PASPCR Newsletter

Volume 8 Number 3 **September, 2000**

Introduction . . .

The **PASPCR** ***Newsletter*** is published quarterly and is intended to serve as a means of communication for the members of our Society. As such, we invite our membership to actively contribute to it. If you attend a scientific meeting and heard results which you think will be of interest to the membership of the **PASPCR**, please write a few paragraphs summarizing what was presented and share it with us. Any information on up-coming meetings of interest will also be included. We also want to note any change of affiliation or address that you may have had to help us keep our membership list up-to-date. This is **your** *Newsletter*, and we depend upon you to help us make sure it best serves the Society's needs. Contributions and comments can be sent to Bill Oetting, preferably by Email, to bill@lenti.med.umn.edu.

The **PASPCR Web** page is the major, up-to-date source of current information for the PASPCR membership. The URL address to our home page is **http://www.cbc.umn.edu/paspcr**. The PASPCR Web page contains information about the PASPCR including the goals, ByLaws and Rules of the Society, future meetings, past issues of the PASPCR *Newsletter* as well as links to other related sites including the InterPig DataBase, the International Federation of Pigment Cell Societies (IFPCS) and the regional Pigment Cell Societies from Europe and Japan. In addition, an updated PASPCR membership directory is available on the PASPCR Web page; please notify us if you wish any or all of your information to be modified or deleted on that site. The PASPCR home page also includes positions available and positions wanted. Postings for **Positions Available** are open to all individuals so long as the position is related to pigment cell research. Postings for **Positions Wanted** will be open only to members of the PASPCR or its sister societies (JSPCR and ESPCR). Please provide an expiration data for any submitted postings. If there is additional information that you wish to have added to this web page, please let us know. Send any comments and/or suggestions to the PASPCR WebMaster, Bill Oetting at bill@lenti.med.umn.edu.

Note: The IFPCS webpage has a new URL address at http://www.cbc.umn.edu/ifpcs.

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PanAmerican Society for Pigment Cell Research

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Calendar of Events :

Sept 28 - Oct 1, 2000 9th Annual Meeting of the European Society for Pigment Cell Research, to be held in Ulm, Germany
Contact: Prof. R.U. Peter, University of Ulm (BWK) Dept of Dermatology Oberer Eselsberg 40 D - 89081 ULM
Tel: 49-731 502-3770
Fax: 49-731 502-3772
E-mail: ralf.peter@medizin.uni-ulm.de

Dec 5-6, 2000 13th Meeting of the Japanese Society for Pigment Cell Research, to be held in Sapporo, Japan,
Contact*:* K Jimbow

Feb 28, March 3, 2001 5th World Conference on Melanoma : Venice, Italy, February 28 - March 3
Contact: Dr Mario Santinami 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

Jun 25 - 28, 2001 Xth Annual Meeting of the PanAmerican Society for Pigment Cell Research, to be held in Minneapolis, MN
Contact: Dr. Richard A. King, Department of Medicine, Box 485 Mayo, 420 Delaware St. S.E., Minneapolis, MN 55455;
Phone: (612) 624-0144
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Email: king@mail.ahc.umn.edu.

2002 The XVIIIth International Pigment Cell Conference, to be held in The Hague, Holland.
Contact: Dr. Stan Pavel, President ESPCR, University Hospital Leiden, Dept of Dermatology, PO Box 9600, NL - 2300 RC LEIDEN
Phone: 31-(71) 526 1952
Fax: 31-(71) 524 8106;
E-mail: SPavel@algemeen.azl.nl

Sept 3-7, 2003 XIth Annual Meeting of the PanAmerican Society for Pigment Cell Research, to be held in Wood's Hole, MA.

Welcome to New Members

by James J Nordlund

We welcome the following new member to the **PASPCR** . . .

Lidia Kos, Ph.D. of the Florida International University

James A. Lister, Ph.D. of the University of Washington

If anyone is interested in joining our Society or wishes to sponsor a member, application forms can be obtained from Dr. James J. Nordlund at the PASPCR Secretary/Treasurer's office.

Corporate Sponsors

by James J Nordlund

The PASPCR would like to acknowledge and thank our Corporate Sponsors; the list below reflects contributions over the past 2 years. Financial gifts from these sponsors have allowed our Society to increase benefits to the membership far out of proportion to the actual dues collected from members. Monies contributed by these sponsors have been used over the years to support various PASPCR functions including our Young Investigator Award program, meeting travel stipends, annual meeting expenses and this Newsletter.

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From the Editor - *Pigment Cell Research*

Vince Hearing, *Editor*

Announcement - Award for Publication Excellence - 2000

At its recent business lunch held during the PASPCR Meeting in College Station, the Editorial Board of ***Pigment Cell Research*** established an annual award for **The Most Outstanding Contribution** published in ***Pigment Cell Research*** each year. The top paper published this year 2000 (Volume 13) will be determined and awarded following distribution of the last issue this year. All Original Research Articles will be considered for the Award, which will be decided by the Editorial Board. The winner will be announced early in 2001 and will receive a year's free subscription to the journal as well as an Award of Achievement. The winner will also be featured in a brief article in the journal next Spring that will present a summary of the victorious study and the reasons behind its selection as the best in Volume 13. This **Outstanding Contribution Award** is dedicated in this Inaugural year to the memory of Profs. Yoshiaki Hori and Bengt Larsson, two ardent supporters of ***Pigment Cell Research*** over the years. Good Luck to all authors.

And now for the rest of the story.

I have always been interested in how a particular line of research began. Was it well planned out, did it come to the investigator in a dream, or was it just serendipity? In this section of the PASPCR Newsletter I plan to publish stories on the background of discoveries in pigment research. In this issue, Dr. Robert (Bob) Smyth talkes about the origin of the Smyth chicken. I hope that you will enjoy these stories. If you wish to know how a particular line of investigation got started, please email me at bill@lenti.med.umn.edu, and I will try to get **the rest of the story**.

Origin and Development of the Smyth Chicken Line: A Model for Autoimmune Vitiligo

by Professor Emeritus J. Robert Smyth
University of Massachusetts
Amherst, MA 01003

It was a grey day in November, 1971, hardly one that held promise of any excitement since the chore that day was to make up reproduction matings for the next generation of some of my purelines. No segregating progeny groups were expected here. Simply select healthy birds of desired phenotype for the matings. A white hen in an otherwise pen of brown colored chickens (Massachusetts Brown line) raised little interest as farm workers frequently throw pen escapees into the most convenient nearby enclosure. The wing band number of the odd white hen, however, indicated that she was supposed to be a Brown line bird. Furthermore, the records showed that her down color at hatching had been a typical brown stripe. All of a sudden, she became an object of extreme interest as she had lost her feather melanins during the three feather changes prior to the attainment of her adult plumage at 20-22 weeks of age.

To determine whether the altered expression of feather melanin had a genetic basis, matings were made between the amelanotic female and normal Brown line males. Since the original white female came from a non-pedigreed multiple male mating, she was artificially inseminated with pooled semen from 5 randomly selected males. At hatching, all 45 progeny had brown striped down typical of the Brown Line. One of these, a male, developed white plumage by sexual maturity. Unlike his mother who was blind at sexual maturity, he remained fully sighted throughout his life. We had now established that the vitiligo-like pigment defect was heritable. Subsequent matings between the vitiliginous mother and son and between them and normally pigmented Brown line birds indicated that the abnormality was not inherited in a simple Mendelian fashion. This was further substantiated by outcrosses to 3 unrelated lines for genetic study and to provide a few outcross amelanotics to broaden the genetic base of a new vitiligo line. The absence of vitiligo among F_2 progeny and the low incidence among the backcross offspring suggested multiple interacting genes present in the vitiliginous Brown line birds.

The base population that was used to develop the new vitiligo line (Smyth line) originated from generation 3, intra-Brown line vitiliginous birds plus a few backcross progeny from the F_1 outcross birds x Brown Line amelanotics. In the following generation the brown plumage pattern was refixed in the new line. The only marker gene still segregating in the Smyth lines is a sex-linked gene ($\underline{id}^+$) that allows dermal eumelanin to be deposited in

the scaly shanks and a few other connective tissues of the bird. Although it is not possible to ascertain precisely the genetic contribution of the 3 original outcrosses, there is little evidence that it was but little. Selection for posthatch feather amelanosis was continued thereafter. The incidences of vitiligo and blindness were 59.8% and 25.6% respectively in the G_4 generation and 85.6% and 39.6% respectively in the G_5 generation. The incidences changed but little after that even though only vitiliginous birds were used for line reproduction.

During the early years of the development of the Smyth line, a considerable body of knowledge on the basis for the pigment defect was accumulated. Its many similarities to human vitiligo were observed including a higher than expected incidence of Hashimoto's thyroiditis, an alopecia-areata like feathering defect, retinal pigment changes, remelanization, etc. Earliest co-workers included Dr. Kay Fite, an ocular biologist here at UMass, and Dr. Ray Boissy, who studied the Smyth line vitiligo for both his M.S. and Ph.D. theses. They were soon followed by Dr. Susan Lamont, a postdoc in my laboratory who took the leadership in our studies of the role of the immune response in our mutant pigment defect. These were followed by other graduate students and postdocs, but the above played the major role in our key early studies.

One of the factors we found to play a role in the expression of the vitiligo was the MHC (major histocompatibility complex). It is now known that the MHC and other histocompatibility genes typically play a role in the expression of autoimmune defects. With the aid of Dr. Elwood Briles and his wife at Northern Illinois University we established that the Smyth line and its Brown parent line were polymorphic for 3 different MHC haplotypes (designated B101, B102 and B103). The haplotypes were further found to be associated with different levels of expression of the pigment defect. For example, the B101 haplotype had the earliest onset of vitiligo, the most complete pigment loss and a high incidence of blindness. The B102 and B103 haplotypes had a later onset of pigment loss and a low incidence of blindness. Because of the random occurrence of these 3 haplotypes in both the Smyth line and its Brown line control, it was decided to establish sublines that would each be homozygous for one of the MHC haplotypes. Both MHC-matched vitiliginous and parental Brown line controls were established. These were designated as SL101, SL102 and SL103 (Smyth lines) and their respective parental controls, BL101, BL102 and BL103. Not unexpectedly, the SL sublines showed similar relationships to the expression of the vitiligo first seen in the segregating MHC haplotypes of the original Smyth line population. The total incidence of vitiligo rarely differs, however, among the 3 SL sublines.

More recent studies have concentrated on the following areas:

(1) Autoantibodies (Drs. Lisa Austin and Ray Boissy, University of Cincinnati)
(2) Role of T-cell subsets in various tissues in SL vitiligo (Dr. Gisela Erf, University of Arkansas)
(3) Roles of exogenous and endogenous viruses in the expression of SL vitiligo
(Drs. Sreekumar, Lakshmanan, UMass and G. Erf, University of Arkansas)
(4) Molecular genetics and characterization of the SL
(Ponce de Leon, Sreekumar and Lakshmanan, UMass)

At present, there are no more SL birds at UMass. SL101 and 102 sublines and their controls are with Dr. Gisela Erf at the University of Arkansas (Telephone: 515/575-8664) and SL101 and SL103 are with Dr. F. Abel Ponce de Leon at the University of Minnesota (Telephone: 612/624-1205).

The most complete and detailed review of the Smyth line through 1989 appeared in Critical Reviews in Poultry Biology, 2: 1-19, 1989. I will be happy to provide reprints for interested people (J. R. Smyth, Ph.D., 307A Stockbridge Hall, University of Massachusetts, Amherst, MA 01003-7250).

Report on the IX Annual Meeting of the PanAmerican Society for Pigment Cell Research

Below are reports on various sessions of the PASPCR IX Annual Meeting. I would like to thank those individuals who wrote the excellent reviews on the sessions that they attended for this newsletter. I am sure that you will agree that the meeting in Texas hosted by Lynn Lamoreux and Estela Medrano was an excellent meeting filled with science covering a broad range of topics in the pigment field.

Symposium on Signal Transduction, UV Radiation, and Pigmentation

Report by Zalfa Abdel-Malek

Hee-Young Park was the invited speaker at this symposium. She discussed recent results from her laboratory demonstrating the expression of RACK (Receptor for Activated Kinase) on melanosomes, and providing further evidence for the role of PKCβ in regulating pigmentation. She concluded that stimulation of cAMP formation leads to PKC β activation, which in turn phosphorylates tyrosinase and increases its activity.

Zalfa Abdel-Malek discussed two distinct signaling pathways activated by mitogens and UVB, respectively. The mitogen activated pathway involves the activation of the MAP kinase ERK1/2, and subsequently $p90^{rsk}$ and the transcription factor CREB. Mitogens such as TPA, bFGF, and endothelin-1, but not α-MSH, activate this pathway, which is dependent on increasing intracellular Ca^{+2} mobilization and activation of PKC and tyrosine kinases, but not cAMP formation. The UVB induced signaling pathway involves phosphorylation of CREB via a pathway independent of ERK1/2 or $p90^{rsk}$, but possibly dependent on the MAP kinase p38.

Sheila Mac Neil presented on the role of α-MSH in the defense of the skin against inflammation and oxidative stress. Earlier work from her laboratory showed that α-MSH inhibits the TNF-α induced ICAM-1 expression and NFKβ activity. Recently, she and her coworkers reported that α-MSH inhibits the TNF-α and H_2O_2 induced activation of glutathione peroxidase. She concluded that several types of cells in the skin are capable of responding to α-MSH to overcome oxidative stress.

Elizabeth Pereira reported the results she has obtained in Zalfa Abdel-Malek's laboratory on the signaling pathways induced by UVB radiation or arsenic in human melanocytes and keratinocytes. UVB radiation induced a dose-dependent and prolonged accumulation of p53, and an increase in the expression of the cyclin-cdk inhibitor p21. Arsenic caused only a slight increase in p53, but extensive expression of p21. UVB radiation did not activate ERK1/2, while arsenic augmented the activation of ERK1/2 by mitogens. UVB radiation, but not arsenic induced the phosphorylation of p38.

Sumayah Jamal presented on the regulation of expression of the adhesion molecule E-cadherin by endothelin-1, a melanocyte mitogen that is secreted by keratinocytes. Loss of expression of E-cadherin results in dedifferentiation of melanocytes and expression of melanoma associated adhesion molecules, as well as loss of contact with keratinocytes. Endothelin-1 activates endothelin-B receptors on melanocytes and down-regulates E-cadherin by inducing its association with caspase-8. The ability of endothelin-1 to down-regulate E-cadherin, a tumor invasion suppressor, suggests a role for this hormone in enhancing melanoma invasion.

Masaki Yoshida described the mechanism by which histamine stimulates melanogenesis. In the brown guinea pig, topical application of famotidine, a H2 antagonist, reduced the pigmentary effect of UVB radiation. Application of histamine, however, did not stimulate pigmentation. Irradiation of cultured human melanocytes with UVB radiation or treatment with histamine did not stimulate tyrosinase activity. Simultaneous treatment of melanocytes with UVB radiation and histamine stimulated tyrosinase activity. He concluded that UVB radiation might sensitize melanocytes to histamine.

Achim H-P Krauss reported on the responses of choroidal and cutaneous melanocytes to endothelin-1 and prostaglandin F2α. Both types of melanocytes expressed the mRNA for the endothlelin B receptor and for the FP receptors. The latter receptors were also observed by immunocytochemistry, and the $G_q\alpha$, the G protein coupled to endothelin B and FP receptors, was detected by Western blotting. Surprisingly, only endothelin-1, but not PGF2α, induced mobilization of intracellular Ca^{+2} and increased DOPA oxidase activity and increased tyrosinase protein levels.

Peter Parsons presented on the effects of sunscreen chemicals on skin cells. He presented the results of a study that was conducted to determine the effects of such chemicals on the efficacy of sunscreens. Three sunscreens tested inhibited the proliferation of human skin cells but did not alter the expression of certain genes. UVA photosensitized o-PABA in melanocytes but not keratinocytes. The relevance of these *in vitro* findings on the action of sunscreens *in vivo* is to be determined.

Patrick Riley described a mathematical model that explains the kinetics of tyrosinase. The model explains the autoactivation kinetics of tyrosinase, whereby the enzyme with copper atoms in the active site in the Cu (II) state. The kinetics are consistent with the kinetics of mushroom tyrosinase *in vitro* and with the effects of known enzyme substrates.

Lessons Learned from Vitiligo.

Report by Caroline Le Poole Ph.D.

Our session opended with a brief discussion of vitiligo per se, highlighting the progressive loss of melanocytes, a varied age of onset, the infrequency of repigmentation and the existence of multiple clinical subforms.potentially reflecting different etiopathologies The latter viewpoint was supported by quotes generously provided by vitiligo researchers not present at the meeting, stating that vitiligo is a disease deserving of further attention among pigment cell biologists and dermatologists alike. A current hypothesis was presented in which genetically compromised melanocytes are hampered in their response to environmentally imposed injury. Stressed melanocytes subsequently initiate intensive cross-talk with neighbouring keratinocytes, both cell types participating in the recruitment of an inflammatory infiltrate. Such infiltrates contains lymphocytes specifically reactive with melanocyte antigens, leading to premature apoptosis of target cells and thus to depigmentation.

Dr. Pranab Das (Amsterdam University) continued with compelling evidence in support of a Th1 mediated immune response in progressive margins of generalized vitiligo skin. The cytotoxic ability of infiltrating T cells was supported by granzyme/perforin staining of marginal skin sections. It was pointed out that most T cells will infiltrate the skin as Th0 cells, polarizing to a Th1 or Th2 phenotype in response to conditions encountered in the skin. On average 30% of T cells cloned from marginal skin biopsies were shown to be reactive with melanocytes, underscoring their importance for the diisease process. A high frequency of Th1 cytokine profiles was noted

among these T cell clones. Interestingly, T cells cloned from marginal skin revealed a 2-10 fold increase in incidence of MART1-reactive T cells over PBL.

Dr. Gisela Erf (University of Arkansas) discussed the potential of the Smyth line chicken to study the autoimmune response in vitiligo. In this model, feather depigmentation will develop 6-12 weeks post-hatch. Among 3 haplotypes available, B101 appears the most useful for vitiligo research with a reported incidence of depigmentation of >70%. Consistent development of vitiligo implies the involvement of a genetic component. Interestingly, the cell-mediated immune component of the disease is well represented in the chicken model. Also, a susceptability to environmental factors has been demonstrated by an increased incidence of vitiligo following immunization with live Marek's disease virus. The incidence of depigmentation decreases in the order SL, (parental line) BL and LBL. In BL chickens, depigmentation following exposure to 5-azacytidine increases to approximately 70%. This agent will inhibit DNA methylation and has been extensively shown to induce autoimmune disease. The agent was shown to increase the CD4/CD8 ratio in exposed birds (independent of depigmentation) and infiltrating T cells frequently express TCR2 rather than TCR1 or TCR3.

By way of introduction to the involvement of the immune response in loss of melanocytes, a short review was given into the role of melanocytes within the skin immune system. The phagocytic capacity of melancoytes in combination with a potential for antigen presentation suggest that participation of melanocytes in elimination of infectious agents penetrating the skin could lead to depigmentation, as often observed in tuberculoid leprosy. For example, melanocytes presenting immunodominant M. leprae HSP65 peptides may be killed by proliferative as well as cytotoxic CD4+T cells as innocent bystander cells. In the second part of his presentation, Dr. Das continued with a description of events leading to elimination of melanocytes by cytotoxic T cells. The process initiates by injury inflicted on melanocytes. Cells that die as a direct consequence are phagocytized by professional antigen presenting cells, that migrate to the lymph nodes, where cellular cross-talk is initiated involving cytokines and costimulatory molecules, which will determine the type of response to follow. Specific effector cells (T cells and/or B cells) then migrate to the initiating lesion where remaining melanocytes are subsequently killed.

The undersigned (**Dr. Caroline Le Poole,** Loyola University Chicago) then presented data relating to CDw60 expression in marginal skin from expanding generalized vitiligo lesions. Gangliosides expressing the CDw60 epitope are membrane molecules involved in multiple cell functions. In normal skin, expression is restricted to melanocytes and some neighbouring keratinocytes. Interestingly, it has previously been demonstrated that CDw60 expression by keratinocytes is regulated by Th1 versus Th2 cytokines. Here, data were shown indicating that expression by melanocytes is not regulated by exposure to such cytokines. Rather, melanocytes appear to secrete an autocrine and paracrine factor that will induce expression by neighbouring keratinocytes. The nature of this factor is under investigation. Interestingly, in 3 out of 5 patients examined, loss of melanocytes was accompanied by T cell infiltrates (indicative of progressive depigmentation) as well as by downregulation of epidermal CDw60 expression and induction of epidermal HLA-DR, both reflecting an active Th1 mediated immune response.

Dr Jim Nordlund (University of Cincinnati) ended the session with a discussion of his findings during an extensive stay in Moshi, Tanzania. There, Dr. Nordlund has seen patients with OCA-2 that exhibited UV-induced skin damage from 6 months of age. In the US , albinos typically develop BCCs or SCCs but curiously, not melanoma. Interestingly, several OCA-2 patients had developed solar lentigines. The nature of the mutation or mutations responsible for such reversion to melanogenesis are currently under investigation.

By contrast, patients with vitiligo exhibited marked solar elastosis but no actinic damage. Among patients with vitiligo, 69% had generalized vitiligo, 20% segmental, 6% localized and 5% showed involvement of the mucous membranes only. The mean age of onset was 29 years of age, one must however take into account that life expectancies are different from the US. In conclusion, the last word has not been said on the protective value of melanin against sun-induced skin damage and the lack thereof in vitligo lesional skin.

In addition to the issues discussed in the vitiligo session, many interesting posters relating to the subject of vitiligo were on display throughout the meeting. There is obviously enough material for continued discussion on the subject of vitiligo. Perhaps at the next meeting?

Melanoma: New Genes, Gene Therapy and Animal Models I

Report by Meenhard Herlyn

The symposium "Melanoma: New Genes, Gene Therapy and Animal Models I" on June 26, 2000, at the Ninth Annual Meeting of the PASPCR in College Station, Texas, was co-chaired by Meenhard Herlyn and Dennis Roop. Dr. Herlyn opened the session by giving an overview of all animal models for melanoma published to date. In these models, melanomas develop spontaneously or after induction by chemical carcinogens (e.g. DMBA) and/or UV light. More recently transgenic mouse models for melanoma have been developed. As for human melanoma *in vivo,* the human skin xenograft/ immunodeficient mouse model gave rise to melanoma after topical DMBA treatment and chronic UVB irradiation of human foreskin. However, recent data of human adult skin grafts show that, in contrast to actinic non-melanoma skin cancer lesions, the incidence of melanocytic lesions is too low within the given protocol and time frame to make the model suitable for routine experimental studies.

Recently established organotypic cultures of pigmented human skin provide a new promising model for human melanoma *in vitro* and *in vivo* after grafting to immunodeficient animals. Genetic manipulation of the different types of normal skin cells as well as the inclusion of melanoma cells from different progression stages in these skin reconstructs enables well-defined and controlled long-term studies of melanoma development and progression in context with the microenvironment of skin.

Invited speaker **Lynda Chin** presented her progress on the transgenic mouse melanoma model in which the transgene H-RAS is expressed with the aid of melanocyte-specific tyrosinase gene promoter (Tyr-RAS) in mice with null mutations for the p16INK4α and p19ARF gene (INK4αD2/3 mutant). The lack of p53 mutations in these murine melanomas parallels the low incidence of p53 mutations in human melanomas. The importance of H-Ras expression for melanoma maintenance was shown by a doxycycline-inducible H-RasV12G INK4α null mouse model. Withdrawal of doxycycline with concomitant down-regulation of H-Ras expression resulted in melanoma regression which could be converted upon new addition of doxycyline. The regression was characterized by apoptosis of tumor and endothelial cells. Although the regulation of VEGF was found to be Ras-dependent *in vitro, in situ* hybridization studies demonstrated an increase in VEGF expression in areas with reduced Ras expression.

The hypothesis that melanoma metastasis is initiated by the formation of host x tumor hybrids through fusion of macrophages with early stage melanoma cells was presented by **John Pawelek**. Motility and invasive capacity of metastatic melanoma cells resemble features of macrophages. Evidence for these hybrid cells comes from the analysis of a mouse lung metastasis found after implantation of murine Cloudman S91 melanoma cells in the tail of

a BALB/c mouse. This metastasis showed an increase in DNA content, a genotype of tyrosinase characteristic of both host and donor of the melanoma, more pigmentation, and more motility. The next objective is to prove the hypothesis in a human model.

Studies of the molecular genetics of melanoma in the Xiphophorus fish model were introduced by **Steven Kazianis**. Backcross hybrids homozygous for Xmrk2, a sex-linked tyrosine kinase related to EGF-Receptor, were generated and genetic mapping studies performed. The hybrid crosses enable also induction studies of melanoma by UV irradiation or chemical carcinogens like methyl-nitrosurea. A tumor suppressor candidate CDKN2X, similar to the mammalian CDKN2 gene family including the loci for p16 and p15, was cloned, sequenced and characterized. In contrast to the human CDKN2A locus in melanoma, the fish CDKN2X locus is overexpressed in melanoma.

A new model of early transformation of human melanocytes *in vivo* was demonstrated by **Carola Berking**. Overexpression of βFGF in fibroblasts in human skin xenografts via intradermal injections of adenoviral vectors for the βFGF gene led to melanocyte proliferation and activation with increased pigmentation and melanocytic hyperplasia. The combination of βFGF expression and UVB irradiation induced a melanoma *in situ*-like lesion. Gene expression array studies of βFGF-transduced fibroblasts in collagen gels revealed an induction of ET-3 production which may have been the paracrine (co-)factor responsible for the melanocyte changes.

Mohamed Gamei presented a new clinical therapy for pigmented lesions. He treated acquired melanocytic nevi with a Q-switched ruby laser and had best cosmetic results in flat lesions with complete response and no recurrence. The laser targets the pigment in melanosomes and seems most suitable for junctional melanocytic nevi.

Latest technologies for melanoma gene expression analysis were employed by **Zhiqiang Wang**. A *Monodelphis domestica* non-metastatic melanocytic cell line was compared with a metastatic melanoma cell line by cDNA microarray and differential display. Metastasis-related genes of the metalloproteinase family were up-regulated and tissue inhibitors of metalloproteinases were down-regulated. Dysregulation of melanogenesis-specific genes were associated with activation of Ras-related oncogenes.

An overview of the melanoma cell-cell and cell-stroma interactions was given by **Meenhard Herlyn**. A wide variety of growth factors and cytokines function in an autocrine and/or paracrine manner to stimulate tumor growth, angiogenesis, stroma formation, adhesion, motility and invasion. Among these factors are βFGF, IGF-1, VEGF, PDGF, MCP-1, and IL-8. While IGF-1 is not produced by melanoma cells themselves, they express the IGF receptor and receive paracrine stimulation via IGF-1 secretion by surrounding fibroblasts. The angiogenic potency of IL-8, VEGF, βFGF, PDGF, and also MCP-1 is well established, however, experimental studies reveal that the amount of production is of utmost importance. While low concentrations of MCP-1 promote tumor growth and angiogenesis, higher expression levels can induce tumor necrosis due to macrophage infiltration. Similarly, too high IL-8 levels may induces neutrophil infiltrations with toxic effects on the tumor.

The importance of cell adhesion molecules in melanoma progression can be demonstrated in skin reconstructs. Early stage non-invasive melanoma cells become aggressive and invade into the dermis after experimental overexpression of melanoma cell adhesion molecule, Mel-CAM/Muc-18. Disruption of melanoma cell-melanoma cell and melanoma cell-fibroblast interactions by down-regulation of N-cadherin and the re-expression of the keratinocyte-melanocyte adhesion molecule E-cadherin in melanoma cells can abrogate malignant properties and reestablish a homeostatic balance between the different cell types.

The Sinclair swine model of melanoma was introduced by invited speaker **Max Amoss**. At least three loci have been linked to the inheritance and expression of melanoma in this model. The swines develop heritable melanoma at early age and either die from complications of multisystemic metastasis or survive due to spontaneous tumor regression. This regression is accompanied by depigmentation, i.e. vitiligo, and may be mediated by infiltrating macrophages and CD8+ T-lymphocytes, however, the exact mechanism is not yet understood.

Symposium: Melanoma: New Genes, Gene Therapy and Animal Models II

Report by Dot Bennett

The expansion of this topic into a second session gave us two more invited and 7 platform talks on a variety of interesting themes. In his invited talk, **Menashe Bar-Eli** (MD Anderson Center) gave a fascinating update on the potential role of transcription factor AP2 in melanoma progression. AP2 is expressed in neural crest and its products including melanocytes, but is commonly lost in metastatic melanomas. AP2 regulates several genes important in melanoma, including upregulation of the SCF receptor KIT (also commonly lost in advanced melanomas), and downregulation of adhesion molecule MUC18/Mel-CAM (consistently overexpressed in metastatic melanomas). Re-expression of exogenous AP2 in melanoma cells was associated with reduced tumor growth and lung colony formation. Thus AP2 downregulation may be a central event in melanoma progression. (So what regulates AP2?)

Keynote speaker **Osvaldo Podhajcer** (Buenos Aires) discussed the role of SPARC (**s**ecreted **p**rotein, **a**cidic and **r**ich in **c**ysteine) in melanoma. SPARC is a matrix-associated protein found in growing or remodelling tissues including some carcinomas. He reported a remarkable association of SPARC with melanoma, namely expression in all tested melanomas of all stages, but in no melanocytes. Expression of a SPARC antisense sequence in a highly malignant melanoma line was followed by reduced tumor growth with altered cytokine levels and massive infiltration by neutrophils. Evidence was mentioned for melanoma cell killing by neutrophils in culture, and this was suggested as a mechanism for tumor cell destruction. (Again, one wonders what regulates SPARC.)

The first three platform talks were interrelated. **S. Pavel** (Leiden), discussed a multiple melanoma patient with not only 2 copies of a deletion in the melanoma susceptibility gene *INK4A /CDKN2A* but also a glucose-6-phosphate dehydrogenase (G6PD) deficiency. Since another member of the family also had homozygous deletions in *CDKN2A*, but no melanoma, it was suggested that the G6PD deficiency, expected to increase oxidative stress in cells, might contribute to the occurrence of melanoma. Other genetic contributing factors were not excluded. Next, **D. Bandopadhyay** (Houston) reviewed changes in gene expression seen in senescent melanocytes. She then described attempts to immortalize human melanocytes with hTERT (telomerase catalytic subunit), as reported for fibroblasts. Some hTERT-expressing clones apparently senesced, while others grew and appeared immortal. The growing clones may have undergone spontaneous genetic change, as downregulation of p16, one product of the *CDKN2A* locus, was observed. Moreover, **D. Bennett** (London) then reported that two human melanocyte strains null for p16 function, one from Dr Pavel's patient, could be immortalized en masse by hTERT, although not by tested viral oncogenes. Both talks suggested that deficiency of the p16/RB pathway was necessary as well as hTERT expression for human melanocyte immortalization - a step in melanoma development.

The other four talks were concerned with abnormalities of gene and protein expression in melanoma. Such information may lead to new therapeutic targets as well as aiding our understanding of the disease. **C. LePoole**

(Chicago) discussed potential immune escape by downregulation of antigenic melanosomal proteins like gp100 /SILV, MART1 and TYRP1 by melanoma cells. Interestingly such downregulation resulted experimentally on exposure to interferon α, with reduced cell lysis by a MART1 specific T-cell clone. **P. Das** (Amsterdam) mentioned that dysplastic but not benign nevi also show antigen loss. He then reported the use of PCR-based subtractive hybridization to look for transcriptional differences between nevi and malignant melanoma cells. Candidate sequences included Rab5a, which may be downregulated during progression. **D. Easty** (Dublin) introduced a new RT-PCR survey of protein tyrosine phosphatases (PTPs) expressed in melanocytes and melanoma. Many oncogenes are protein tyrosine kinases, so PTPs are potential tumor suppressor genes. 15 PTPs were identified in pigment cells, and two related PTPs appeared to be downregulated in some melanomas, including PTPκ, which maps to chromosome 6q, a site of consistent chromosomal aberrations in melanoma. Lastly, **W. Xu** (Houston) gave a potential functional explanation for consistent overexpression of transcriptional repressor SKI in melanoma. Interaction of SKI with Smad proteins was reported. Smads are transcriptional activators and mediators of TGFβ responses. TGFβ inhibits growth of melanocytes, but SKI overexpression prevented this growth inhibition. This potentially explains why melanoma cell growth is often not repressed by TGFβ.

Melanosomal Genesis, function and transfer.

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 hematopeoitc 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. Raymond Boissy summarized our current understanding of Hermansky Pudlak (HP) syndrome as well as his most recent findings regarding melanosome biogenesis. Melanosome biogenesis involves the formation of coated vesicles that tether, dock and fuse to late endosomes. The gene for HP syndrome type I has been mapped to chromasome 10q23.1-3, but the function of the protein is unknown. The protein forms a 200 kDa and sometimes 500 kDa complex, is not a glycoprotein and is not a transmembrane protein. HP syndrome type II is due to a defect in the adaptor _3 subunit. In melanocytes from patients with HPS type I Dr. Boissy and colleagues have observed the presence of a "membranous complex" by electron microscopy. Melanocytes from patients with HPS II exhibit many late endosomes. There appears to be impaired trafficking into or out of this late endosome. Thus, HP syndrome may be understood as a disorder in melanosome biogenesis through at least 2 different pathways.

Dr. Miri Seiberg presented data to show that protease activated receptor-2 (PAR-2) is involved in melanosome transfer to keratinocytes through increased keratinocyte phagocytosis. PAR-2 is activated by cleavage by serine proteases. In epidermal equivalents agonists of PAR-2 induced pigmentation through increased uptake of melanosomes by keratinocytes. Conversely, they showed that inhibitors of PAR-2 reduced pigmentation through inhibition of melanosome uptake. PAR-2 inhibition also functions as a feedback loop to inhibit TRP-1 transcription, and to unregulate TRP-2 transcription. Finally, Dr. Seiberg also showed that PAR-2 activation alters the actin cytoskeleton in keratinocytes, and that PAR-2 activation results in protease secretion.

Dr. Ruth Halaban presented data showing that tyrosinase-negative albinism is due to endoplasmic reticulum retention of tyrosinase. Dr. Halaban and colleagues analyzed several mutant tyrosinases and followed the intracellular localization by tagging these mutants with green fluorescent protein in murine melanocytes. They also examined the enzymatic properties of the mutant proteins and their subcellular localization. They found that the albino mutant tyrosinase is retained in the ER and is a misfolded protein that is associated with the chaperone proteins calnexin and calreticulin.

Dr. Toyofuku presented data showing that mutant tyrosinases from patients with OCA IA or IB, when transfected into COS7 cells, were retained in the ER stably associated with calnexin. These mutant tyrosinases were also shown to be degraded faster than wild type tyrosinase.

Dr. Virador presented data analyzing the process of melanosome transfer to keratinocytes. Dr. Virador and colleagues used several different model systems to analyze this process. Using fluorescent beads they showed that bead uptake by keratinocytes is determined by the size of the bead. Using time lapse video microscopy they showed that one method of melanosome transfer involves insertion of the melanocyte dendrite under the keratinocyte. Finally, treatment of melanocytes with MSH resulted in release of melanosomes into the media within 6 hours and increased blebbing of the melanocyte membrane.

Dr. Manga presented data analyzing the function of the pink eyed dilution locus in melanosome biogenesis. Mutations in the p locus are a major cause of albinism. Dr. Manga and colleagues used EM to study the localization of tyrosinase in murine p-null cells (melan-p1) and wild type (melan-a) cells. By EM they show that in the absence of p, tyrosinase accumulates in small vesicles throughout the cell. They also show that tyrosinase is secreted in melan-p1 melanocytes and that the observed secretion is due to proteolysis of the abnormally processed tyrosinase. Dr. Manga and colleagues hypothesize that the p protein may function to stabilize the melanosomal complex and aid in transport of melanosomal proteins.

Dr. Manwalla examined the role of lectins and neoglycoproteins in melanosome transfer to keratinocytes. Lectins are involved in membrane recognition events, and lectin expression is upregulated by UV light. They used co-cultures of melanocytes and keratinocytes and a dye (CFDA) as a marker for melanosomes. Using this model system they showed that neoglycoproteins were more effective in inhibiting dye transfer to keratinocytes than lectins. They also counted the number of melanosomes in the cytoplasm of keratinocytes in the presence of inhibitors of lectins or neoglycoproteins and showed similar results. Finally, they analyzed the factors that contribute to localization of melanosomes in the keratinocytes and show that it is the keratinocyte (i.e. black Vs white) that determines melanosome localization rather than the size of the melanosome granule.

Ms. MC Scott presented data on the regulation of expression of the MC1 receptor in melanocytes from different pigmentary phenotypes. They showed that the increase in MC1R mRNA levels in response to MSH was more pronounced in melanocytes with low melanin content than in dark melanocytes. Basic FGF upregulated MC1R mRNA levels in dark melanocytes but reduced levels in melanocytes with low melanin content. They also showed differences in MC1R mRNA levels in lightly pigmented Vs darkly pigmented melanocytes in response to TPA, and to combinations of estrogen and MSH. These differences MC1R regulation in dark and light melanocytes suggests differential regulation of MC1R gene expression in people of different pigmentary phenotypes.

Positions - Wanted and Available :

Principal Scientist- Clinical Research - Skin Science Research

Unilever employs over 200 scientists at our New Jersey Laboratory who are dedicated to innovative and scientifically rigorous skin research programs. Our world sales exceed $40 billion so our programs have solid financial funding allowing for an innovative and challenging research culture. We currently have a full time opening that provides a unique opportunity to apply your basic science skills to human studies that impact the condition of skin for hundreds of millions people worldwide. We are seeking an expert in pigment biology or photobiology who can advance our knowledge and link laboratory research to clinically defined improvements of consumer skin problems. As a member of our skin research team, you will have an opportunity to work with other scientific experts in many fields including cell biology, biochemistry, measurement science and physical chemistry. You will also be encouraged to establish and maintain close ties to research in academic and government research communities.

We offer a competitive salary, benefits including tuition assistance and relocation, and a dynamic environment filled with learning and discovery beyond conventional scientific boundaries. Applicants must be authorized to work in the USA. For consideration please forward your CV to: Human Resources, Dept. CR-SID, Unilever Research US, 45 River Road, Edgewater, NJ 07020 or E-Mail: job.mca@unilever.com . Please place only the letters "CR-SID" as the subject of your e-mail. Unilever is an Equal Opportunity Employer m/f/d/v.

Postdoctoral Fellows - Cancer and Developmental Biology - Two NIH-funded positions are available for fellows interested in studying the Hedgehog signaling pathway in development and disease using skin as a model system. One project centers on defining the function of the Hedgehog pathway during skin appendage morphogenesis (Dev. Biol. 205: 1-9, 1999); a second project focuses on understanding how deregulated activation of this pathway gives rise to basal cell carcinomas (Nature Genet. 24: 216-7, 2000). Applicants should have a solid background in molecular and cell biology, with experience in transgenic animal models desirable but not required. Interested individuals should send a CV, letter of interest, and names of three references to: Dr. Andrzej Dlugosz, University of Michigan, Department of Dermatology and Comprehensive Cancer Center, 3310 CCGC, Box 0932, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0932 Email: dlugosza@umich.edu. The University of Michigan is an Equal Opportunity Employer.

Postdoctoral Research Associate - Position available to study the biology of human inherited disorders of pigmentation using gene transfer technology. The successful applicant will have a Ph.D. and/or M.D. with experience in cell biology and molecular biology. Experience in gene transfer/genome manipulation is preferred. Please send curriculum vitae along with the names of three references to Dr. Richard King, Division of Genetics, Department of Medicine, Box 485 Mayo, 420 Delaware St. S.E., University of Minnesota, Minneapolis, MN 55455. Equal Opportunity Employer.

Postdoctoral Position - Ph.D. in molecular biology, biophysics, genetics or biochemistry. Position available to conduct research on molecular mechanisms of cellular response to oxidative stress in human melanocytes and melanoma cells and its regulation for preventive and therapeutic indications. Contact Dr. Frank L. Meyskens Jr., Director, University of California-Irvine, Chao Family Clinical Cancer Research Center, 101 The City Drive, Orange, CA 92668, USA. Fax (714) 456-5039 Email flmeyske@uci.edu

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The Bibliography published in this issue covers the period May through July, 2000. If you notice a paper that was not detected by this search that should be included, please send it to us and we will include it in the next issue. By its very nature, assignment of a reference to a particular category is arbitrary and we urge you to read through all categories to make sure you don't miss any pertinent to your field. We have attempted to highlight any publications which include a member of the PASPCR with a star (*sorry if we missed you but let us know and you'll get a free marked repeat in the next issue*).

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