PANAMERICAN SOCIETY for PIGMENT CELL RESEARCH

PASPCR Newsletter

Volume 4 Number 1 **March,1996**

Introduction . . . **by the Publications Committee**

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 the *Newsletter*; help us to update the Job Listings, Calendar of Events, Meeting Reports, Abstracts in press and other items of general membership interest. If you attend a scientific meeting at which you heard about work 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. If you should have a change of affiliation or address, we'd like to know that, too. 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 any of the members of the Publications Committee.

NEW! A WorldWideWeb Page for the PASPCR. The PASPCR now has its own **WWW** page. We plan this to be a major source of current information for the PASPCR membership. The address for the page is: **http://lenti.med.umn.edu/paspcr**. This site contains information on the goals of the society, future meetings, council information, past issues of the PASPCR newsletter as well as links to other sites including the InterPig data base and the International Federation of Pigment Cell Societies (IFPCS). We will be also finalizing the membership directory. You should soon receive a mailing that will allow you to state what information about yourself that you want included in this directory. This will be an important part of this web site, in that changes in the membership directory can be easily made, providing access to up to date membership information. The PASPCR WWW page is still under construction and we want to know if there is any other information you would like located on this site.

Please check out the PASPCR web site and send any comments and/or suggestions to either Bill Oetting at bill@lenti.med.umn.edu or Vince Hearing at hearingv@dc37a.nci.nih.gov.

IN THIS ISSUE . . . . .

enclosure: **IPCC Travel Stipend Form**

PanAmerican Society for Pigment Cell Research

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Calendar of Events :

April 20 - 24, 1996 87th Annual Meeting of the American Association for Cancer Research, to be held in Washington DC (contact: AACR Office, Public Ledger Bldg, suite 816, 150 South Independence Mall West, Philadelphia PA 19106-3483; FAX: 215/440-9313)

May 1 - 5, 1996 57th Annual Meeting of the Society for Investigative Dermatology, to be held in Washington DC (contact: SID Office, Suite 500A, 11001 Cedar Ave, Cleveland, OH 44106; FAX: 216/844-6810)

June 2 - 6, 1996 Annual Meeting of the American Society for Biochemistry and Molecular Biology and the American Association of Immunologists, to be held in New Orleans, LA (contact: FASEB Office, 9650 Rockville Pike, Bethesda, MD 20814-3998; FAX: 301/530-7014)

Oct 29- Nov 3, 1996 XVIth International Pigment Cell Conference, to be held in Anaheim, California, (contact: MMC/UCI Center for Health Education, PO Box 1428, Long Beach, CA 90801-1428, FAX: 310/933-2012)

Dec 7 - 11, 1996 36th Annual Meeting of the American Society for Cell Biology and 6th International Congress on Cell Biology, to be held in San Francisco, CA, (contact: ASCB Secretariat, 9650 Rockville Pike, Bethesda, MD 20814-3992; FAX: 301/530-7139)

Jun 15- 18, 1997 VIIth PASPCR Annual Meeting, to be held in Providence, RI (contact: Dr. Walter C Quevedo, Jr., Brown University, Division of Biology and Medicine, Providence, RI 02912; FAX: 401/863-1971)

Welcome to New Members

by James J Nordlund / Richard A King

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

Yiqing Xu

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 / Richard A King

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.

GOLD Corporate Patrons	*SILVER Corporate Patrons*	*Corporate Patrons*
ICN Pharmaceuticals, Inc	Avon Products, Inc	Galderma Laboratories, Inc
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Ortho Pharmaceutical Corp	Procter and Gamble Co	
	Shiseido Co, Ltd	

1995 PASPCR Elections

by Richard A King

The PASPCR elections are complete. Following are the results.

President-Elect:	Richard A. King		
Secretary/Treasurer:	James J. Nordlund		
Council members:	Kenneth Mason	Frank Meyskens	David A. Norris

In Memoriam . . . Dr. Takuji Takeuchi

by Shosuke Ito and Jiro Matsumoto

Dr. Takuji Takeuchi, Editor-in-Chief for ***Pigment Cell Research***, died of liver failure on January 2, 1996. He was in the position of President of Nihon Gene Research Laboratories in Sendai and Professor of Ishinomaki Senshu University in Ishinomaki, Japan.

He was born in Kagawa Prefecture, Japan in 1930, graduated from Tohoku University in Sendai in 1955 and, via the Graduate School of Science there, appointed as Assistant Professor of Fukushima Prefectural Medical College in Fukushima in 1958. He stayed in the Department of Zoology at Rochester University from 1960 - 1961 and at the Department of Genetics (Professor M. Foster's laboratory) at the University of Michigan from 1961 - 1963.

After his return to Japan, he became Associate Professor of Miyagi University of Education in Sendai in 1965 and moved to Tohoku University in 1970. He was promoted to Professor in 1979 and became Professor of Animal Embryology there in 1985. In 1993 he retired at the age of 63 according to the age-limit system of Tohoku University.

Dr. Takeuchi dedicated himself to the organization of the XIth IPCC held in Sendai in 1980, together with the late Professor Makoto Seiji. He also organized the Symposium on Molecular Biology of Pigment Cells which was held in Sendai in 1990. He received the Seiji Award in 1984, the Zoological Society Prize from the Zoological Society of Japan in 1992, and the Myron Gordon Award from the IFPCS in 1993.

Dr. Takeuchi was a member of the Board of Associate Editors after the foundation of ***Pigment Cell Research*** in 1987 and was nominated as the successor of Professor Joseph T. Bagnara, the founding Editor-in-Chief, by recommendation of the IFPCS. The Japanese Government conferred the Order of Secret Treasure (Gold) with Neck Ribbon on him after his death.

XVIth IPCC (International Pigment Cell Conference) by Roger Bowers, Frank Meyskens

The XVIth International Pigment Cell Conference will be held from October 29th to November 3rd, 1996 at the Disneyland Hotel in Anaheim, California. Frank Meyskens is the Organizer of this meeting with Roger Bowers and Alistair Cochran serving as co-chairs of the Organizing Committee. The PASPCR has established a Web page that contains relevant information for this meeting; take a look at: "**http://lenti.med.umn.edu/paspcr/ipcc.htm**". Travel stipends for PASPCR members will be available as usual and a Travel Stipend Application Form with Instructions and Eligibility Requirements is included within this Newsletter. Following is the tentative Program of the IPCC:

XVIth International Pigment Cell Conference - Tentative Conference Agenda

Tuesday, October 29, 1996
3:00 - 7:00 pm Pre-registration/View Exhibits
7:00 - 10:00 pm Welcome Reception: Fashion Show: "***Safe and Sexy in the Sun***"

Wednesday, October 30, 1996 Conference Attendees
7:00 - 8:00 am Registration/Continental Breakfast/View Exhibits
8:00 - 8:05 am Welcome: Chairman, Frank L. Meyskens, Jr.
Introduction: Laurel Wilkening, Chancellor, Univ of California, Irvine
8:05 - 8:35 am ***Special Lecture***, R. Sherwood Rowland, Nobel Laureate, 1995, Chemistry
"***Ozone Depletion, Ultraviolet Light, and the Pigment Cell***"

Symposium I: Economic and Societal Implications of Melanin amd Melanogenesis
8:35 - 9:00 am Keynote Speaker
9:00 - 10:30 am Invited and Competitive Abstract Speakers
10:30 - 11:00 am Break
11:00 - 12:30 pm ***Workshop A: "Extracutaneous Melanin"***
Posters and Discussion #1 TBN* (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)
12:30 - 2:00 pm Lunch on your own

Symposium II: Molecular Biology of Pigment Cells
2:00 - 2:30 pm Keynote Speaker
2:30 - 4:00 pm Invited and Competitive Abstract Speakers
4:00 - 4:05 pm IFPCS InterPig Database on the WorldWideWeb: Vincent Hearing
4:05 - 4:15 pm Break
4:15 - 6:15 pm ***Workshop B: "Regulating Mechanisms of Melanocyte Proliferation"***
4:15 - 6:00 pm Posters and Discussion #2 TBN* (4:15 - 5:30 Viewing; 5:30 - 6:00 Discussion)
5:30 - 7:00 pm ***Workshop C: "Biophysics of Melanin"***
6:15 pm Adjourn Free evening

Accompanying Guests (8:00 - 11:00 am) Welcome/Introduction: Buffet Breakfast;
(12:00 - 5:00 pm) Group Activity

Thursday, October 31, 1996
7:00 - 8:00 am Continental Breakfast/View Exhibits
8:00 - 8:30 am ***Seiji Lectureship:*** Introduction: Giuseppe Prota, President, IFPCS

Symposium III: Melanoma Research: Basic and Applied
8:30 - 9:00 am Keynote Speaker
9:00 - 10:30 am Invited and Competitive Abstract Speakers
10:30 - 11:00 am Break

11:00 - 12:30 pm ***Workshop D: "Control of Melanogenesis"***
11:00 - 12:30 pm Simultaneous Business Meetings of Regional Societies
12:30 - 2:00 pm Lunch on your own

Symposium IV: Photobiology of Melanocytes: Etiology and Prevention
2:00 - 2:30 pm Keynote Speaker
2:30 - 4:00 pm Invited and Competitive Abstract Speakers
4:00 - 7:00 pm ***Workshop E: The "Blues" Symposium***
4:00 - 7:00 pm Poster Viewing
Adjourn - Free evening

Friday, November 1, 1996
7:00 - 8:00 am Continental Breakfast/View Exhibits
8:00 - 8:30 am Introduction: Sally Frost-Mason, *President,* PASPCR
***Gelb Lectureship*:** Seth Orlow

Symposium V: Melanogenesis and Pigmentary Disorders
8:30 - 9:00 am Keynote Speaker
9:00 - 10:30 am Invited and Competitive Abstract Speakers
10:30 - 11:00 am Break
11:00 - 12:30 pm ***Workshop F: "Biology and Biochemistry of Melanosomes"***
11:00 - 12:30 pm Posters and Discussion #3 TBN* (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)
12:30 -1:15 pm ***Controversy Session:*** "Semiquinone Radicals are <u>not</u> Important during Melanin Synthesis"
Pro: Patrick Riley Con: Stan Pavel
1:15 pm Adjourn Scientific Session
1:15 - 6:30 pm Break
6:30 - 7:30 pm Reception
7:30 - midnight Banquet, Awards and Dancing

Saturday, November 2, 1996
7:00 - 8:00 am Continental Breakfast/View Exhibits
8:00 - 8:30 am ***Presidential Address:*** Giuseppe Prota, President IFPCS

Symposium VI: Comparative Developmental Biology of Pigment Cells
8:30 - 9:00 am Keynote Speaker
9:00 - 10:30 am Invited and Competitive Abstract Speakers
10:30 - 11:00 am Break
11:00 - 12:30 pm ***Workshop G: "Genetic Aspects of Albinism"***
11:00 - 12:30 pm Posters and Discussion #4 TBN* (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)
12:30 - 2:00 pm Lunch on your own
2:00 - 4:00 pm ***Educational Forum: "Living with the Sun"***
4:00 - 6:00 pm Family Farewell Reception and Wine Tasting

Sunday, November 3, 1996
8:00 - 5:00 pm
1. *Satellite Meeting (all day):* ***Classification of Cutaneous Melanoma***: Alistair Cochran
2. *Satellite Meeting (3 hours):* ***Safety of Sunscreens and Tanning Parlors*:** J.P. Césarini, et al.
3. *Satellite Meeting* (*3 hours*): ***Ocular Melanin*:** Giuseppe Prota

Workshops and poster and poster discussion sessions will be simultaneous. The poster sessions and discussions will feature areas that do not overlap with the workshop. The chairs of these sessions will be selected from submitted competitive abstracts and the Chairman in turn will organize this session with help from the Organizing Committee.

A Letter from the President : **by Sally Frost-Mason**

Dear Members,

Please accept my heartfelt best wishes for a happy, safe, and productive new year. With the new year come changes in the Society. Vince Hearing who has so ably and unselfishly served three years as President, now assumes the role of Past President. Dick King, who has capably managed the Society's treasury since its inception, will be succeeded by Jim Nordlund. Dick will not be without responsibilities as he begins a term as President-Elect. New Council members include Frank

Meyskens, David Norris, and Ken Mason. Clearly, the Society remains in good hands as we look toward the millenium.

This year will be a year in which we celebrate our own successes at the International Pigment Cell Meeting in Anaheim, California in the fall. Frank Meyskens, Alistair Cochran, Roger Bowers and others have worked hard to ensure an outstanding scientific platform in one of the world's foremost playgrounds. Our Society can be pleased and proud of the quality that our meetings have always achieved, and we must all support the efforts in Anaheim as hosts to the rest of the Federation.

In the summer of 1997 we can look forward to PanAmerican meeting in Providence, Rhode Island. Walt Quevedo has already begun the planning process, so be sure and set aside time now to visit beautiful New England in June 1997.

A Society is only as viable as its membership. We have indeed been blessed with a loyal and hard-working membership. It is my hope that our membership can continue to grow in the coming years, and one of my primary goals will be to continue to find ways to both encourage and reward our young investigators, including faculty, postdocs, and students.

Finally, as we look toward the uncertainty of funding for basic research especially at the federal level, we must be ever vigilant in our efforts to support each other. I believe it is this spirit of cooperative endeavor that has sustained the vitality of our Society, and I know from speaking with visitors to our meetings that the mutual respect and support that we all derive from each other is both envied and greatly appreciated.

Once again, I thank you all for your support and I look forward to serving as your president for the next three years.

Sincerely, Sally Frost-Mason

PASPCR Secretary / Treasurer's Report : by Richard A King

Following is a synopsis of the PASPCR Council Meeting held by teleconference on October 19, 1995 . . .

Hearing opened the meeting and a quorum was declared present. The minutes of the June 25, 1995, council meeting were unanimously approved.

Oetting, chair of the Publications Committee, discussed the WWW information site for the PASPCR and the newsletter, and reviewed the potential data that could be included in the web site including the name, method of contact and a short paragraph of research and publications. A questionnaire will be included with the upcoming ballot and dues notice surveying the membership on potential participation in the PASPCR web site. The Council discussed the lack of development of the membership brochure; this project will be turned over to a new committee.

Hearing reviewed the recent discussions and concerns regarding the 1996 IPCC Anaheim meeting. He noted that the officers of the society had met by telephone conference with Frank Meyskens, Roger Bowers and Alistair Cochran to express their interest in having continued regional society involvement in the development of the meeting, and Meyskens reassured the officers that this was planned. The IFPCS council will meet in Japan in early December and further discussion of regional society involvement in the development of the 1996 IPCC is expected.

Quevedo reviewed the plans for the 1997 PASPCR meeting in Providence, Rhode Island. Harold Swartz and Quevedo are organizing the program committee, and they have outlined three potential themes for the meeting: Cell suicide and apoptosis, development regulation and function of ocular pigment, and molecular genetics of melanoma models. Fund raising for this meeting continues to be a concern.

The minutes have been prepared by R. King, Secretary-Treasurer

Positions - Wanted and Available :

Predoctoral Position - Opportunity available to do graduate studies towards a doctoral degree at the University of Cincinnati College of Medicine. Graduate program is through the Department of Cell Biology, Neurobiology, & Anatomy. Dissertation project would focus on molecular biology of the melanocyte physiology and pigmentary diseases. For information contact: Raymond E. Boissy, Ph.D., Department of Dermatology, University of Cincinnati College of Medicine, 231 Bethesda Avenue ML-592, Cincinnati, Ohio 45267-0592; (513)558-6242 [TEL]; (513)558-0198 [FAX]; boissyre@ucbeh.san.uc.edu [eMAIL].

Faculty Position - Massachusetts General Hospital, Harvard Medical School, Cutaneous Biology Research Center. The Cutaneous Biology Research Center (CBRC) seeks a molecular, cellular or developmental biologist to establish a program in fundamental research relevant to skin pigmentation. Areas of reseearch can include but are not limited to pigment synthesis and transfer in melanocytes, genetics of mouse coat color and development/migration of neural crest cells. Applicants must have a Ph.D. and/or M.D. degree and relevant postdoctoral experience. Only applicants with a strong research record and the potential to develop extramurally supported research programs will be considered. Individuals with a demonstrated ability to develop imaginative approaches to important biological questions are particularly encouraged to apply. Rank/salary/start-up funds and space are negotiable depending on experience and qualifications. The CBRC occupies 45,000 square feet of fully equipped laboratory space in a new multidisciplinary research facility. Interested individuals should send curriculum vitae, reprints, a statement of research and future directions, along with the names, addresses and telephone numbers of three references to: Dr. Paul F. Goetinck, Chair, Faculty Search Committee, Cutaneous Biology Research Center, Massachusetts General Hospital - East, Building 149, 13th Street, Charlestown, MA 02129

Director, Section of Basic Science Research - the Department of Dermatology and the Research Institute of the Cleveland Clinic Foundation are seeking applications for a full-time research scientist. Candidates should possess an M.D. and/or Ph.D. degree and have research experience in one of the following areas: wound healing, keratin/collagen biochemistry, or cancer immunology/molecular biology. The successful candidtate will have the ability to work as an independent investigator and be the focus for dermatological research in a dynamic, multi-disciplinary and supportive environment. Preference will be given to individuals who have demonstrated prior success in obtaining extramural support. Salary and title will be commensurate with training and experience. A University affiliation is available. Send C.V. to: Charles Camisa, M.D., Vice-Chair, Department of Dermatology, A61, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195-5032.

INTERPIG DataBase Update

by Vincent Hearing

The INTERPIG database is on the InterNet! You can now access the InterPig DataBase at the following address: **http://lenti.med.umn.edu/paspcr/interpig.html**. Please note that as of this time, I estimate that less than 5% of the various IFPCS members have contributed entries. Think of how useful and complete this list would be if everyone took the time to supply their own information. Please take a moment to fill out the database data entry form and send it back to Dr. Hearing (a copy of the entry form is inserted into this Newletter, and it can be photocopied).

Meeting Report

by Koichiro Kameyama

10th Annual Meeting of the J S P C R Osaka, Japan December 9-10, 1995

This meeting was organized by Osaka University School of Medicine with Prof. Morita as Chairman; it was composed of six special lectures by council members of IFPCS and 25 papers.

Special lecture "Modulation of expression and activity of tyrosinase, tyrosinase-related proteins and other melanosomal proteins in murine melanocytes treated with MSH or agouti signal protein" Dr. Vincent J. Hearing discussed the interaction of tyrosinase gene family products and MSH or agouti signal protein (ASP). Recently ASP has been reported to antagonize the ability of MSH using a cloned MSH receptor, and

ASP and MSH are key substances of the switching between eumelanogenesis and pheomelanogenesis. To further characterize the switch between eumelanogenesis and pheomelanogenesis, Dr. Hearing studied the responses of cultured melan-a black melanocytes exposed to MSH and/or recombinant ASP. After exposure to ASP, the color of the cell pellets changed from black to light brown, the number of melanosomes decreased, they became pheomelanosome-like at the ultrastructural level, and the amount of total melanin or eumelanin was much decreased, while the amount of pheomelanin was slightly but significantly increased. At the same time the amount of tyrosinase, TRP1 or TRP2 were decreased at mRNA level or protein synthesis level. When MSH was added to the culture, the amount of tyrosinase was increased at transcriptional level or translational level, and total melanin was increased due to increasing eumelanin, while the amount of pheomelanin was not changed significantly. Interestingly, in cells treated with MSH and ASP, the level of tyrosinase, TRP1, TRP2 and melanin production was increased. These data suggest that ASP suppress tyrosinase, TRP1 and TRP2 expression primary at the level of transcription and that the effects of ASP are not mediated solely by inhibition of MSH binding. After his lecture, there was one interesting question which asked the mechanism of the interaction between ASP and MSH. He answered that agouti is a functional antagonist of MSH binding and the possible mechanism is that; 1) via competitive inhibition of MSH binding, 2) via distinct agouti receptor, 3) via distinct effect on MSH receptor, and to resolve the question completely, further study such as binding study is necessary.

Special lecture " The genetic complexities of human oculocutaneous albinism: a model of melanin regulation" Dr. Richard King reported the model system for the study of normal and abnormal human pigmentation in oculocutaneous albinism (OCA); he identified four major genes responsible. Mutations of the tyrosinase gene produce OCA1 or tyrosinase related OCA, and at least 100 different mutations of this gene have been identified. Mutations of the P gene are responsible for OCA2 or tyrosinase positive OCA, the most common type of OCA in humans. He analyzed P gene mutation on individuals with a phenotype compatible with OCA2. One common 2.7 kb deletion mutation has been found in African-American and African individuals. A mutation in the TRP1 gene has been found in one individual with OCA3 or brown OCA. Cultured melanocytes from this individual have no TRP1 mRNA and demonstrate a significant reduction but not a total loss of DHICA oxidase function. After his presentation there were several interesting questions. One question asked if there is a relationship between tyrosinase gene mutation and P gene mutation or not. His answer was yes and he mentioned two possible mechanisms; one possibility is that the two genes exist on very close site, and another possibility is that P gene plays an important role on the management of promoter of tyrosinase gene. One another question asked the presence of the patient with TRP2 mutation. His answer was no.

Analysis of melanin - Dr. Shosuke Ito developed microanalytical methods to qualify eumelanin (EM) and pheomelanin (PM) based on the HPLC analysis of their specific degradation products pyrrole-2,3,5-tricarboxylic acid (PTCA) and aminohydroxyphenylalanine. He showed that 1) total melanin (EM+PM) can be dissolved in hot Soluene-350, 2) EM can be dissolved in $NaOH\text{-}H_2O_2$ after removal of PM by heating in hydriodic acid, and 3) PM can be preferentially dissolved in NaOH. He also mentioned that to see the total melanin, spectrophotometric method using Soluene-350 is the best, and this method is available for cultured cells. He also commented that an important point is that Soluene-350 is the trade name, to check the total melanin, he uses 500 nm not 350 nm. Dr. Ozeki Hiroyuki in Dr. Ito's group presented a comparison of spectrophotometric methods (Sp. EM)/spectrophotometric method using Soluene-350 (total M). He found that the comparison of Sp. EM/total M ratio of brown eumelanin in mice hair was about a half that of black eumelanin, although the PTCA/total M ratio indicates that the proportions of DHICA-derived units were similar. He also showed that 1) Sp. EM/total M ratio correlates to the percentage content of DHICA-derived units, 2) the Sp. EM/total M ratio of DHICA melanin increases as the polymerization proceeds, and 3) the Sp. EM/total M ratio is a parameter as useful as the PTCA/total M ratio in distinguishing chemical properties of eumelanins.

Adult T cell leukemia-derived factor (ADF) / thioredoxin - Dr. Yoko Funasaka in Kobe University studied the effect of ADF/ human homologue of thioredoxin in cultured human keratinocytes. She has already reported that UVB irradiation induces the strong expression of ADF in cultured human keratinocytes. To study the effect of ADF on UVB-induced melanogenesis, she analyzed the MSH receptor binding activity using ^{125}I labeled MSH, the expression of MSH receptor mRNA using the melanocortin 1 receptor (MC1-R) cDNA and ^{3}H-thymidine uptake in normal human melanocytes. She showed that ADF increased MSH receptor binding activity of MSH and MC1-R mRNA expression. ADF has not stimulated DNA synthesis neither alone nor with endothelin-1, stem cell factor, hepatocyte growth

factor, and basic fibroblast growth factor, but ADF increased MSH-induced DNA synthesis. These results indicate that ADF is one of the stimulatory factors for UVB induced melanogenesis via upregulating MSH receptor binding activity with increasing MC1-R mRNA expression and increasing the DNA synthesis by MSH stimulation. After her presentation, there was a question which asked the mechanism of increasing of MSH binding activity, so that, increasing the number of MSH receptor or increasing the affinity of them. Her answer was the mechanism is still not clear.

Tyrosinase related proteins - The enzymatic functions of tyrosinase, TRP1 and TRP2 are already becoming clear. TRP1 can oxidize DHICA produced by TRP2. However the effect of those enzymes on each other is still not clear. Dr. Kobayashi studied the stabilizing function of the b-locus protein on tyrosinase. He investigated the effect of mutations at the brown locus on stability of tyrosinase in cells. Pulse labeling and chase experiments showed that tyrosinase degraded more quickly in melan-b melanocytes, homozygous for the brown mutation than in melan-a melanocytes, the wild type. Less stability of tyrosinase in melan-b cells was partly rescued by transfection of the wild-type TRP1 gene along with phenotypic rescues. His results clearly showed that TRP1 could stabilize tyrosinase in melanocytes and might also contribute to the polymerization of DHI into melanin.

Melanogenic inhibitor - Dr. Yokota and Dr. Kameyama reported the new melanogenic inhibitor, ascorbic acid 2-phosphate sodium salt (APS). They reported that APS is an ascorbic acid derivative that is stable in aqueous solution unlike ascorbic acid. $8X10^{-3}$ M APS decreased the total amount of melanin approximately 30% , and suppressed ^{14}C-thiouracil uptake approximately 50% without suppression of ^{3}H-thymidine uptake in cultured B16 melanoma cells. Relatively high concentration of APS ($2.7X10^{-2}$M) suppressed the activities of tyrosinase, TRP1 and TRP2 significantly on cultured melan-a cells. Then 3% APS cream was applied to the patients with hyperpigmented disorders such as melasma. The results showed that APS cream was effective on 9 out of 20 patients. These findings clearly indicated that APS application can be useful for some patients with hyperpigmented diseases. After their presentation there was a question which ask the absorption of APS. Dr. Kameyama answered although they don't have the experimental data of APS, similar ascorbic acid derivative magnesium L-ascorbyl-2-phosphate showed that approximately 2% was absorbed into epidermis or dermis on diffusion cell chamber assay.

Bibliography :

The Bibliography published in this issue covers the period November, 1995 through January, 1996. 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. We have attempted to highlight any publications which include a member of the PASPCR with a star.

MELANINS, MELANOGENS & MELANOGENESIS

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Awards Presented at the 6th ESPCR Meeting in Lausanne

by Ghanem Ghanem

The Golden Melanocyte Award

Melanin pigmentation plays an essential role in protecting the skin from the damaging effects of ultraviolet radiation (UVR)

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In mammals, there are at least two types of melanin, the red/yellow phaeomelanin and the brown/black eumelanin, both of which are present in human skin (1). Of these two types of melanin, eumelanin has the more significant role in protection against UVR whereas phaeomelanin, because of its greater potential to produce free radicals in response to UVR (2), which are capable of inflicting cell injury, may actually contribute to UV-induced skin damage. Thus, the susceptibility to photocarcinogenesis and possibly the tanning ability may depend not simply on the amount of melanin in the skin but also upon the type of melanin produced in the melanocytes.

In mice and other mammals the relative proportions of phaeomelanin and eumelanin are regulated by melanocyte-stimulating hormone (MSH), which acts via its receptor (MC1R), on melanocytes, to increase the synthesis of eumelanin (3,4) and the product of the agouti locus which antagonises this action (5). In mice, mutations at either the MC1R or agouti genes affect the pattern of melanin synthesis resulting in coat colour changes (6,7).

The purpose of the present study was to determine whether MC1R variants occur in humans as in other mammals and whether they are related to their pigmentation phenotype.

Because of the polygenic nature of human pigmentation, 60 British or Irish Caucasians were selected from individuals showing extremes of hair colour, that is either red or black. Genomic DNA was extracted from blood or mouthwash samples and the coding region of the MC1R gene analysed by nested PCR followed by direct cycle sequencing. This methodology enabled us to determine that the 30 dark-haired individuals that we examined had no variations respect to the sequence previously published for the coding region of the MC1R gene (8). In contrast, 70 % of the red-haired population had at least one substitution in this gene. In total, 9 different heterozygous variations were identified (A64S, F76Y, D84E, V92M, T95M, V97I, A103V, L106Q, D294H). 8 of them were located in a region of 42 aminoacids between the first cytoplasmic loop and the first extracellular loop spanning the second transmembrane domain. This location was very similar to that previously found for the dominant mutations of the MC1R gene in mice (9). On the other hand, the substitution at codon 294 was located in the seventh transmembrane domain. The D294H change was the most common (53 %) followed by the variation at codon 92 (27 %). However, from the functional point of view, the variations at codons 84, 106 and 294 may be the most important, since these positions are highly conserved in the melanocortin receptor family (8,10,11).

Since we only found variations in the MC1R gene sequence from the red-haired individuals as opposed to those from the dark-haired ones, we examined whether these substitutions were related to a specific shade of red hair. To do this, we subclassified the red-haired population according to their shades of red hair as light-red, deep-red and auburn or brownish-red. The variation at codon 294 was found in all three groups, the substitution at codon 92 occurred in light-red and deep-red haired individuals, while all the other changes were seen either in light-red or deep-red haired ones. Moreover, 8 of these individuals with light-red or deep-red hair had a combination of two, three or even four substitutions in their MC1R gene (D294H & T95M; D294H & V97I; V92M & L106Q; two with D294H & V92M; D294H, V92M & A64S; D294H, V92M & D84E; D294H, V92M, A64S & D84E). The

cloning of their PCR products and the subsequent sequencing of several clones showed that only one had the variations at the same allele of the gene (D294H & V97I) while the remaining 7 were compound heterozygous. These results pointed to a complex relationship between the red hair phenotype and the MC1R genotype. On the other hand, these data did not discount the possibility that the MC1R variants were present in individuals with intermediate hair phenotype between the extremes red and black. We therefore analysed a new group of 75 volunteers with different hair colours ranging from one extreme to the other. The results obtained from these 75 individuals were pooled whith those the 60 previously examined. 82 % of light-red/deep-red haired persons had changes in one or both alleles, compared with 22 % auburns, 33 % of fair or blondes and less than 20 % of the brown or blacks. The ocurrence of some subtitutions (only at codons 92, 103 or 294) in some individuals with intermediate hair phenotype and even with black hair indicated that the MC1R variants were not exclusively associated with red hair. It was possible however that they were related to the poor tanning ability which characterises the red-haired population. To examine this possibility, we classified the individuals' skin type by using the Fitzpatrick classification and found the highest frequency of any MC1R variation in individuals with skin type I (76.5 %), followed by those with skin type II (46.5 %). No individuals with skin type IV and only 5 % with skin type III had changes. In addition, only persons with skin type I or II, who also had light-red or deep-red hair had more than one substitution or substitutions at both alleles of the gene.

Our findings suggest that in humans as in other mammals, MC1R may be a control point in the regulation of pigmentation phenotype, and more importantly, that variations in this protein are associated with a poor tanning in humans. Functional and mapping studies are now in progress to determine whether MC1R may be used in the future as a marker for the study of human population genetics and to clarify the wide variations in pigmentation and the susceptibility to skin cancer in humans.

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The Silver Melanocyte Award

Expression of apoptosis related antigens in cultured melanocytes

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During the last six years the investigators at AMC-UvA concentrated their studies on the role of melanocytes (MC) within the Skin Immune System (SIS), particularly in relation to the pathology of vitiligo. From in vitro investigations it has become clear that MC can have both an immune effector function as well as can become a target of the immune system (1). In situ immunohistochemical analysis of lesional, peri-lesional and non-lesional vitiligo skin showed the presence of interacting cellular infiltrates composed of T-cells and macrophages. Particularly in peri-lesional skin CD68 positive macrophages seem to constitute the major part of these infiltrates. These in situ data indicate that the interaction between MC, T-cells and macrophages is important in destruction of MC as seen in vitiligo. We hypothesize that immune mediated destruction of MC plays an important role at least in some of the vitiligo patients (2).

Numerous studies on the biology of MC indicate that toxic intermediates are formed during the process of melanin synthesis (3). Necrotic spill over of these substances by dying MC might damage the surrounding cells. Since tissue damage is scarcely observed in hypopigmentation we hypothesize that MC in vitiligo are induced to die by apoptosis. In this form of cell death the cell itself actively participates by activating a suicide program. This process finally leads to the formation of morphologically distinct apoptotic bodies that can be cleared by phagocytic cells like the infiltrating macrophages found in the peri-lesional vitiligo skin (4).

In order to substantiate such hypothesis an essential pre-requisite is to demonstrate that MC are able to undergo apoptosis. With the use of UVB we confirmed that MC in vitro are vulnerable to an apoptotic process. After UVB irradiation cells were incubated with the DNA binding dyes "Propidium Iodide" and "Hoechst 33342" and examined by epifluorescence microscopy, that allows discrimination of healthy, apoptotic and necrotic cells by morphological criteria. The results indicate that at 48 hours after UVB irradiation (40 J/m2) apoptosis was induced in 30% of MC whereas necrosis occurred in less than 2% of the cells. Free radical species are considered to be one of the major intracellular mediators of apoptosis. Interestingly, antioxidant treatment of vitiligo patients has been reported a suitable therapeutic strategy by several groups (6,7). This suggests that vitiligenous MC either have an intrinsic defect in antioxidant defences or cannot overcome the extracellular oxidative stress as may be executed by the observed immune infiltrates. A possible intrinsic defect and the subsequent excess of intracellular Reactive Oxigen Species (ROS) may lead directly to ROS-mediated damage and apoptosis. Alternatively damage caused by ROS may lead to altered antigenicity of MC, this may render MC a target of the immune system.

The investigations were extended to study possible differences in baseline expression levels of Bcl-2, BAX, p53, p21 and FAS on MC. The Bcl-2 molecule has been suggested to function in an antioxidant pathway and plays an important role in cell resistance to apoptosis. BAX can form hetero-dimers with Bcl-2 and herewith inhibit its function; high levels of BAX as compared to Bcl-2 will therefore render cells more vulnerable to certain apoptotic stimuli. On the other hand the tumor suppressor p53 directly regulates gene expression of both Bcl-2 and BAX (8). In addition p53 can regulate cell cycle arrest via p21 (9) and recent publications suggest that the cell membrane expressed FAS molecule can be upregulated by p53. FAS/FAS-ligand interaction is an essential for one of the two pathways by which cytotoxic T-cells can induce apoptosis in target cells (10). To inventory baseline expression levels of the above mentioned molecules melanocytic cell cultures of differentiation stages were grouped as follows: fetal (n=3D4), neonatal (n=3D5), normal adult (n=3D5) and adult naevus (n=3D5) as well as non-lesional adult vitiligo MC (n=3D5) and were investigated by FACS.

Donor to donor differences exist for all markers, also within the different groups of cells. Differences in average expression levels between groups are discussed below. Examining Bcl-2:BAX ratios a trend can be seen in which the ratio is low in both fetal and neonatal cells and equally high in normal adult and non-lesional vitiligo MC. The Bcl-2:BAX ratio in adult naevus cells however resembles that of fetal and neonatal cells. Focussing on the BCL-2 defence system alone these results suggest that adult naevus cells as well as fetal and neonatal cells are more prone to be affected by certain apoptotic stimuly. One has to bear in mind though that although relative BCL-2:BAX expression levels are similar in normal adult and non-lesional vitiligo cells this does not exclude possible functional differences.

p53 expression levels are somewhat lower in fetal and naevus cells as compared to neonatal, non-lesional vitiligo and normal adult MC. This does not reflect the differences found in Bcl-2:BAX ratios as it has been reported that p53 upregulates BAX and downregulates Bcl-2 expression. At present it is not kown wether Bcl-2 and BAX levels can be regulated via other mechanisms. The range of p21 expression levels is similar in all cultures except for naevus cells which have an increased p21 expression. In view of the relatively low p53 expression by naevus cells this result may be explained by a pathway for p53 independent induction of p21 as has been reported by others (9).

Average FAS expression is slightly upregulated in fetal cells whereas the naevus cells express almost three times as much FAS than cells in other differentiation stages. Therefore these cells are possibly more susceptible to FAS mediated induction of apoptosis by cytotoxic T-cells. From the above mentioned investigation it can be speculated that fetal and naevus cells and not non-lesional vitiligo MC will be more prone to actually enter apoptosis after a stimulus has been applied. To unravel the possible role of MC apoptosis in hypopigmentation, further studies are in progress.

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The Bronze Melanocyte Award

Comparison of attachment of human ocular melanocytes and melanoma cells to extracellular matrix (ECM) proteins

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The aim of our study was to investigate whether the intracellular signalling systems used by the uveal melanoma cell differed significantly from those used by the uveal melanocyte in their attachment to ECM proteins. Any differences identified could be used to select a pharmacological approach to affect neoplastic but not normal cell attachment. First of all we compared the substrate preference of the normal versus the neoplastic cell and found that the melanocyte expressed a clear preference for fibronectin over the other substrates studied (collagen I, collagen III, collagen IV, laminin and plastic). In contrast to this, the melanoma cells showed an equal preference for collagens I,III,IV and fibronectin. We then compared the timecourse of attachment of both cell types to fibronectin, but found that both normal and transformed cells attached similarly showing rapid attachment within 20 - 30 minutes. In this study, we considered the post receptor events and examined which intracellular signalling systems which were important in mediating the early stages of cell attachment to matrix proteins. All initial studies were conducted using melanocytes in their mitogen rich medium that has one-tenth the normal physiological calcium concentration. Under these conditions we found that by manipulating the cyclic AMP system using dideoxyadenosine and, the protein kinase C system, using forskolin and phorbol 12-myristate 13-acetate and staurosporine, had little or no effects on both cell types. Similarly, we found that inhibition of the calmodulin system using either the experimental calmodulin antagonist drug J8 or tamoxifen produced a profound inhibition of attachment. Both melanocyte and melanoma cells did not differ in their sensitivity to either drug.

We were surprised to discover that by manipulating the intracellular calcium system using ionomycin and TMB8 was initially without effects on the attachment of the ocular melanocyte, while they inhibited the ocular melanoma cell attachment. As it may have offered an approach for selective pharmacological intervention we examined this result further. In these further studies the melanocytes were transferred to medium containing physiological concentrations of calcium for two days prior to experimentation. These experiments revealed that the lack of response of the melanocytes to ionomycin was dependent upon the calcium concentration of the medium as all other additions to the medium were kept constant. We also confirmed that melanocyte and melanoma cells responded equally well to an acute addition of ionomycin with a rapid increase in intracellular calcium. However, the action of ionomycin is entirely dependent on the concentration of extracellular calcium and therefore our results lead us to conclude that if uveal melanocytes are grown under concentrations of low extracellular calcium then ionomycin is probably unable to elevate intracellular to a level which will inhibit cell attachment.

To conclude, we found that both normal uveal melanocytes and transformed uveal melanoma cells use the same intracellular signalling systems in their initial stages of attachment to ECM proteins This signalling involves calcium and calmodulin to a large extent, accordingly any drugs which affect calcium or calmodulin activity could reduce the likelihood of the attachment of either cell. We can also state that the ocular melanocyte and melanoma cells show a requirement for a calcium and calmodulin sensitive intracellular signal within the first few minutes of adhesion suggesting that this is unlikely to be specific to a particular cell or even to any particular adhesion molecule/ECM substrate interaction.

In summary, while we are unable to identify a pharmacological target for intracellular signalling which would allow one to focus on the transformed rather than the normal cell, it is still possible that drugs affecting calcium or inhibiting calmodulin could be used in preventing adhesion of any unattached cells and this could be of value in preventing metastatic spread.

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