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DEBATE

A recent paper authored by Ness et al. that appeared in the July issue of British Medical Journal and relayed by reports in Newspapers in many European Countries, raised a debate about "Reducing exposure to sunlight" as a public health advice, under the title: "Are we really dying for a tan?".

Despite the fact that the paper was published in the "Education and debate "Section of the Journal, News to the public were brought under completely erroneous titles not reflecting the essence and the goal of the authors at all; such as the one appearing in the "Le Soir" newspaper of the 10/07/99 in Belgium with the heading (translated from french): "Heavy exposure to the sun is harmless, suggested by a british study" this is an illustration of how a scientific debate nowadays may break to the public hampering the efforts made through numerous prevention campaigns.
Below is the full text of the paper as well as a response by ESPCR member Prof. R. MacKie.
Full text and detailed related answers can be found on BMJ URL:
http://www.BMJ.com/cgi/content/full/319/7202/114

ESPCR Bulletin Editor

Are we really dying for a tan?

Ness AR, Frankel SJ, Gunnell DJ, Smith GD.
BMJ 1999;319:114-116 (10 July)

Professionals in health care and health promotion have embraced the notion that sunlight (particularly in doses that lead to sunburn) is bad for health.[1-3] This was not always the case. In the early years of this century sunlight was regarded as an effective treatment for tuberculosis of the skin, and was also thought to be generally beneficial to health (box). Even today there are health resorts around the world offering heliotherapyparticularly for diseases of the skin such as psoriasis. The public have been slow to accept the message that exposure to sunlight is bad for health. Many people still sunbathe. In a survey carried out in England in 1995, 40% of those aged 16-24 reported being unburnt in the preceding year, and just under 40% regarded a tan as being important to them.[4] Furthermore, qualitative research with people in Scotland aged 20-35 who regularly travelled abroad for pleasure, suggested that at least part of the attraction of a tan was the perceived feeling of healthiness.[5]

Summary points:
- There is discordance between the health message to reduce exposure to sunlight and the health beliefs and behaviour of the public
- The health promotion message aims to reduce skin cancer incidence and mortality
- Increased exposure to sunlight may have beneficial effects on other diseases
- Health educators should weigh up explicitly the potential risks and benefits of reduced exposure to sunlight to ensure that the health education message is appropriate

Prevention paradox

The prevention paradox, as described by Rose, arises because many interventions that aim to improve health have relatively small influences on the health of most people. Thus, for one person to benefit, many people will have to change their behaviour and receive no benefit from these changes. Perhaps

sensing that public awareness of this paradox might act as a disincentive to adopting behaviours that will protect health, the strength of the purported benefits of population programmes tends to have been exaggerated. For example, advocates of cervical screening have not made it clear how rare the disease is, nor have they advertised the disadvantagesthe unnecessary anxiety and the cost of managing of false positive cases. It is evident, however, that people judge for themselves the plausibility of these official exhortations by comparing them with the evidence of their own experience.

In this system of lay epidemiology, people interpret health risks by integrating information from observation and discussion of cases of illness and death in personal networks and the public arena with formal and informal evidence provided by the media, health educational materials, and other relevant sources of information.[6] The evidence of people's attitudes and behaviour suggests that, on balance, lay epidemiology holds that exposure to sunlight is beneficial rather than harmful. This belief contrasts sharply with the current health education message. But before we dismiss this discordance between lay epidemiology and health education messages as ignorance on the part of the public, we should re-examine the scientific evidence on the overall balance of the benefits and harm of sun exposure.

Harm from sun exposure

Malignant melanoma

The main rationale for the health message reduce exposure to sunlight, and, in particular, avoid sunburnhas been the belief that exposure contributes to the increasing incidence of malignant melanoma.[2] However, the exact nature of the association between malignant melanoma and exposure to sunlight has yet to be determined.[7] A recent systematic review of case-control studies confirmed that intermittent sun exposure (odds ratio 1.71; 95% confidence interval 1.54 to 1.90) and sunburn at all ages (1.91; 1.69 to 2.17) were associated with an increased risk of melanoma. It also showed, however, that people exposed to sun through their work were at a reduced risk (0.86; 0.76 to 0.96).[8] Even if reducing exposure to sunlight reduces the incidence of melanoma, its effect on overall mortality will be slight, as the number of deaths postponed will be small. In 1995, the deaths of 697 men and 698 women in England and Wales were attributed to malignant melanoma.[9] Even the most forceful campaign could be expected to prevent only a few of these deaths. There may also be effective options for reducing mortality from melanoma that do not require reducing exposure to sunlight for example, by increasing awareness of the diagnosis and access to treatment.

Other adverse effects

Increased rates of other more benign forms of skin cancer (such as squamous cell carcinoma and basal cell carcinoma), cataracts, and skin ageing are associated with either intermittent or cumulative exposure to sunlight. [2,10] While these diseases are important causes of morbidity, they are usually amenable to treatment, and are not generally fatal. In 1995, the deaths of 264 men and 175 women in England and Wales were attributed to non-melanoma skin cancer.[9] More recently, it has been suggested that exposure to sunlight increases the incidence of non-Hodgkin's lymphoma.[11] This hypothesis is speculative; it has not yet been confirmed or refuted.[12]

The solarium at Jamnagar

The widespread belief that sunlight was beneficial to health was shared by physicians working in many areas of medicine. Sunlight exposure was recommended for several disorders, and ways of increasing exposure were contrived. The solarium at Jamnagar, Gujarat, India (see URL above), is an impressive example of this. The solarium was built on the initiative of the ruler of Nawanagar state, the cricketer Ranjitsinghi. It was designed by a French engineer, Dr Jean Saidman (who built three of these solariums), and was operational from 1934. The Jamnagar solarium is 40 feet tall and the treatment rooms are located in the rotating top section, which is 114 feet long and takes an hour to rotate fully. Maximal light exposure can be ensured by rotation. Some treatment rooms are equipped with filters which allow through only rays of wavelengths considered suitable for the various diseases treated in the solarium, and lenses concentrate the light to two and a half times its natural intensity. The solarium no longer works because most of the lenses and concentrators were broken during a cyclone and replacements cannot be found. A detailed photographic library provides before and after views of people treated for various conditions, including lymphoid hyperplasias, tuberculosis, and several skin conditions.

Benefits of sun exposure

Coronary heart disease

There are seasonal patterns in cardiovascular mortality and in cardiovascular risk factors that may be partly explained by reduced exposure to sunlight in the winter months.[13-15] Some studies have reported a protective association for vitamin D (a marker of sunlight exposure).[16-18] For example, Scragg et al, in a case-control study of acute myocardial infarction, reported an odds ratio of 0.43 (95% CI 0.27 to 0.69) for subjects with 25-hydroxycholcalciferol concentrations equal to or above the median compared with subjects whose concentrations were below the median.[16] These findings are tentative, and might be explained by bias inherent in the case-control design or confounding by exposures such as physical activity. Nevertheless, as coronary disease is such an important cause of death (in 1995 the deaths of 73 129 men and 60 732 women in England and Wales were attributed to ischaemic heart disease[9]), even a modest protective effect of exposure to sunlight could result in a substantial reduction in mortality.

Mental health

People find lying or sitting in the sun enjoyable and relaxing.[3] This subjective sense of wellbeing may be important in itself in improving the quality of a person's life. Seasonal variations in sunlight exposure may underlie a proportion of episodes of depression those attributed to seasonal affective disorder.[19] Furthermore, the well documented increases in suicidal behaviour in early spring may also be related to patterns of day length and sun exposure, although other explanations for this phenomenon exist.[20] Psychiatric illness is an important factor in population health, and any beneficial effect of increased exposure to sunlight might reduce appreciably the population burden of disease.

Other diseases

Exposure to sunlight increases vitamin D production and reduces the risk of rickets in childhood and of osteomalacia and fractures in adulthood.[21] In addition, ultraviolet light (both from natural and medical sources) is used to treat some skin conditions such as psoriasis, which affects 2% of people of European ancestry. It has also been suggested on the basis of the ecological association with latitude, that exposure to sunlight reduces the incidence of multiple sclerosis.[22]

Conclusions

There is evidence that the potential benefits of exposure to sunlight may outweigh the widely publicised adverse effects on the incidence of skin cancer. Advice aimed at reducing the frequency of episodes of sunburn may have the net effect of reducing the population's mean exposure to sunlight. For example, in one study the use of sunscreens was shown to reduced vitamin D concentrations.[23] No population data are available on long term trends in exposure to sunlight in Britain to confirm that such a reduction has taken place. Reduced exposure to sunlight could have adverse effects, but we believe that any advice to increase exposure to sunlight is premature given the tentative nature of our review and concerns about the changing nature of sunlight exposure with the thinning of the ozone layer.[24] However, we suggest that the basis for current advice to reduce exposure to sunlight should be reviewed in a formal and quantitative manner so that the potential benefits and harm from exposure to sunlight can be conveyed to the public. The risk:benefit ratio will differ between individuals; for many people the small absolute increase in risk of melanoma could easily be outweighed by the effect of reduced sunlight on mood. A recent article in *Vogue* suggests that lay understanding is, perhaps again, ahead of medical thinking in attempting to weigh up factors for and against exposure to sunlight.[25] Perhaps, while we await the conclusions of such formal analyses, those of us who enjoy spending time in the sun can rest (on our deck chair, sun lounger ... or whatever) assured that the chance that we will be one of the people dying from our tan is small.

Acknowledgments

We thank Mr Sunil Thakar of the M P Shah Hospital, Jamnagar, for arranging a visit to the solarium and providing details of its history.

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Letter responses to this article:
(Also available from BMD web site)

Hazards of sun exposure Rona M MacKie FRCP, Professor of Dermatology, Glasgow University UK

Sir,

The article by Ness et al[1] may have created publicity for the BMJ but is it really this type of publicity that the BMJ needs? The article is in the section 'Education and Debate'. No debate is encouraged in that only the opinions of Ness et al are given journal space. To my knowledge none of those involved in advocating a safe sun approach to life have been asked to offer their opinion in an adjacent article. It is also important to note that Ness and colleagues have contributed absolutely no original data to this debate at any time. No original data from the Bristol Group is reported in the article and they themselves have never published in this field. Thus reports in the tabloids on Friday on "new research from Bristol" are meaningless.

Furthermore they have misquoted others. Those of us who have an interest in prevention and early detection of skin cancer, particularly malignant melanoma, have a longstanding interest in vitamin D levels particularly when we are working with small children and older individuals. We regularly offer the public a balanced view and have also carried out studies to determine whether or not sensible use of sunscreen is associated with the drop in vitamin D levels. I find it a very disturbing aspect of this article that Ness and colleagues have totally misquoted the work of Robin Marks and his colleagues

from Australia[2] who, in 1995, carried out an excellent study in older individuals and published in the Archives of Dermatology reassuring evidence that use of sunscreen was not associated with a fall in vitamin D levels. This is incorrectly reported in Ness et al's paper as giving worrying concern of falling vitamin D levels in patients using sunscreen. This is a major error which obviously should have been spotted by the authors themselves and preferably also by an informed referee. The data on coronary disease levels and vitamin D is irrelevant until it is recorded that safe sun advice is associated with a significant fall in vitamin D levels.

The UK public could do without this kind of so-called debate at the present time.
We are currently even in Scotland enjoying a period of extremely warm sunny weather. I have just returned from my children's clinic where I saw a 4 month old baby who required hospital admission at the weekend because of sunburn and blisters on the child's forehead.
When asked about the problem, the mother quoted the fact that she had read in her newspaper on Friday that it was now proven that sun was good for small children. I would be interested in Ness et al's comments.

Yours sincerely

Rona M MacKie CBE, FRCP

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Vitamin D and Sunscreens Professor Robin Marks, Professor of Dermatology , *St. Vincents Hospital*

Risks and benefits of the sun Sam Gibbs, Publich Health Fellow/Dermatology Registrar, *Public Health Dept, Norfolk Health Authority*

Society is suspicious of public health Paul Pharoah, CRC Clinical Research Fellow, *Strangeways Research Laboratories, Cambridge*

The anti-tanning lobby should change its tune Joseph A. Levy, executive director, *International Smart Tan Network*

Dying and suffering for a tan Dr. Dafydd Roberts, Consultant Dermatologist. Chairman, UK Skin Cancer Working Group p, *Singleton Hospital, Swansea*

Wellbeing associated with Tanning is not a Medical Matter Salvador Vale, Research Psychiatrist, *Antiguo Hospital Concepción Beistegui*

Sunlight and health: it depends where you live David C Whiteman, Nuffield Medical Research Fellow, *University of Oxford*

Sunlight is essential, ultraviolet exposure of the skin is harmful, so avoid it ! J L M Hawk BSc MD FRCP, Professor of Dermatological Photobiology and Consultant Dermatologist, *St John's Institute of Dermatology, St Thomas' Hospital, London SE1 7EH*

'Are we really dying for a tan?' - striving for an informed debate Jane Melia, Epidemiologist, *Cancer Screening Evaluation Unit, Institute of Cancer Research, Sutton, UK*

Sunlight may protect against internal cancer Dr. Derek Pheby, Director, Unit of Applied Epidemiology, *Frenchay Campus, University of the West of England, Coldharbour Lane, Bristol, BS16 1QY*

Sunlight-exposure in health education Jean Coope, Retired general practitioner, Bollington, Macclesfield, Cheshire

Sunlight and cancer: more good than harm? Peter Selby, Lecturer in Medicine/Hon Consultant Physician, *University of Manchester*

2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

Van Belle and al. Investigated the expression of the beta-3 integrin in paraffin-embedded specimens of human nevi and melanomas, using different monoclonal antibodies. Expression was not observed in melanocytes and was absent or low in most nevi. In those nevi that reacted focally, the reactivity tended to occur in the dermal component of neurotized nevi and in Spitz nevi the expression was stronger and diffuse. Dysplastic nevi showed focal reactivity of the junctional component. In primary melanomas, expression was absent or low in the radial growth phase whereas it was higher in the vertical growth phase compartment. In all melanomas expression of beta –3 integrin increased with thickness. Their results suggest that expression of this marker may have prognostic value; however, its consistent and strong expression in Spitz nevi limited the diagnostic utility of this marker.

A standardized method has been developed, by **Virador and al.**, to screen compounds with potential effects on pigmentation. In this protocol (termed STOPR, for standardized testing of pigmentation regulators) compounds are initially screened using purified tyrosinase and are then tested on melanocyte cultures. After treatment of melanocytes with potentially bio-active compounds, cell proliferation and viability, total melanin accumulated and melanogenic potential are measured. When bio-active candidate compounds are identified, tests may proceed for pharmacological or otherwise commercial applications in co-culture and/or organ culture models followed by in vivo testing.

Meyskens and al. measured the levels of the two major redox response transcription factors, nuclear factor-kappa B (NF-kB) and activator protein-1 (AP-1), in metastatic melanoma cells and normal melanocytes and their response to oxidative stress. The basal DNA-binding activity of NF-kB, was increased 4-fold in melanoma cells compared with that of normal melanocytes. The level of binding was paralleled by a 1.5-to 4-fold increase in the expression of p50 (NF-kappaB1), p65 (Rel-A) and IkappaB-alpha. In contrast, the expression of p75 (c-rel) was markedly decreased (60%) in melanoma cells compared with normal melanocytes. Following oxidative stress, NF-kB binding activity increased 1.5-2.5 fold in melanoma cells but only slightly in normal melanocytes. In contrast activator protein-1 binding activity was unaffected or increased in normal melanocytes in response to oxidative stress, but was either unaffected or decreased in melanoma cells. These results suggest that the redox regulation of melanoma cells at the molecular level is fundamentally different from normal melanocytes and may offer a unique avenue for preventive or therapeutic intervention as well as new insights into the pathogenesis of melanocyte transformation.

After the successful introduction of functional melanocytes into the epidermal reconstructs, the integration of Langerhans cells remains an important challenge, particularly because after the isolation from human epidermis, these cells cannot be sub-cultured and do not integrate into the reconstructing epidermis. **Regnier and co-workers** show that cord blood derived and CD34+ progenitors isolated from the peripheral blood give rise to residential Langerhans cells when co-seeded with normal human keratinocytes.

Using Comet assay, **Marrot and co-workers** analyzed the induction of DNA breaks by UVA (320-400 nm) in the nucleus of normal human melanocytes in culture. Endogenous pigment and/or melanin related molecules were found to enhanced DNA breakage: comet assay were more intense in cells with high melanin content or after stimulation of melanogenesis by supplying tyrosine in the culture medium. After UVA doses, were strong comets were observed, neither cytotoxicity nor stimulation of tyrosinase activity were detected. However, the accumulation of p53 protein suggest that cells reacted to genotoxic stress under these experimental conditions. The results present in this paper suggest that human melanocytes may be used as a target cell to evidence broad-spectrum photo-protection. Moreover, these data could be helpful in understanding the role of sunlight in the initiating steps of melanocyte transformation.

Laminin-5 is a component of anchoring filaments of the lamina lucida of the epidermal basement membrane. **Scott and al.** suggest that laminin-5 may be a ligand for normal human melanocytes in the basement membrane and that loss of laminin-5 production, by melanoma cells, may be a marker for malignant transformation.

Normal human melanocytes have been shown to respond to the signal peptide endothelin by increased proliferation and melanin formation. Moreover it was not clear whether malignant cells differ from their normal precursors in this respect. **Eberle and al.** showed that expression of Endothelin B receptor (EDNRB) is typical for melanocytic cells and that down-regulation seems to be an important characteristic of melanoma cells possibly related to malignancy or apoptosis.

Opdecamp and co-workers analysed the discrete steps at which Endothelins exert their functions in melanocyte development in mouse neural crest cell cultures. They observed that Endothelin 2 was potent in promoting the maturation of melanoblasts and that Endothelin 1 and 3 stimulated the generation of melanoblasts and of pigmented cells to an even greater extent. They demonstrated also that the all three Endothelins activate signal through the endothelin B receptor. The results indicate that endothelins are potent stimulators of melanoblast proliferation and differentiation.

Transforming growth factor-beta 1 (TGF-beta1) acts as an autocrine growth inhibitor on normal human melanocytes, while melanoma cells may not respond to this stimulus. The role of other TGF-beta isoforms TGF-beta-2 and TGF-beta-3 was analysed by **Krasagakis and co-workers**. The expression of the three isoforms was analysed in human melanoma cell lines and in cultures of normal human melanocytes in vitro. mRNA expression of TGF-beta1, 2 and 3 varied considerably in melanoma cells, whereas it was very low in melanocytes. In melanoma cells secreted amounts of the three iso-forms were found increased in comparison to normal melanocytes. Although TGF-beta secretion increased, the proliferation of

melanoma cells was found to be moderately inhibited by TGF-beta iso-forms, in contrast to its strong anti-proliferative effect on normal human melanocytes. In addition, TGF-beta-dependent growth inhibition of melanoma cells from primary tumors vs. cells from metastases showed a trend for further decreased response for the metastatic populations.
The data show loss of responsiveness of melanoma cells to the growth-inhibitory function of TGF-beta iso-forms but not of melanocytes. Although melanoma cells are not growth-inhibited by all the three TGF-beta iso-forms, they secrete significantly higher levels of TGF-beta, as compared to melanocytes. The reduced response indicates their escape from TGF-beta surveillance with ongoing tumor progression.
The protein c-KIT and its ligand, stem cell factor (SCF) play a crucial role in the development of melanocytes from their precursor. Using a monoclonal anti-c-kit antibody, ACK2, which is an antagonistic blocker of c-KIT function, **Ito and al.** demonstrated that mouse melanocytes disappeared with the injection of ACK2 during certain period of embryonic and postnatal life. Because melanocytes disappeared without any inflammation, in these in vivo studies, the authors suspected that apoptosis was the mechanism of cell death. A significant increase of apoptosis was detected after removal of SCF from the culture medium and was further increased with the addition of ACK2 during the SCF-dependent period. Immuno-histochemical analyses confirmed that the apoptotic cells were c-KIT positive and the electron microscopy showed that these apoptotic cells were melanocytes precursors. It was therefore demonstrated that apoptosis was induced in the SCF-dependent c-KIT positive melanocytes in vitro when the SCF7C-KIT interaction was obstructed. These data co9ntribute to elucidate the mechanism of the regulation of melanocyte development, and the survival and proliferation of these precursor cells by SCF-c-Kit interactons.
Apoptosis mediated by TRAIL (TNF-related apoptosis-inducing ligand) is regulated by the expression of two death receptors, respectively TRAIL-R1 and TRAIL-R2 and two decoy receptors TRAIL-R3 and TRAIL-R4 that inhibit apoptosis. TRAIL, but not other members of tumor necrosis factor family, induces apotosis in many melanoma cell lines. **Zhang and co-workers** try to find a possible correlation between TRAIL-R expression and sensitivity to TRAIL-induced apoptosis. Cell lines that were insensitive to TRAIL, or were devoid of genes or failed to express death receptors. Other cell lines, despite the presence of mRNA for the TRAIL-R, failed to express TRAIL-R protein on their surface. Studies on permeabilized cells revealed that the receptors were located within the cytoplasm and redistribution from the cytoplasm may represent a post translational control mechanism. Surface expression of TRAIL-R1 and -R2 (but not TRAIL-R3 and TRAIL-R4) showed an overall correlation with TRAIL-induced apoptosis. This results appear to have important implications for future clinical studies on TRAIL.
Pheo-melanin synthesis, in the process of mammalian melanogenesis, requires the incorporation of thiol-containing compound(s) These molecules must across the membrane barrier from the cytosol to melanosome interior. Cysteine and/or Glutathione were proposed as suitable thiol donors but uptake of these compounds into melanosomes was not previously characterised. **Potterf and al.** showed that Cysteine uptake in melanosomes from murine melanocytes, results from a carrier-mediate mechanism and is a temperature and concentration-dependent process. This study is the first demonstration of melanosomal membrane transport of cysteine, and it strongly suggests that free cysteine is the thiol source utilised for pheomelanin synthesis in mammalian melanocytes.
Protein kinase C-beta is required for activation of tyrosinase. In vivo phosphorylation experiments by **Park and co-workers** revealed that tyrosinase is phosphorylated through the PKC-dependent pathway and that introduction of PKC-beta into non pigmented human melanoma cells, lacking PKC-beta, leads to the phosphorylation and activation of tyrosinase. Pre-incubation of intact melanosomes with purified active PKC-beta in vitro increased tyrosinase activity ~3 fold. By immuno-electron microscopy PKC-beta but not PKC-alpha was closely associated with tyrosinase on the outer surface of melanosomes. Only the extra-melanosomal domain of tyrosinase, which contains two serines but no threonines was phosphorylated and both serines were phosphorylated. The authors concluded that PKC-beta activates tyrosinase directly by phosphorylating serine residues in the cytoplasmic domain of this melanosome-associated protein.
Growth-related oncogene-alpha (GROalpha), interleukin-8 (IL-8) and other members of the alpha-chemokine super-family have mitogenic and angiogenic effects on melanoma cells. **Fujisawa and al.** try to determine if inhibition of the alpha-chemokine receptor would e effective in inhibiting the tumour growth and pulmonary metastasis of human melanoma cells. The proliferation of two high metastatic melanoma cell lines was significantly increased by human recombinant GROalpha and inhibited by anti human GROalpha monoclonal antibody. Antileukinate, a potent inhibitor of alpha-chemokine receptor binding, inhibits the tumour growth and pulmonary metastasis of human melanoma cells in nude mice. This results suggest a possible use of alpha-chemokine receptor inhibitors such as antileukinate in the treatment of malignant melanoma.

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Progression-related expression of beta-3 integrin in melanoma and nevi. Human Pathol 30: 562-7, 1999.

Virador VM, Koayashi N, Matsnaga J, Hearing VJ.
A standardised protocol for assessing regulators of pigmentation. Anal Biochem 270:107-19, 1999.

- Zhang XD, Franco A., Myers K, Gray C, Nguyen T, Hersey P.
Relation of TNF-related apoptosis-inducing ligand (TRAIL) receptor and FLICE-inhibitory protein expression to TRAIL-induced apoptosis of melanoma. Cancer Res 59: 2747-53, 1999.

3. MSH, MCH, other hormones, differentiation

(Dr. B. Loir)

- Abe K., Shoji M., Chen J., Bierhaus A., Danave I., Micko C., Casper K., Dillehay DL., Nawroth PP., Rickles FR.
Regulation of vascular endothelial growth factor production and angiogenesis by the cytoplasmic tail of tissue factor. Proc. Natl. Acad. Sci. USA 96(15):8663-8, 1999.
Commentary: The authors have "demonstrated a significant correlation between TF and vascular endothelial growth factor (VEGF) production in 13 human malignant melanoma cell lines". They also have investigated the structure-function relationship of TF and VEGF by transfection of TF cDNA (full-length sequence and various mutants) in a low TF and VEGF producer melanoma cell line.

- Alanko T., Rosenberg M., Saksela O.
FGF expression allows nevus cells to survive in three-dimensional collagen gel under conditions that induce apoptosis in normal human melanocytes. J. Invest. Dermatol. 113(1):111-6, 1999.

- Chakraborty AK., Funasaka Y., Pawelek JM., Nagahama M., Ito A., Ichihashi M.
Enhanced expression of melanocortin-1 receptor (MC1-R) in normal human keratinocytes during differentiation: evidence for increased expression of POMC peptides near suprabasal layer of epidermis. J. Invest. Dermatol. 112(6):853-60, 1999.

- Chambers J., Ames RS., Bergsma D., Muir A., Fitzgerald LR., Hervieu G., Dytko GM., Foley JJ., Martin J., Liu WS., Park J., Ellis C., Ganguly S., Konchar S., Cluderay J., Leslie R., Wilson S., Sarau HM.
Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. Nature 400(6741):261-5, 1999.

- Chin L., Tam A., Pomerantz J., Wong M., Holash J., Bardeesy N., Shen Q., O'Hagan R., Pantginis J., Zhou H., Horner JW. 2nd, Cordon-Cardo C., Yancopoulos GD., DePinho RA.
Essential role for oncogenic Ras in tumour maintenance. Nature 400(6743):468-72, 1999.

Shortened abstract: The authors "show that melanoma genesis and maintenance are strictly dependent upon expression of H-RasV12G in a doxycycline-inducible H-Ras12G mouse melanoma model null for the tumour suppressor INK4a. Withdrawal of doxycycline and H-RasV12G down-regulation resulted in clinical and histological regression of primary and explanted tumours. The initial stages of regression involved marked apoptosis in the tumour cells and host-derived endothelial cells. Although the regulation of vascular endothelial growth factor (VEGF) was found to be Ras-dependent in vitro, the failure of persistent endogenous and enforced VEGF expression to sustain tumour viability indicates that the tumour-maintaining actions of activated Ras extend beyond the regulation of VEGF expression in vivo".

- Drozdz R., Hintermann E., Tanner H., Zumsteg U., Eberle AN.
(D-(p-benzoylphenylalanine)13, tyrosine19)-melanin-concentrating hormone, a potent analogue for MCH receptor crosslinking. J. Pept. Sci. 5(5):234-42, 1999.
Commentary: The authors have designed a photoreactive analogue of human MCH and used it for competitition binding analysis and size determination of MCH receptors in melanoma cells and other cell types.
Very recently, MCH was identified as the natural ligand for the 353-amino-acid human orphan G-protein-coupled receptor (GPCR) SLC-1: Chambers et al, Saito et al, and Shimomura et al have identified and characterized the MCH receptor (see references herewith).

- Reed JA., Finnerty B., Albino AP.
Divergent cellular differentiation pathways during the invasive stage of cutaneous malignant melanoma progression. Am. J. Pathol. 155(2):549-55, 1999.

- Saito Y., Nothacker HP., Wang Z., Lin SH., Leslie F., Civelli O.
Molecular characterization of the melanin-concentrating-hormone receptor. Nature 400(6741):265-9, 1999.

- Schioth HB., Phillips SR., Rudzish R., Birch-Machin MA., Wikberg JE., Rees JL.
Loss of function mutations of the human melanocortin 1 receptor are common and are associated with red hair. Biochem. Biophys. Res. Commun. 260(2):488-91, 1999.
Summary: The authors have determined the functional significance of the Val60Leu, Arg142His, Arg151Cys, Arg160Trp, and Asp294His point mutations in the human MC1 receptor. In comparison to the wild type receptor, the binding affinity was slightly reduced for the Arg142His and Asp294His mutant receptors, and the ability to stimulate cAMP production in response to alpha-MSH stimulation was reduced for all of them.

- Shimomura Y., Mori M., Sugo T., Ishibashi Y., Abe M., Kurokawa T., Onda H., Nishimura O., Sumino Y., Fujino M.
Isolation and identification of Melanin-Concentrating Hormone as the endogenous ligand of the SLC-1 receptor. Biochem. Biophys. Res. Commun. 261(3):622-626, 1999.

- Walch ET., Albino AP., Marchetti D.
Correlation of overexpression of the low-affinity p75 neurotrophin receptor with augmented invasion and heparanase production in human malignant melanoma cells. Int. J. Cancer 82(1):112-20, 1999.

5. Neuromelanins

(Prof. M. d'Ischia)

Four papers dealing more or less peripherally with the subject of neuromelanin have appeared during the last months. Ma et al. investigated in detail the relationship between dopamine transporter (DAT) immunoreactive neurons and age in the human substantia nigra. The results indicated a faster decrease in intensely stained neurons (11.2% per decade) compared to the total number of nigral neurons (6.7% per decade). Relative to young subjects, there were 75% and 88% reductions in DAT positive neurons in the middle-aged and aged groups, respectively, which contrasts with the 35% and 41% reductions in total number of neuromelanin-containing neurons seen in middle-aged and aged groups, respectively. The finding that DAT positive neurons decline at faster pace than nigral neurons may be of particular interest for the understanding of the functional significance of DAT and its relation to neuromelanin and aging.
In a study aimed at investigating the possible existence of circuits between neurons which synthesize corticotropic-releasing hormone and serotonin and their implication in the pathophysiology of major depression and suicide, Ruggiero et al. delineated complex maps relating the different neural pathways and highlighted, inter alia, the presence of corticotropic-releasing hormone projections to noradrenergic neurons containing neuromelanin in the locus ceruleus. On this basis, the authors suggest that corticotropic-releasing hormone may influence the activity of two major monoaminergic cell systems implicated in mental illness, through neural and humoral mechanisms.
Of particular interest is also a paper by Vymazal et al showing the potential of magnetic resonance imaging for identification and quantification of brain iron in healthy subjects, patients with Parkinson disease, and patients with multiple system atrophy. By measuring regional variations in relaxation times T1 and T2 the authors demonstrate a good correlation between T1 and T2 and regional changes in iron content and form, pointing to the potential of these parameters as indicators of such changes.
Finally Baumann et al. investigated both the numbers and distribution of neuromelanin-containing neurones in the locus

coeruleus (LC) in the brainstem of 12 patients with bipolar disorder (n = 6) or major depression (n = 6), and 12 normal comparison subjects. The results suggest significant differences of innervation arising from the LC of bipolar patients as compared to patients with major depression.

- Baumann B, Danos P, Krell D, Diekmann S, Wurthmann C, Bielau H, Bernstein HG, Bogerts B.
Unipolar-bipolar dichotomy of mood disorders is supported by noradrenergic brainstem system morphology. J Affect Disord. 54(1-2):217-24, 1999.

- Ma SY, Ciliax BJ, Stebbins G, Jaffar S, Joyce JN, Cochran EJ, Kordower JH, Mash DC, Levey AI, Mufson EJ.
Dopamine transporter-immunoreactive neurons decrease with age in the human substantia nigra. J Comp Neurol. 409(1):25-37, 1999.

- Ruggiero DA, Underwood MD, Rice PM, Mann JJ, Arango V.
Corticotropic-releasing hormone and serotonin interact in the human brainstem: behavioral implications. Neuroscience. 91(4):1343-54, 1999.

- Vymazal J, Righini A, Brooks RA, Canesi M, Mariani C, Leonardi M, Pezzoli G.
T1 and T2 in the brain of healthy subjects, patients with Parkinson disease, and patients with multiple system atrophy: relation to iron content. Radiology. 211(2):489-95, 1999.

6. Genetics, molecular biology

(Dr. F. Beermann)

- Beermann F, Hunziker A, Foletti A.
Transgenic mouse models for tumors of melanocytes and retinal pigment epithelium [Review]. Pigment Cell Research 12(2):71-80, 1999.

- Brizzi MF, Dentelli P, Rosso A, Yarden Y, Pegoraro L.
STAT protein recruitment and activation in c-Kit deletion mutants. Journal of Biological Chemistry 274(24):16965-16972, 1999.

- Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, Shen Q, O'Hagan R, Pantginis J, Zhou H, Horner II J, Cordon-Cardo C, Yancopoulos G, DePinho R.
Essential role for oncogenic ras in tumor maintenance. Nature 400:468-472, 1999.
Summary: The authors use transgenic mice expressing the reverse tetracycline transactivator (rtTA) in melanocytes (by use of tyrosinase regulatory sequences). Combination of this transgenic line with transgenic lines carrying oncogenic Ha-ras (V12G) controlled by the tet operator allows to induce ras expression by feeding doxycycline drinking water. To obtain melanomas, the mice were kept on a INK4a -/- background. When Ha-ras V12G expression was downregulated (by withdrawal of doxycycline) primary and explanted tumors regressed, thus suggesting that oncogenic ras is not only important in genesis of tumors, but also in maintenance.

- Danielson KG, Siracusa LD, Donovan PJ, Iozzo RV.
Decorin, epiphycan, and lumican genes are closely linked on murine chromosome 10 and are deleted in lethal steel mutants. Mammalian Genome 10(2):201-203, 1999.

- De Sepulveda, P, Okkenhaug K, La RJ, Hawley RG, Dubreuil P, Rottapel R.
Socs1 birds to multiple signalling proteins and suppresses Steel factor-dependent proliferation. Embo Journal 18(4):904-915, 1999.

- Eberle J, Weitmann S, Thieck O, Pech H, Paul M, Orfanos CE.
Downregulation of endothelin B receptor in human melanoma cell lines parallel to differentiation genes. Journal of Investigative Dermatology 112(6):925-932, 1999.

- Fang D, Setaluri V.
Role of microphthalmia transcription factor in regulation of melanocyte differentiation marker TRP-1. Biochemical & Biophysical Research Communications 256(3):657-663, 1999.

- Kaufmann D, Gruener S, Braun F, Stark M, Griesser J, Hoffmeyer S, Bartelt B.
EVI2B, a gene lying in an intron of the neurofibromatosis type 1 (NF1) gene, is as the NF1 gene involved in differentiation of melanocytes and keratinocytes and is overexpressed in cells derived from NF1 neurofibromas. DNA & Cell Biology 18(5):345-356, 1999.

- Kim DK, Morii E, Ogihara H, Lee YM, Jippo T, Adachi S, Maeyama K, Kim HM, Kitamura Y.
Different effect of various mutant MITF encoded by mi, Mi(or), or Mi(wh) allele on phenotype of murine mast

cells. Blood 93(12):4179-4186, 1999.

- London CA, Galli SJ, Yuuki T, Hu ZQ, Helfand SC, Geissler EN.
Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. Experimental Hematology 27(4):689-697, 1999.

- Opdecamp K, Kos L, Arnheiter H, Pavan WJ.
Endothelin signalling in the development of neural crest-derived melanocytes. Biochemistry & Cell Biology Biochimie & Biologie Cellulaire 76(6):1093-1099, 1998.

- Parichy DM, Stigson M, Vass SR.
Genetic analysis of steel and the PG-M/versican-encoding gene AxPG as candidates for the white (d) pigmentation mutant in the salamander Ambystoma mexicanum. Development Genes & Evolution 209(6):349-356, 1999.

- Park BJ, Brown CK, Hu Y, Alexander HR, Horti J, Raje S, Figg WD, Bartlett DL.
Augmentation of melanoma-specific gene expression using a tandem melanocyte-specific enhancer results in increased cytotoxicity of the purine nucleoside phosphorylase gene in melanoma. Human Gene Therapy 10(6):889-898, 1999.

- Petit J, Boisseau P, Taine L, Gauthier B, Arveiler B.
A YAC contig encompassing the 11q14.3 breakpoint of a translocation associated with schizophrenia, and including the tyrosinase gene. Mammalian Genome 10(6):649-652, 1999.

- Porter SD, Hu JC, Gilks CB.
Distal upstream tyrosinase S/MAR-containing sequence has regulatory properties specific to subsets of melanocytes. Developmental Genetics 25(1):40-48, 1999.
Comment: The authors have continued their analysis of the mouse tyrosinase enhancer region, focusing on the scaffold / matrix attachment region (S/MAR) situated upstream of the core enhancer. They generated transgenic mouse lines, where the S/MAR was linked to a mouse tyrosinase promoter / human tyrosinase cDNA construct. From the expression data, they conclude that the S/MAR provides position-independent transgene expression in cutaneous melanocytes and in RPE pigment cells, but not in other neural-crest derived melanocytes like in choroid.

- Rehli M, Den EN, Cassady AI, Ostrowski MC, Hume DA.
Cloning and characterization of the murine genes for bHLH-ZIP transcription factors TFEC and TFEB reveal a common gene organization for all MiT subfamily members. Genomics 56(1):111-120, 1999.

- Rehli M, Lichanska A, Cassady AI, Ostrowski MC, Hume DA.
TFEC is a macrophage-restricted member of the microphthalmia-TFE subfamily of basic helix-loop-helix leucine zipper transcription factors. Journal of Immunology 162(3):1559-1565, 1999.

- Rios M, Habecker B, Sasaoka T, Eisenhofer G, Tian H, Landis S, Chikaraishi D, Roffler TS.
Catecholamine synthesis is mediated by tyrosinase in the absence of tyrosine hydroxylase. Journal of Neuroscience 19(9):3519-3526, 1999.
Summary: This paper provides a further argument for a function of tyrosinase in neuronal cells, by contributing to the synthesis of catecholamines, and partially rescuing tyrosine hydroxylase (TH) deficiency. TH deficiency is lethal before birth, but can be treated by administration of catecholamines. Once born, TH-null pups can survive without further treatment until weaning. Even though deficient, low levels of catecholamines were detected, but absent in postnatal TH-null albino mice that lack tyrosinase. In contrast to the pigmented TH-null mice, catecholamine histofluorescence is undetectable in postnatal albino mutants, and the catecholamine content of TH-null pups lacking tyrosinase is 18% or less than that of TH-null mice with tyrosinase. The authors suggest that tyrosinase serves as an alternative pathway to supply catecholamines.

- Rosbotham JL, Malik NM, Syrris P, Jeffery S, Bedlow A, Gharraie S, Murday VA, Holden CA, Carter ND.
Lack of c-kit mutation in familial urticaria pigmentosa. British Journal of Dermatology 140(5):849-852, 1999.

- Rusciano D, Lorenzoni P, Burger MM.
Regulation of c-met expression in B16 murine melanoma cells by melanocyte stimulating hormone. Journal of Cell Science 112(5):623-630, 1999.

- Sato M, Morii E, Takebayashi SK, Yasui N, Ochi T, Kitamura Y, Nomura S.
Microphthalmia-associated transcription factor interacts with PU.1 and c-Fos: Determination of their subcellular localization. Biochemical & Biophysical Research Communications 254(2):384-387, 1999.

- Sato S, Toyoda R, Katsuyama Y, Saiga H, Numakunai T, Ikeo K, Gojobori T, Yajima I, Yamamoto H.
Structure and developmental expression of the ascidian TRP gene: Insights into the evolution of pigment cell-specific gene expression. Developmental Dynamics 215:225-237, 1999.

- Seitz JJ, Schmutz SM, Thue TD, Buchanan FC.
A missense mutation in the bovine MGF gene is associated with the roan phenotype in Belgian Blue and Shorthorn cattle. Mammalian Genome 10(7):710-712, 1999.

- Tan CP, McKee KK, Weinberg DH, MacNeil T, Palyha OC, Feighner SD, Hreniuk DL, Van, der, Ploeg, Lht, MacNeil DJ, Howard AD.
Molecular analysis of a new splice variant of the human melanocortin-1 receptor. Febs Letters 451(2):137-141, 1999.

- Tripathi RK, Flanders DJ, Young TL, Oetting WS, Ramaiah A, King RA, Boissy RE, Nordlund JJ.
Microphthalmia-associated transcription factor (MITF) locus lacks linkage to human vitiligo or osteopetrosis: An evaluation. Pigment Cell Research 12(3):187-192, 1999.

- Wehrle HB, Weston JA.
Altered cell-surface targeting of stem cell factor causes loss of melanocyte precursors in Steel(17H) mutant mice. Developmental Biology 210(1):71-86, 1999.
Summary: The Steel mutant *Sl<17H>* encodes for a stem cell factor which has an altered cytoplasmic domain (due to a defect in splicing), and leads to complete absence of coat pigmentation. The authors show that melanocyte precursor migration is altered, thus suggesting a defect in interaction of stem cell factor with its ligand, c-kit. They provide evidence that, in keratinocytes, the *Sl<17H>* stem cell factor is mislocated to the apical surface of the epithelial cells, instead of the basolateral compartment, thus preventing correct interaction with migrating melanocyte precursors.

- Zheng BH, Mills AA, Bradley A.
A system for rapid generation of coat color-tagged knockouts and defined chromosomal rearrangements in mice. Nucleic Acids Research 27(11):2354-2360, 1999.
Comment: In this methods paper, the authors have constructed genomic libraries containing either a tyrosinase or an agouti minigene. Following generation of knock-out mice using either of the libraries, genomic arrangements (for example using Cre-loxP) might be followed by taking advantage of the coat color transgenes incorporated.

7. Tyrosinase, TRP1, TRP2 and other enzymes
(Prof. J.C. Garcia-Borron)

- Birkle S, Ren S, Slominski A, Zeng G, Gao L, Yu RK.
Down-regulation of the expression of O-acetyl-GD3 by the O-acetylesterase cDNA in hamster melanoma cells: effects on cellular proliferation, differentiation, and melanogenesis. JNeurochem. 72(3):954-61, 1999.

- Blarzino C, Mosca L, Foppoli C, Coccia R, De Marco C, Rosei MA.
Lipoxygenase/H2O2-catalyzed oxidation of dihdroxyindoles: synthesis of melanin pigments and study of their antioxidant properties. Free Radic Biol Med. 26(3-4):446-53, 1999.
Abstract: 5,6-Dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), which are important intermediates in melanogenesis, can be converted into the corresponding melanin pigments by the action of the lipoxygenase/H2O2 system. Kinetic and HPLC analyses indicate that both DHI and DHICA are good substrates for this enzymatic system. Enzyme activity on both substrates was measured in comparison with peroxidase and tyrosinase; the oxidizing behaviour of lipoxygenase is more similar to that of peroxidase rather than that of tyrosinase. The antioxidant properties of DHI- and DHICA-melanins have been investigated in comparison with other kinds of melanins. DHICA-melanin shows a more pronounced antioxidant effect than that of DHI-melanin and this behaviour can be ascribed to the different structure and solubility of the two pigments. The mixed polymer synthesized from DHI and DHICA is the most effective one. Some implications about the possible explanation of the above mentioned behaviour are discussed.

- Bubacco L, Salgado J, Tepper AW, Vijgenboom E, Canters GW.
1H NMR spectroscopy of the binuclear Cu(II) active site of Streptomyces antibioticus tyrosinase. FEBS Lett. 442(2-3): 215-20, 1999.
Abstract: The 600 MHz 1H NMR spectrum of tyrosinase (31 kDa) of Streptomyces antibioticus in the oxidized, chloride-bound form is reported. The downfield part of the spectrum (15-55 ppm) exhibits a large number of paramagnetically shifted signals. The paramagnetism is ascribed to a thermally populated triplet state. The signals derive from six histidines binding to the metals through their Nepsilon atoms. There is no evidence for endogenous bridges. The exchange coupling, -2J, amounts to 298 cm(-1). In the absence of chloride the peaks broaden. This is ascribed to a slowing down of the electronic relaxation. The exchange coupling decreases to -2J=103 cm(-1).

- Castelli C, Tarsini P, Mazzocchi A, Rini F, Rivoltini L, Ravagnani F, Gallino F, Belli F, Parmiani G.
Novel HLA-Cw8-restricted T cell epitopes derived from tyrosinase-related protein-2 and gp100 melanoma antigens. J Immunol. 162(3):1739-48, 1999.

Abstract: The identification of T cell epitopes presented by alternative HLA-B and -C alleles may provide a means to counteract the tumor escape mechanism based on the selection of tumor cells no longer susceptible to HLA-A-restricted T cell recognition. Several T cell clones and lines were obtained from T lymphocytes purified from melanoma-infiltrated or noninfiltrated lymph nodes of a patient who remained disease free 8 yr after surgery. Selected T cells recognized the autologous melanoma as evaluated by direct cytolysis and production of cytokines. These effectors were directed against the tyrosinase-related protein-2 (TRP-2) and gp100 melanoma epitopes restricted by HLA-Cw8. The nonamer and decamer peptides containing the sequence ANDPIFVVL (residues 387-395) of TRP-2 and the octamer, nonamer, and decamer peptides containing the sequence SNDGPTLI (residues 71-78) of gp100 reconstituted the epitope for TRP-2- and gp100-specific T cell lines and clones, respectively. However, only the nonameric form of TRP-2 and the nonameric and octameric forms of gp100 were able to induce peptide-specific T cells recognizing the autologous tumor in an HLA-class I-restricted fashion from PBMC of the melanoma patient studied. Together these data indicate that HLA-Cw8 can restrict the recognition of gp100 and TRP-2 epitopes by CTL, and that such peptides could stimulate a patient's PBL, suggesting that these Ags could have contributed to a systemic immunity against melanoma.

- Curto EV, Kwong C, Hermersdorfer H, Glatt H, Santis C, Virador V, Hearing V.J. Jr, Dooley TP.
Inhibitors of mammalian melanocyte tyrosinase: in vitro comparisons of alkyl esters of gentisic acid with other putative inhibitors. Biochem-Pharmacol. 57(6):663-72, 1999.
Abstract: To discover safe and effective topical skin-lightening agents, we have evaluated alkyl esters of the natural product gentisic acid (GA), which is related to our lead compound methyl gentisate (MG), and four putative tyrosinase inhibitors, utilizing mammalian melanocyte cell cultures and cell-free extracts. Desirable characteristics include the ability to inhibit melanogenesis in cells (IC50 < 100 microg/mL) without cytotoxicity, preferably due to tyrosinase inhibition. Of the six esters synthesized, the smaller esters (e.g. methyl and ethyl) were more effective enzyme inhibitors (IC50 approximately 11 and 20 microg/mL, respectively). For comparison, hydroquinone (HQ), a commercial skin "bleaching" agent, was a less effective enzyme inhibitor (IC50 approximately 72 microg/mL), and was highly cytotoxic to melanocytes in vitro at concentrations substantially lower than the IC50 for enzymatic inhibition. Kojic acid was a potent inhibitor of the mammalian enzyme (IC50 approximately 6 microg/mL), but did not reduce pigmentation in cells. Both arbutin and magnesium ascorbyl phosphate were ineffective in the cell-free and cell-based assays. MG at 100 microg/mL exhibited a minimal inhibitory effect on DHICA oxidase (TRP 1) and no effect on DOPAchrome tautomerase (TRP-2), suggesting that MG inhibits melanogenesis primarily via tyrosinase inhibition. MG and GA were non-mutagenic at the hprt locus in V79 Chinese hamster cells, whereas HQ was highly mutagenic and cytotoxic. The properties of MG in vitro, including (1) pigmentation inhibition in melanocytes, (2) tyrosinase inhibition and selectivity, (3) reduced cytotoxicity relative to HQ, and (4) lack of mutagenic potential in mammalian cells, establish MG as a superior candidate skin-lightening agent.

- Day BW, Tyurin VA, Tyurina YY, Liu M, Facey JA, Carta G, Kisin ER, Dubey RK, Kagan VE.
Peroxidase-catalyzed pro- versus antioxidant effects of 4-hydroxytamoxifen: enzyme specificity and biochemical sequelae. Chem Res Toxicol. 12(1):28-37, 1999.

- Englaro W, Bahadoran P, Bertolotto C, Busca R, Derijard B, Livolsi A, Peyron JF, Ortonne JP, Ballotti R.
Tumor necrosis factor alpha-mediated inhibition of melanogenesis is dependent on nuclear factor kappa B activation. Oncogene. 18(8):1553-9, 1999.

- Fang D, Setaluri V.
Role of microphthalmia transcription factor in regulation of melanocyte differentiation marker TRP-1. Biochem Biophys Res Commun. 256(3):657-63, 1999.

- Jin EJ, Thibaudeau G.
Effects of lithium on pigmentation in the embryonic zebrafish (Brachydanio rerio). Biochim Biophys Acta. 1449(1):93-9, 1999.

- Kubo I, Kinst Hori I.
2-Hydroxy-4-methoxybenzaldehyde: a potent tyrosinase inhibitor from African medicinal plants. Planta Med. 65(1):19-22, 1999.
Abstract: By bioassay-guided fractionation using mushroom tyrosinase (EC 1.14.18.1), 2-hydroxy-4-methoxybenzaldehyde was characterized as the principal tyrosinase inhibitor from three East African medicinal plants, the root of Mondia whitei (Hook) Skeels (Asclepiaceae), the root of Rhus vulgaris Meikle (Anacardiaceae), and the bark of Sclerocarya caffra Sond (Anacardiaceae). It inhibited the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA) by mushroom tyrosinase with an ID50 of 4.3 micrograms/ml (0.03 mM). The inhibition kinetics analyzed by a Lineweaver-Burk plot found this simple benzaldehyde derivative to be a mixed type inhibitor for this oxidation and affects on the enzyme in several ways. Based on finding this potent tyrosinase inhibitor, various related analogues were also tested in order to gain new insights into their inhibitory functions on a molecular basis.

- Oetting WS, King RA.
Molecular basis of albinism: mutations and polymorphisms of pigmentation genes associated with albinism. Hum Mutat. 13(2):99-115, 1999.
Abstract: Albinism, caused by a deficiency of melanin pigment in the skin, hair, and eye (oculocutaneous albinism

[OCA]), or primarily in the eye (ocular albinism [OA]), results from mutations in genes involved in the biosynthesis of melanin pigment. The lack of melanin pigment in the developing eye leads to fovea hypoplasia and abnormal routing of the optic nerves. These changes are responsible for the nystagmus, strabismus, and reduced visual acuity common to all types of albinism. Mutations in six genes have been reported to be responsible for different types of oculocutaneous and ocular albinism, including the tyrosinase gene (TYR) and OCA1 (MIM# 203100), the OCA2 gene and OCA2 (MIM# 203200), the tyrosinase-related protein-1 gene (TYRP1) and OCA3 (MIM# 203290), the HPS gene and Hermansky-Pudlak syndrome (MIM# 203300), the CHS gene (CHS1), and Chediak-Higashi syndrome (MIM# 214500), and the X-linked ocular albinism gene and OA1 (MIM#300500). The function of only two of the gene products is known tyrosinase and tyrosinase-related protein-1 both of which are enzymes in the melanin biosynthetic pathway. Continued mutational analysis coupled with function/structure studies should aid our understanding of the function of the remaining genes and their role in albinism. Mutation and polymorphism data on these genes are available from the International Albinism Center Albinism Database web site (http://www.cbc.umn.edu/tad).

- Rompel A, Fischer H, Meiwes D, Buldt Karentzopoulos K, Magrini A, Eicken C, Gerdemann C, Krebs B.
Substrate specificity of catechol oxidase from Lycopus europaeus and characterization of the bioproducts of enzymic caffeic acid oxidation. FEBS-Lett. 445(1):103-10, 1999.

- Schmidt A, Tief K, Yavuzer U, Beermann F.
Ectopic expression of RET results in microphthalmia and tumors in the retinal pigment epithelium. Int J Cancer. 80(4):600-5, 1999.
Abstract: The retinal pigment epithelium (RPE) is essential for eye development by interacting with the overlaying neuroepithelium. Regulatory sequences of the gene encoding for tyrosinase-related protein 1 (TRP-1), linked to the lacZ reporter gene, lead to strong and specific beta-galactosidase expression in the RPE. We asked how the oncogene ret would affect this epithelial cell type during mouse development. We used the TRP-1 promoter to express ret in the developing RPE, and obtained transgenic mouse lines, which showed mild to severe microphthalmia. During development, the RPE changed to a stratified epithelium with reduced or absent pigmentation from E10.5 onward. In addition, proliferation of RPE cells and tumor formation were observed from E12.5 onward. These early events prevent closure of choroid fissure and lead to microphthalmia and secondary malformations after birth. We conclude that ret transgene expression in the RPE prevents normal differentiation of this epithelial layer and induces proliferation and tumor formation. The appearance of the microphthalmic phenotype underlines the requirement of a normally developed RPE for eye development.

- Schmittel A, Keilholz U, Max R, Thiel E, Scheibenbogen C.
Induction of tyrosinase-reactive T cells by treatment with dacarbazine, cisplatin, interferon-alpha +/- interleukin-2 in patients with metastatic melanoma. Int J Cancer. 80(1):39-43, 1999.
Abstract: We have shown the presence of tyrosinase-reactive T cells in the peripheral blood of melanoma patients, who had been in remission after treatment with IL-2-containing regimens. In this consecutive study, we analyzed the T-cell response to various peptides derived from tyrosinase in serial blood samples obtained from 7 stage-IV melanoma patients before, during and following treatment. All patients were treated within a randomized trial (EORTC 18951) with cisplatin (CDDP), dacarbazine (DTIC), interferon-alpha (IFN-alpha) +/- interleukin-2 (IL-2). Using an ELISPOT assay detecting peptide-specific IFN-gamma release, we measured the T-cell response to 4 different HLA class I-binding peptide epitopes derived from tyrosinase containing an HLA-A2.1-, HLA-A24- or HLA-B44-binding motif in peripheral-blood mononuclear cells (PBMC). In one patient, tyrosinase-reactive T cells were detected before therapy. In 4 out of 7 patients, tyrosinase-reactive T cells against both HLA-A2.1-binding peptides and the B44-binding peptide became detectable at frequencies of up to 30 in 5 x 10(5) lymphocytes following treatment. These patients received CDDP, DTIC and IFN-alpha, 2 of them without IL-2 and 2 with IL-2, resulting in one complete remission and 3 partial remissions. Two patients relapsed 8 and 9 months after treatment. At the time of relapse, no T cells reactive with tyrosinase were detectable. Our results show that high frequencies of tyrosinase-reactive T cells in the peripheral blood of melanoma patients can be induced by chemotherapy in combination with IFN-alpha, regardless of concomitant IL-2 administration.

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Photoinduced dermal pigmentation in patients taking tricyclic antidepressants: histology, electron microscopy, and energy dispersive spectroscopy. J Am Acad Dermatol. 40(2 Pt 2):290-3, 1999.

- Zhao X, Wakamatsu Y, Shibahara M, Nomura N, Geltinger C, Nakahara T, Murata T, Yokoyama KK.
Mannosylerythritol lipid is a potent inducer of apoptosis and differentiation of mouse melanoma cells in culture. Cancer Res. 59(2):482-6, 1999.

Calendar of events

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

1999 **Symposium: New directions in Melanoma Primary Prevention**
30th Anniversary of the EORTC Melanoma Co-operative Group
Rotterdam, September 24
Contact: Mrs. Renée SCHRIJVER
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1999 **XVIIth International Pigment Cell Conference (IPCC): Nagoya Congress Center, Japan, October 30 - November 3**
Organizer: Prof. S. Ito
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Contact: Kazumasa WAKAMATSU, Ph.D.
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2000 **IXth Annual Meeting of the Pan American Society for Pigment Cell Research**
College Station, TX, June 25 - 28
Contact: Dr. Lynn LAMOREUX
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2000 **IXth Annual ESPCR Meeting: Krakow, PL**
Contact: Dr T. SARNA
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2001 5th World Conference on Melanoma
Venice, February 28 - March 3
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