D. N. Hu, S. Ito, K. Jimbow, Y. Kawakami, P.B. Koch, S. MacNeil, Y. Mishima, M. Mizoguchi, N. Oshima, T. Saida, S. Shibahara, F. Solano, R.T. Swank, A. Taieb, A.J. Thody, and Y. Tomita.

If you did not subscribe to the journal or did not attend the IPCC, you may reserve and order a copy (cost: 5,000 yen approximately \$50 per copy), please contact Dr. K. Wakamatsu at: kwaka@fujita-hu.ac.jp

"The treatments developed in the Netherlands Institute for Pigmentary Disorders" (1994 - 1999)

Wiete Westerhof, Editor

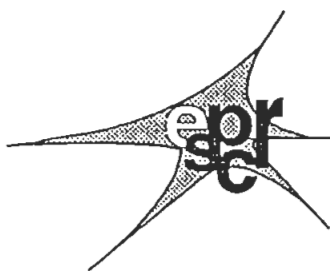
Publisher: SNIP-Pers, Amsterdam, The Netherlands, 1999

CONTENT :

Preface

Introduction

- Chapter 1 A left-right comparison study of fluticasone propionate + ultraviolet-A versus fluticasone propionate and ultraviolet-A for the long-term treatment of vitiligo.
- Chapter 2 Treatment of vitiligo with UV-B radiation vs topical psoralen plus UV-A.
- Chapter 3 Repigmentation in vitiligo vulgaris by autologous minigrafting: Results in nineteen patients.
- Chapter 4 Repigmentation of leukodermic defects in piebaldism by dermabrasion and thin split-thickness skin grafting in combination with minigrafting.
- Chapter 5 Depigmentation therapy in vitiligo universalis using topical 4-methoxyphenol and the Q-switched ruby laser.
- Chapter 6 Laser treatment for further depigmentation in vitiligo.
- Chapter 7 Effective removal of certain pigmented skin macules (lentiginos) using the 'Q-switched' ruby laser.
- Chapter 8 Treatment of acquired melanocytic nevi by Q-switched Ruby laser.
- Chapter 9 Shave excision of benign melanocytic nevi.
- Chapter 10 N-acetyl cysteine as a bleaching agent in the treatment of melasma.



Pigment Cell Research - into the next Millennium by Vince Hearing, Editor

Hopefully by now many of you will already have seen the new format of our journal *Pigment Cell Research*. The journal has had an interior and exterior face-lift that goes beyond the cosmetic changes immediately visible and embraces dramatic changes in editorial policy. I would invite each of you to visit our Web Site (www.pigment.org), which I hope will develop into a focal point for everyone in the field. Click on the 'Register' button and sign up for 'The PCR Primer' (cf below) or to volunteer to help review manuscripts. There is a 'Search' page where you can search all the archived issues of *Pigment Cell Research* for articles published on your favorite topic(s). We have a 'Hot Links' page that lists various scientific DataBases of interest to pigment researchers, an 'Archives' page that lists all papers published to date in the journal (and their abstracts). Even better, the 'Current Issue' page shows the contents and abstracts of the issue that just came out, and the 'In Press' page lists papers that have been recently accepted and are in press. The publisher (Munksgaard) will soon have *Pigment Cell Research* online (details forthcoming), but at this time there are no plans to publish articles from past years electronically and our Web page will be the only source for that information (unless you've been subscribing all this time).

I'd encourage each of you to sign up for 'The PCR Primer', an Email notification of journal activities, which will be published bimonthly as each issue is released, or as necessary when other breaking news develops. You can sign up for that by clicking the 'Register' button (you can also take your name off the Email list there if you wish). You can send comments to the Editor and/or register to review papers submitted to the journal from that same page.

You might be surprised at the quality of papers you'll see being published in the journal this year, particularly as issues come out later this year that have been handled by the current board of Associate Editors. I picked my Associate Editors based on their fields of expertise, their active research in those fields, their energy levels and their opinionated views on how a top-notch scientific journal should be run at the Editorial level. We've been working together closely this past year getting ready and you'll see the fruits of our efforts in the spectacular line-up in store for you this coming year. For example, each issue will contain a Major Invited Review, a Review on a specific Pigment Gene and its associated Disease, and a Review on an Innovative Technology being developed that has application in our fields. The titles and authors of those reviews for this year are listed on the Web Site. Increasing the quality and speed of published articles has also become a top priority, and this has come at an expense, notably an increase in the rejection rate for the journal which is rapidly approaching 50%. We invite you to submit your articles to *Pigment Cell Research*, but please make them your best ones or you may have an unpleasant surprise in store.

So the next time you're surfing, stop by and check us out - all suggestions for improvement of the Web Site, or the journal, are welcomed.