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CONTENTS

Literature highlights 1042

Review of the literature
2. Biology of pigment cells and pigmentary disorders 1043
3. MSH, MCH, other hormones, differentiation 1044
4. Photobiology 1045
5. Neur melanins 1046
6. Genetics, molecular biology 1046
7. Tyrosinase, TRP1, TRP2, and other enzymes 1049
8. Melanosomes 1052
9. Melanoma experimental, Cell culture
    A: Melanoma cytotoxicity, experimental 1053
    B: Culture systems to study melanocytes 1054

Announcements and related activities 1056

National Editorial Board: J.M. Niepoort (RUG, State Univ. of Gen), D. Roseeuw
(VUB, Free Univ. of Brussels), K. Deraemaeker, B. Lour, F. Sulis (VUB, Free Univ. of Brussels).
HIGHLIGHTS

Large deletions of chromosome 9p in cutaneous malignant melanoma identify patients with a high risk of developing metastases


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published in Melanoma Research 2000, 10, pp.231-236.

ABSTRACT

Cutaneous malignant melanoma (CMM) is an aggressive tumour with a high metastatic potential. Deletions of chromosome 9p have been detected in CMM, some of which involve the CDKN2A/p14ARF genes. Loss of heterozygosity (LOH) of 16 microsatellite markers on 9p and mutations in the CDKN2A/p14ARF genes had been previously studied in 32 melanoma patients by our group. 9p deletions were detected in 15 primary tumours (45.5%) and are here correlated with the clinical outcome over 5 years and compared with classical prognostic factors. Eight of the 32 patients developed metastases (25%). The metastases were all detected within 768 days of the initial diagnosis. The patients without metastases were last monitored at least 1621 days after diagnosis. None of the 21 patients with more than eight microsatellites conserved developed metastases, whereas all of the eight patients who developed metastases had eight or more markers deleted. The sensitivity of this analysis to predict metastases was 100% (specificity 84%), whereas the sensitivity for the same sample using a Breslow thickness > 3 mm was 62.5% (specificity 68%). LOH of eight or more of the 9p microsatellite markers is therefore a useful prognostic factor to predict the development of metastases in the first 4.4-6.3 years (1621-2294 days).
2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

Several authors have investigated the regulatory mechanism of melanoma progression, migration and invasion in vitro. Toschi E et al hypothesized that, in addition to its pro-apoptotic and autocrine/paracrine effect, p53 overexpression in human melanoma cell lines can activate intracellular pathways through modulation of matrix metalloproteinase-2 and 9. The authors demonstrated that the p53 overexpression gene, obtained by a recombinant vector, in human cell line carrying mutated gene caused a reduced invasiveness associated with a decreased matrix metalloproteinase-2 level without mRNA level modification. In addition, no further modulation of some wild-type p53-regulated pathways can be achieved by introduction of additional copies of the gene. The authors found a modification of metalloproteinase-9 level after p53 overexpression. Thus, Toschi suggests a novel regulatory mechanism for p53 rough modification of metalloproteinase-2 secretion.

In the number of June 2000 of Pigment Cell Research several reviews focused on the melanocytes metabolism. Setaburi V. focused the attention on the cellular sorting machinery that recognizes specific sorting signals and regulates the entry and exit of proteins through intracellular compartments on route to melanosome. As regard to tyrosinase, the biosynthesis appears to be regulated by quality-control event at endoplasmic reticulum. The di-leucine motif of cysteine tail of melanosomal proteins and its interaction with adaptor protein complexes (i.e APs) are critical. Defects in sorting signals cause several marine coat color phenotypes and human pigmentary disorders. Rest JL reviews the available data on the "loss of function" mutations in melanocortin 1 receptor (MCIR) which is associated, in animal and man, with a switch from eumelanin to phaeomelanin production. The MCIR seems to be an important determinant of sun sensitivity and a genetic risk factor for melanoma and non melanoma skin cancer. The world wide pattern of MCIR diversity seems to be compatible with functional constraint operating in Africa, whereas the greater allelic diversity seen in non-African populations would be consistent with neutral predictions rather than selection. The gene expression profile analysis was recently performed in pigment cells to investigate, on a genomic scale, the pigment cell function and development, as reported in the review from Loftus SK and Pavann WJ.

The Suzuki Minoru group have evaluated the tyrosinase gene expression within 24 h of NO-induced melanogenesis and found that mRNA expression for tyrosinase was induced 2h after and suppressed by a cGMP-dependent protein kinase inhibitor, suggesting that this enhancement of tyrosinase gene expression is an important mechanism for NO-induced melanogenesis.

Recent work showed that m-MSH or chole toxin (CT) can activate a CAMP pathway that elicits proliferative arrest and senescence in normal melanocytes. Because senescence may be defence against malignant transformation, Bandopadhay D and Medrano EE have examined the different responses of melanocytes derived from light and dark skin to CT. The authors demonstrated that in melanocytes from dark skin the CT-induced melanogenesis was associated with accumulation of the tumor suppressor p16INK4a and decreased expression of E2F1. On the contrary, melanocytes from light skin accumulate smaller amount of melanin under the same condition and they continued to proliferate. This delayed senescence may be the consequence of a reduced association of p16 with CDK4 and steady levels of cyclin e and E2F1. In the last number of J Invest Dermatol, Schallreuter KU respond to Fuller BB (same Journal, 114(2): 268-76, 2000) about the regulation of tyrosinase activity.


3. MSH, MCH, other hormones, differentiation

(De B. Lour)


**Summary:** The authors have assessed the mRNA expression of the angiogenic placenta growth factor and its receptor neuropilin-1 by RT-PCR as well as the secretion of this growth factor. Their findings demonstrated that melanoma progression is accompanied by decreased, constitutive placenta growth factor expression. Placenta growth factor, however, serves no apparent autocrine role in melanoma proliferation.


**Summary:** The authors have generated rabbit antibody against the carboxyl terminus sequence of ASP. Then, they characterized the expression of ASP in the skin of newborn nonagouti (A/aj), agouti (A+a+) and lethal yellow (A/y/y) mice. In the last one, the expression pattern suggests that ASP is delivered quickly and efficiently to melanocytes and to hair matrix cells in the hair bulbs where it regulates melanin production.
- Mazurkiewicz JE, Cortis D, Slominski A. Spatiotemporal expression, distribution, and processing of POMC and POMC-derived peptides in murine skin. J Histochem Cytochem. 48(7):905-14, 2000. Summary: After dermal-induced anagen, the authors found cell-specific variances in the expression of POMC mRNA that were consistent with immunoactivities for POMC-derived peptides. Based on their results, they can also infer that the activities of PC1 and PC2 are responsible for the cell-specific differential processing of POMC in murine skin.


- Shellman YG, Chapman JT, Fujita M, Norris DA, Maxwll IH. Expression of activated n-ras in a primary melanoma cell line counteracts growth inhibition by transforming growth factor-beta. J Invest Dermatol. 114(6):1300-4, 2000. Summary: "These data suggest that activated Ras plays an important part in melanoma progression from the radial growth phase to the vertical growth phase by counteracting inhibition by cytokines such as transforming growth factor-beta, thus providing a growth advantage."

5. Neuromelansins


6. Genetics, molecular biology

(Dr. F. Boerman)

Two new coat color genes have been identified and cloned in mouse and human. NEMO (NF-κB essential modulator) is a X-linked gene. Mutations of the gene are found in the human syndromecontinentia pigmenti. When NEMO is mutated in transgenic mice by homologous recombination, symptoms similar to those of incontinentia pigmenti are observed (Makris et al., Schmidt-Jöppl et al., Smahi et al.). 


Carreira S, Lu B, Goding C. The gene encoding the T-box factor Tbx2 is a target for the microphthalmia-associated transcription factor in melanocytes. Journal of Biological Chemistry 275:21920-21927, 2000. Summary: The T-box transcription factor family comprises several members and is conserved in evolution. In this paper, the authors show, that one T-box factor, Tbx2, is transcriptionally regulated by Mitf: a Mitf binding site is present in the mouse Tbx2 promoter, the promoter is bound by Mitf in vitro and expression of Tbx2 in melanoma cell lines is correlated to presence of Mitf. They conclude that Tbx2 is one of the first target genes of Mitf, which is not directly involved in pigment synthesis.


Loftus SK, Pavan W. The use of expression profiling to study pigment cell biology and dysfunction [Review]. Pigment Cell Research 1047

Shortened abstract: IKK/NEMO is the essential regulatory subunit of the Ikk kinase (IKK), encoded by an X- linked gene in man and a mouse. It is required for NF-{kappa}B activation and resistance to TNF-induced apoptosis. Female mice heterozygous for Ikk/Nema deficiency develop a unique dermatopathy characterized by keratinocyte hyperproliferation, skin inflammation, hyperkeratosis, and increased apoptosis. Although Ikk+/-- females eventually recover, Ikk{minus} males die in utero. These symptoms and inheritance pattern are very similar to those of incontinentia pigmenti (IP), a human genodermatosis, synecjonic with the IKK/NEMO locus.


Shortened abstract: Griscelli syndrome (GS, MIM 214450), a rare, autosomal recessive disorder, results in pigmentary dilution of the skin and the hair, the presence of large clumps of pigment in hair shafts and an accumulation of melanosomes in melanocytes. We previously mapped the GS locus to chromosome 15q21 and found a mutation in a gene (MY05A) encoding a molecular motor in two patients. Further linkage analysis suggested a second gene associated with GS was in the same chromosome region. We detected mutations in RAB27A, which lies within this interval, in 16 patients with GS. Unlike MY05A, the GTP-binding protein RAB27A appears to be involved in the control of the immune system, as all patients with RAB27A mutations, but none with the MY05A mutation, developed HS (haemaglophagyctosis syndrome).


Abstract: Disruption of the X-linked gene encoding NF-{kappa}B essential modulator (NEMO) produces male embryonic lethality, completely blocks NF-{kappa}B activation by proinflammatory cytokines, and interferes with the generation and/or persistence of lymphocytes. Heterozygous female mice develop patchy skin lesions with massive granulocyte infiltration and hyperproliferation and increased apoptosis of keratinocytes. Diseased animals present severe growth retardation and early mortality. Surviving mice recover almost completely, presumably through clearing the skin of NEMO-deficient keratinocytes. Male lethality and strikingly similar skin lesions in heterozygous females are hallmarks of the human genodermatologic disorder incontinentia pigmenti (IP). Together with the recent discovery that mutations in the human NEMO gene cause IP, our results indicate that we have created a mouse model for that disease.


Abstract: Familial incontinentia pigmenti (IP, MIM 308110) is a genodermatosis that segregates as an X-linked dominant disorder and is usually lethal prenatally in males. In affected females it causes highly variable abnormalities of the skin, hair, nails, teeth, eyes and central nervous system. The prominent skin signs occur in four classic cutaneous stages: perinatal inflammatory vesicles, verrucous patches, a distinctive pattern of hyperpigmentation and dermal scarring. Cells expressing the mutated X chromosome are eliminated selectively around the time of birth, so females with IP exhibit extremely skewed X-inactivation. The reasons for cell death

1048
in females and in utero lethality in males are unknown. The locus for IP has been linked genetically to the factor VIII gene in Xq28. The gene for NEMO (NF-κB essential modulator)/IKK (IKB kinase-γ) has been mapped to a position 200 kilobases proximal to the factor VIII locus. NEMO is required for the activation of the transcription factor NF-κB, which is involved in the inflammatory and apoptotic pathways. Most cases of IP are due to mutations of this locus and that a new genomic rearrangement accounts for 80% of new mutations. As a consequence, NF-κB activation is defective in IP cells.


- Shortened abstract: The melanocyte-specific promoter of the human MITF gene (MITF-M promoter) contains a functional LEF-1-binding site, which is bound in vitro by LEF-1 and confers the preferential expression on a reporter gene in melanocytes and melanoma cells, as judged by the transient transfection assays. Exogenously added Wnt-3a protein also transactivates the MITF-M promoter via the LEF-1-binding site. These results suggest that Wnt-3a signaling recruits b-catenin and LEF-1 to the LEF-1-binding site of the MITF-M promoter. Therefore, the present study identifies MITF/MITF-M as a direct target of Wnt signaling.


- Abstract: The dilute (d), brown (br), and ash (sh) mutations provide a unique model system for studying vesicle transport in mammals. All three mutations produce a lightened coat color because of defects in pigment granule transport. In addition, all three mutations are suppressed by the semidominant dilute-suppressor (dso), providing genetic evidence that these mutations function in the same or overlapping transport pathways. Previous studies showed that d encodes a major vesicle transport motor, myosin-VA, which is mutated in Griscelli syndrome patients. Here, using positional cloning and bacterial artificial chromosome rescue, we show that ash encodes Rab27a, a member of the Rab GTPases family. Rab27A represents the largest branch of the RAS superfamily and is recognized as a key player in vesicular transport and organelle dynamics in endocytic cells. We also show that ash mice have platelet defects resulting in increased bleeding time and a reduction in the number of platelet dense granules. These defects have not been reported for d and br mice. Collectively, our studies identify Rab27a as a critical gene for organelle-specific protein trafficking in melanocytes and platelets and suggest that Rab27a functions in both MyoVA-dependent and independent pathways.

7. Tyrosinase, TRP1, TRP2 and other enzymes

(Pref. J.C. Garcia-Borroto) Five of the references provided in this issue deal with the post-transitional events associated to the processing of tyrosinase and the tyroses. This certainly reflects the increasing interest of this area, which, I think, is due at least to two factors: the biomedical relevance of the process, and the realization that, at least as far as tyrosinases and the tyroses are concerned, the regulation of pigmentation is much more than translational control. Indeed, it is becoming evident that defective processing has important pathological and biomedical implications, including certain forms of albinism and antigen processing in melanocytes. For instance, Halaban et al (Proc Natl Acad Sci U S A. 2000 May 23;97(11):5489-5494) demonstrate that several loss-of-function mutations in tyrosinase, associated with albinism, produce a retention of misfolded protein in the endoplasmic reticulum (ER). They go on to conclude that albinism can be at least partially considered an "ER retention disease." According to the results presented by Wang and Andreouci (Biochem Biophys Res Commun. 2000 Apr 29;271(1):22-7), and to previous work from Ruth Halaban's laboratory already commented in this Bulletin, this retention could result in retrograde transport of tyrosinase from the ER to the cytosol, followed by protease-dependent degradation and presentation of antigenic epitopes to the immune system. On the other hand, the romanian group led by S. Petrucescu continues to provide a wealth of information on the mechanism of processing of tyrosinase and the tyroses (J Biol Chem 2000 Mar 17;275(11):8169-75; Biochemistry. 2000 May 9;39(18):5229-37; J Biol Chem. 2000 Jul 27), in keeping with their outstanding recent scientific production. Thus, the complex pathways
of post-translational processing of the melanogenic enzymes have been already reasonably well delineated, and their details may be definitively solved soon.

On the other hand, several papers describe new forms of regulation of melanogenesis at different levels. Takizuki et al. (Pigment Cell Res. 2000 Apr;13(2):109-15) describe a novel mechanism of tyrosinase gene expression regulation based on the interaction of 3' non coding mRNA sequences with repetitive sequences in the 5' upstream regulatory region of the gene. It will be interesting to assess the relevance "in vivo" of this mechanism. Yoshida et al (J Invest Dermatol 2000 Feb;114(2):334-42) describe the regulation of tyrosinase activity via H2 histamine receptors. Since these receptors are coupled to adenylyl cincase and their activation increases intracellular cAMP levels, it is not surprising that the effects of histamine on melanocytes are reminiscent of those of alpha2MSH, and, apparently, mediated by protein kinase A. In a very interesting and original paper, Fuller et al (J Invest Dermatol. 2000 Jul;115(1):130-1) demonstrate that yohimbine reversibly decreases tyrosinase activity in cultured melanocytes by a still uncharacterized and probably novel mechanism. The compound has no effect on cAMP levels, but blocks the activatory effect of cAMP elevating agents. It has no effect on either substrate availability or tyrosinase abundance, and, therefore, its mode of action is as yet unknown. As the authors point out, it will be interesting to see whether the drug finds clinical applications for the treatment of hyperpigmentary disorders, but, from a basic point of view, it will also be extremely interesting to unravel its probably novel mechanism of action. Finally, Ancas and Thody (FEBS Lett. 2000 Jul 28;478(1-2):57-60) and Hornyak et al (J Invest Dermatol. 2000 Jul;115(1):106-12) deal with two well known regulatory factors, namely, intracellular pH and cell density, and provide new interesting information and , which is also important, a reminder to the pigment cell community of two simple and often forgotten factors influencing melanogenesis, whose accurate control is critical for many experimental setups.

The new series of reviews published by Pigment Cell Research deserves a special commend. These extremely well documented studies on the hottest topics, written by leading experts, will be extremely useful not only for the pigment cell community, but also for scientists from other fields willing to get a clear and updated picture of pigment cell biology. Let's hope that the reviews published thus far will set up a standard of quality and a new tradition for the journal.

- Ancas I, Thody AJ.


- Aigner B, Benesfelder U, Muller M, Brem G.


- Arase S, Yatsumoto K, Takada K, Udoe T, Takahashi K, Shibahara S.


- Benson JE, Frank DW, Calvo PA, Bieler B, Marks MS.


- Busca R, Ballotti R.


- Decker H, Tucek F.


- Fesoli LG, Rodriguez-Lopez JN, Vonar G, Garcia-Raz PA, Garcia-Canoas F, Tufreda J.


- Fuller BB, Drake MA, Spaulding DT, Chaudhry F.


Endogenous reticulun retention is a common defect associated with tyrosinase-negative albinism. Proc Natl
8. Melanosomes

(De. J. Borovansky)

Papers investigating the role of microtubule- and actin-based motor proteins in melanosomes transport have recently flooded the literature. Another large group of reports focused on the characterization of melanosome and pigment granules in tumours. A special attention should be paid to papers by Peters et al. and by Salceda & Sanchez.


Comments: An indirect evidence suggesting that RPE melanosomes might have a role in preventing the cytotoxicity derived from L-DOPA and in regulating the generation of NO.


Comments: Study supporting a major role for kinesin in microtubule-associated anterograde melanosomal transport in human melanocyte dendrites. Ultrastructurally, kinesin molecules were closely associated with melanosomes.


Comments: Adrenal black adenoma (ABA) cells contain numerous pigment granules but the nature of these granules has remained controversial. Electing microscopic study of a ABA case revealed two types of pigmented granules - typical lipofuscin granules and structures resembling stage IV melanosomes. According to histochemical reactions the last had characteristics of lipofuscin. Table of histochemical reactions distinguishing between lipofuscin, melanin and neuromelanin.


Comments: Black thyroid adenoma in woman treated with minocycline. The pigment had histochemical characteristics of melain, was eosinophilic and was deposited within tyrosinates of follicular cells.


Comments: Evidence has been summarized for possible roles of kinesin-II in anterograde transport, melanosome transport (in melanophores), the secretory pathway and during mitosis. Molecular anatomy of kinesin-II molecule.


Comments: EM characterization of melanosomes in tongue melanoma. Lamellar melanosomes with uneven deposition of melanin and often missing limiting membranes were present in the primary tumour, whereas metastatic lesion contained immature abnormal melanosomes frequently fusing together.


Comments: By means of immunohistochemistry, immunogold electron microscopy and western blotting the presence of the entire system for pro-opiomelanocortin processing was demonstrated in the melanosome.

- Reck-Peterson SL, Provance Jr DW, Mooseker MS, Merzer IA.

1052

Comments: Review discussing the emerging evidence that myosin V is a processive actin-based motor that has multiple functions in the cell. Detailed description of myosin V domains and function. Special paragraphs devoted to melanosome movement and to Griscelli syndrome.


Comments: The presence of classic or aberrant melanosomes belongs to the most frequent findings in angiomylolipomas; clear cell ("sugar") tumors, regarded by some authors as a clear cell epithelioid variant of angiomylolipoma, also habitually contains melanosomes. A case of such tumour in kidney consisting of S100 and HMB45 positive cells with Fontana-Masson positive pigment and melanosomes demonstrated by EM is presented.


Comments: Characterization of Ca uptake to and release from melanosomes in the absence and presence of ionophore A23187, nigericin and inhibitors of plasma membrane channels. Demonstration of ryanodine binding sites in melanosomes. Description of isolation method to obtain pure melanosome fractions with intact membranes. Demonstration of the enrichment of acid phosphatase activity within the melanosomal fraction.


Comments: Excellent review concentrating on the functions of myosin classes I, V, VI and VII in membrane trafficking processes and also on the switching between microtubule and actin networks. Genetic disorders mapped to the myosin V locus (Griscelli syndrome, dilute lethal mouse mutation) and mutations in myosin VIIA (shaker mice) are associated with altered distribution of melanosomes.


Comments: Data suggesting that kinesin and its receptor kinesin have an important role in microtubule-based melanosome transport in human melanocytes.

9. Melanoma experimental, Cell culture

A. Melanoma cytotoxicity, experimental


B. Culture systems to study melanocytes


- Denai SH, Buskovic G, Eastham L, Dawson M, Niles RM.


ANNOUNCEMENTS
& RELATED ACTIVITIES

Calendar of events

Also available from address: http://www.ulb.ac.be/edecine/loce/espcr.htm

2000 9th ESPCR Meeting: Ulm, Germany
Sept 28 - Oct 1
Contact: Prof. R.U. Peter
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

2001 5th World Conference on Melanoma: Venice
Feb 28-Mar 3
Contact: Dr. M. Santinami
Secretary General
5th World Conference on Melanoma
Dept. of Surgery - Casa di Cura S. Pio X
Via F. Nava 31
I- 20159 Milano
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2001 8th International Conference on Solar Energy and Applied Photochemistry: Cairo, Egypt
April 3-8
Contact: Prof. M.S.A. Abdel-Motaleb
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2001 Xth Annual Meeting of the PASPCR, Minneapolis, MN
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1056
Dear Colleagues,

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

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

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

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

The first is reported below.

In advance, thank you.

The Bulletin Editor