Experimental Dermatology 2006: 15: 643–648
Blackwell Munksgaard . Printed in Singapore
doi: 10.1111/j.1600-0625.2006.00439.x

EXPERIMENTAL DERMATOLOGY
ISSN 0906-6705

5th Meeting of the 'Endocrine Dermatology' Group of the Arbeitsgemeinschaft Dermatologische Forschung (ADF)

University Hospital Aachen, Aachen, Germany
March 22, 2006

Abstracts

The human basophil – a novel target of the neuropeptide alpha-melanocyte-stimulating hormone

M. Böhm[1], U. Raap[2], T. Scholzen[1], M. Mertens[1], R. Brehler[1], A. Kapp[2] and T. A. Luger[1]
[1]*Department of Dermatology, University of Münster, Münster, Germany;*
[2]*Department of Dermatology and Allergology, Hannover Medical University, Hannover, Germany*

There is increasing evidence that the basophil does not only play an important role in acute allergic reactions but also in the pathogenesis of chronic allergic disorders. Here we show that human basophils express melanocortin receptors (MC-Rs) and respond to alpha-melanocyte-stimulating hormone (alpha-MSH) with regulation of proallergic cytokine expression and modulation of basophil activation markers. Using primers against all known MC-R subtypes we demonstrate that the human basophil cell line KU812 expresses MC-1R. Expression of MC-1R on the surface of KU812 cells was confirmed by FACS analysis using an anti-MC-1R antibody. The MC-1R expressed by KU812 cells was functionally active as alpha-MSH induced intracellular cAMP in a dose-dependent manner. Moreover, alpha-MSH abrogated the effect of calcium ionophore A23187 on IL-4 mRNA expression in these cells. The relevance of the above findings was corroborated by showing that MC-1R surface expression is also detectable in basophils of leukocyte suspensions derived from whole human blood. Most interestingly, alpha-MSH was capable of suppressing the inductive effect of fMLP on surface expression of the basophil activation marker CD63 in leukocyte suspensions of atopic individuals. Likewise, alpha-MSH significantly blocked grass pollen-induced up-regulation of CD63 in leukocyte suspensions of patients with grass pollen allergy. Our findings highlight a novel functional dimension of alpha-MSH. In addition, MSH peptides may become a novel future therapeutic avenue in treating human allergic diseases.

The vitamin D endocrine system of human sebocytes

J. Reichrath[1], CH. Schuler[1], M. Seifert[1], CH. Zouboulis[2] and W. Tilgen[1]
[1]*Department of Dermatology, The Saarland University Hospital, Homburg, Germany;* [2]*Department of Dermatology, Charite-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany*

Locally produced vitamin D metabolites are of high importance for growth regulation in various tissues. Using real-time PCR, we have characterized expression of key components of the vitamin D system vitamin D receptor (VDR), vitamin D-25-hydroxylase (25OHase), 25-hydroxyvitamin D^{-1} α-hydroxylase (1αOHase), and 1,25-dihydroxyvitamin D-24-hydroxylase (24OHase) in the SZ95 human sebaceous gland cell line. Incubation of SZ95 cells with 1,25-dihydroxyvitamin D resulted in a dose-dependent suppression of cell proliferation. RNA levels for VDR, 25OHase, 1αOHase, and 24OHase were characterized in SZ95 cells treated with various vitamin D analogs. In conclusion, our findings demonstrate that key components of the vitamin D system are strongly expressed in the SZ95 human sebaceous gland cell line and that local synthesis or metabolism of vitamin D metabolites may be of importance for growth regulation and various other cellular functions in sebaceous glands. Moreover, we conclude that sebaceous glands represent promising targets for therapy with vitamin D analogs or for pharmacological modulation of calcitriol synthesis/metabolism.

Opioid receptors in skin – link between stress and skin disease?

M. Bigliardi-Qi[1], C. Gaveriaux-Ruff[2], D. Hohl[1] and P. L. Bigliardi[1]
[1]*Departments of Dermatology, CHUV Hôpital Beaumont, Lausanne, Switzerland;*
[2]*Institute de Génétique et Biologie Moléculaire et Cellulaire IGBMC, CNRS/INSERM/ULP, BP 10142, 67404 Illkirch Cedex, France*

Opioid peptides and its receptors are highly conserved and crucial for survival in stress situations. Besides their well-known role in antinociception, they control cell differentiation and proliferation and influence apoptosis in various tissues. We are first to discover their presence in skin and to establish their functions in modulation of chronic itch and wound healing.

μ-Opioid receptor is a key player in induction of chronic itch. This could be confirmed using μ-opiate receptor knockout mice experiments and clinical studies on patients with chronic itch. We have induced a dry skin dermatitis as a model for chronic itching on knockout and wild type mice. μ-Opioid receptor knockout mice revealed significant less scratching behavior,

less epidermal hypertrophy and different density and quality of epidermal nerve endings compared to the wild type mice. In addition, topically applied opioid receptor antagonists relieved significantly chronic pruritus in a double-blind, placebo-controlled, cross-over study in 40 patients with atopic dermatitis.

Several findings suggest that the δ-opioid receptor and its endogenous ligands (enkephalins) are important in cell differentiation and proliferation. We proved the existence of a functional active δ-opioid receptor in different skin cells using RT-PCR, Western blot analysis and migration assays. In addition, δ-opioid receptor knockout mice revealed a phenotype of thinner epidermis and higher expression of cell differentiation marker cytokeratin 10 (CK 10) compared to wild-type mice. In a burn wound model, δ-opioid receptor knockout mice showed significant wound healing delay and a severe epidermal hypertrophy at the wound margin. This can be explained by a change of cell differentiation and migration due to the absence of a functional active δ-opioid receptor.

In summary, these experiments proved that the skin and the nervous system interact with each other in a bi-directional way, i.e. nervous system releases neuropeptides in stress situations affecting skin homeostasis and sensations and vice versa.

Therefore, the opioid receptor system is an excellent model to study the connection between psychological stress and aggravation of different skin diseases.

Human mast cells in the neurohormonal network: expression of POMC, detection of precursor proteases, and evidence for IgE-dependent secretion of alpha-MSH

M. Artuc[1], A. Grützkau[1], T. Zuberbier[1], B. M. Henz[1], T. A. Luger[2] and M. Böhm[2]

[1]Department of Dermatology, Charité, Humboldt University, Berlin, Germany;

[2]Department of Dermatology and Ludwig Boltzmann Institute of Cell Biology and Immunobiology of the Skin, University of Münster, Münster, Germany

Human mast cells have been shown to release histamine in response to the neuropeptide α-melanocyte-stimulating hormone (alpha-MSH), but it is unknown whether these cells express proopiomelanocortin (POMC) or POMC-derived peptides. We therefore examined highly purified human skin mast cells and a leukemic mast cell line (HMC-1) for their ability to express POMC and members of the prohormone convertase (PC) family known to process POMC. Furthermore, we investigated whether these cells store and secrete α-MSH. RT-PCR analysis revealed that both skin mast cells and HMC-1 cells express POMC mRNA and protein. Expression of the POMC gene at the RNA level in HMC-1 cells could be confirmed by Northern blotting. Transcripts for both PC1 and furin convertase were detectable in skin-derived mast cells and HMC-1 cells, as shown by RT-PCR. In contrast, PC2 transcripts were detected only in skin mast cells, while transcripts for PACE4 were present only in HMC-1 cells. Radioimmunoassays performed on cell lysates and cell culture supernatants from human skin-derived mast cells disclosed immunoreactive amounts of alpha-MSH in both fractions. Stimulation with an anti-IgE antibody significantly reduced intracellular alpha-MSH and increased extracellular levels, indicating IgE-mediated secretion of this neuropeptide. Our findings show that human mast cells are active players in the cutaneous POMC system. Mast cell-derived alpha-MSH may contribute to cutaneous hyperpigmentation as seen in patients with urticaria pigmentosa. Moreover, IgE-dependent release of alpha-MSH suggests an immunomodulatory role of this neurohormone during inflammatory and allergic reactions of the skin.

Melatonin metabolism in the skin

T. W. Fischer[1,4], T. W. Sweatman[2], I. Semak[1,5], R. M. Sayre[3], J. Wortsman[6] and A. Slominski[1]

[1]Department of Pathology and Laboratory Medicine, University of Tennessee Health Science Center, Memphis, TN, USA;
[2]Department of Pharmacology, University of Tennessee Health Science Center, Memphis, TN, USA;
[3]Division of Dermatology, University of Tennessee Health Science Center, Memphis, TN, USA;
[4]Department of Dermatology and Allergology, Friedrich-Schiller-University, Jena, Germany;
[5]Department of Biochemistry, Belarus State University, Minsk, Belarus; [6]Department of Internal Medicine, Southern Illinois University, Springfield, IL, USA

The melatoninergic system is fully expressed in the skin, which is an effector organ of melatonin protective effects. The present study aimed at detecting melatonin metabolism in keratinocytes and to identify resulting melatonin metabolites.

Intracellular melatonin and three metabolites were detected by HPLC and LC-MS. The metabolites were 6-hydroxymelatonin, AFMK (N^1-acetyl-N^2-formyl-5-methoxykynuramine) and 2-hydroxymelatonin (the main intermediate between melatonin and AFMK). The intracellular concentrations were highest for melatonin, followed by 6-hydroxymelatonin, AFMK and 2-hydroxymelatonin. More interestingly, over a 24 h time period, melatonin consumption in favour of production of the melatonin metabolites AFMK and 2-hydroxymelatonin was observed, whereas levels of 6-hydroxymelatonin decreased. Finally, this process was enhanced by exposure to UVR. Thus, the cutaneous melatonin metabolism and the known biological activities of melatonin metabolites increase the spectrum of potential actions of the recently identified cutaneous melatoninergic system.

Impact of acetylcholine on sebocyte biology *in vitro*

H. Kurzen[1], C. Fademrecht[1], S. Goerdt[1], H. Seltmann[2], CH. C. Zouboulis[2,3] and A. Gratchev[1]

[1]University Medical Centre Mannheim, Ruprecht-Karls-University of Heidelberg, Department of Dermatology, Venereology and Allergology, 68135 Mannheim, Germany;
[2]Laboratory for Biogerontology, Dermatopharmacology and Dermato-Endocrinology, Charité Universitaetsmedizin Berlin, Campis Benjamin Franklin, Berlin, Germany;
[3]Departments of Dermatology and Immunology, Dessau Medical Center, Dessau, Germany

Extraneuronal acetylcholine (ACh) has been demonstrated to influence a plethora of cutaneous cell functions in an autocrine, paracrine and endocrine fashion. Previously, we could demonstrate a differentiation-specific expression of its nicotinic (nAChR) and muscarinic receptors (mAChR) in human epidermis and its adnexal structures including sebaceous glands. Using an immortalized human sebaceous gland cell line (SZ95 sebocytes), we examined the AChR expression pattern *in vitro*. In proliferating and confluent SZ95 sebocytes, a wide range of AChR subunits could be detected using RT-PCR. In particular, we detected mRNA coding for the α3, α5, α6, α7, α10, β1, β2 and β4 nAChR as well as the M_1, and $M_{3-5.}$ The α1 and M_2 and M_5 subunits could only be detected in confluent SZ95 sebocytes, while in proliferating ones these subunits remained negative. The α2, α4, α9 and β3 nAChR could not be detected. Using functional assays, we assessed the impact of cholinergic agonists and antagonists on sebocyte proliferation and differentiation as evidenced by lipid production. Atropine (inhibition of all mAChR) and himbacine (inhibition of M_2 and M_4) potently inhibited SZ95 sebocyte proliferation in a dose-dependent

manner with a maximum effect at milimolar concentrations, while glycopyrrolate (inhibition of M_1 and M_3) inhibited proliferation only at high concentrations (100 μM). Interestingly also muscarine inhibited SZ95 sebocyte proliferation, however, with a maximum effect (50%) at nanomolar concentrations. The inhibitory effect of mecamylamine was less pronounced (20%). Nicotine strongly induced SZ95 sebocyte proliferation to more than 200% in a dose-dependent manner. Lipid production was increased by nicotine and muscarine in the micromolar range. Inhibition of nAChR by mecamylamine did not significantly influence lipid production, while even nanomolar concentrations of atropine significantly increased lipid production. In conclusion, we could demonstrate highly potent effects of the cholinergic system on sebocyte proliferation and lipid production *in vitro*, mediated by wealth of different AChR subunits present at least at the mRNA level. In particular, promotion of sebocyte proliferation seems to be an attractive explanation for the exacerbation of sebaceous gland–related disorders like acne under the influence of chronic nicotine ingestion. In addition, seborrhea observed after treatment with anticholinergic drugs is well in line with an increase in lipid production that we found after treatment of sebocytes with atropine.

Decline of hormones with increasing age regulates the biological activity of human sebocytes

E. Makrantonaki[1] and C. C. Zouboulis[1,2]

[1]Department of Dermatology, Charité Universitaetsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany; [2]Departments of Dermatology and Immunology, Dessau Medical Center, Dessau, Germany

Hormone deficiency occurring with age leads among other systemic disorders to deterioration of the skin quality. Systemic substitution with estradiol in females and GH in males reversed the observed alterations and retarded the skin aging process in single controlled studies. In order to evaluate the importance of sex steroids and growth factors on skin aging *in vitro*, human sebocytes were treated with GH, IGF-I, 17β-estradiol, DHEA, testosterone and progesterone in combination and as single agents in concentrations corresponding to those circulating in young (f20) and postmenopausal women (f60). Cell proliferation and lipid synthesis were measured by means of the 4-methylumbelliferyl heptanoate fluorescence assay and nile-red microassay/fluorescence microscopy, respectively. Cells incubated with all hormones mentioned above at f60 showed significantly lower content of sebaceous lipids ($P < 0.001$) vs. cells at f20 relating to *in vivo* observations, which have documented a decline of epidermal lipids in aged skin. While progesterone and DHEA, as single agents, showed no effect on lipid synthesis, after treatment with IGF-I for 48 h at f20 and f60 concentrations, a significant dose-dependent increase of neutral ($P < 0.001$) and polar lipids ($P < 0.001$) was observed at both concentrations tested. Cell size was also increased. On the other hand, GH at f20 and f60 enhanced significantly the production of neutral ($P < 0.01$ and $P < 0.05$, accordingly) and polar lipids ($P < 0.05$ and $P < 0.01$, accordingly) in sebocytes but not in such an extent as IGF-I. 17β-estradiol induced only polar lipid synthesis ($P < 0.01$) and cell size. No treatment affected the cell proliferation. In conclusion, sex steroids and growth factors and their decline with age play a key role in the regulation of the lipid synthesis in human sebocytes. Furthermore, among all hormones tested, the GH/IGF-I axis seems to be the most potent regulator of skin lipids.

New frontiers in human hair follicle (neuro-)endocrinology

R. Paus[1], A. Kromminga[1,4], S. Hasse[1], M. Laugsch[2], W. Jelkmann[1], B. E. Wenzel[2] and E. Bodó[1]

[1]Department of Dermatology, University Hospital Schleswig-Holstein, University of Lübeck, Lübeck, Germany; [2]Department of Physiology, University Hospital Schleswig-Holstein, University of Lübeck, Lübeck, Germany
[3]Department of Medicine I, University Hospital Schleswig-Holstein, University of Lübeck, Lübeck, Germany; [4]Institute for Immunology, Clinical Pathology, and Molecular Medicine (IMP), Hamburg, Germany

Recently, we have shown that normal human scalp hair follicles in anagen VI (a) synthesize CRH, ACTH, alpha-MSH and cortisol and display a fully functional equivalent of the hypothalamus–pituitary–adrenal axis (Ito *et al*. FASEB J 2005) (b) synthesize melatonin, up-regulate melatonin synthesis and secretion upon stimulation with noradrenaline (just like in the pineal gland), and express melatonin receptors in a hair cycle-dependent manner (Kobayashi *et al*. FASEB J 2005) and (c) synthesize prolactin, express functional prolactin receptors, and respond to their stimulation by premature catagen induction, which is mediated in part via up-regulation of TGFβ2 (Foitzik *et al*. Am J Pathol, in press). Besides these, by now established, aspects of hair follicle (neuro-) endocrinology, we present preliminary evidence that points to exciting new frontiers in this field: for example, human anagen VI scalp hair follicles also express erythropoietin and its receptor (EPO and EPO-R) on the gene and protein level and up-regulate EPO-R expression under conditions of hypoxia, and that these hair follicles may have established yet another hypothalamus–pituitary axis equivalent, the TRH-TSH system. Studying the effects of calcitonin gene-related peptide (CGRP), a key skin neuropeptide associated, e.g., with stress responses and neurogenic inflammation, on organ-cultured human hair follicles, we also noted that CGRP inhibits both hair matrix keratinocyte proliferation *in situ* and hair follicle pigmentation. After defining the most immediately pressing open questions that remain to be addressed in these areas, our own data are discussed in the context of how the organ-culture of human scalp hair follicles can be exploited for addressing questions of general importance in human dermatoendocrinology.

'Neurotrophology' of the hair follicle: neurotrophins act as auto- and paracrine growth factors and immunomodulators

E. M. J. Peters[1] and R. Paus[2]

[1]Cutaneous Neuroimmunology, Internal Medicine Psychosomatics, University-Medicine Charité, Berlin, Germany; [2]Department of Dermatology, University Hospital Schleswig-Holstein, Campus Lübeck, University of Lübeck, Lübeck, Germany

Neurotrophins are increasingly appreciated as growth factors and immune modulators not only inside but also outside of the nervous system. They are now appreciated to affect a wide variety of epithelial tissues and have become intensely studied in the context of stress-responses. In mouse skin, for example, the prototypic neurotrophin, nerve growth factor (NGF), is produced by hair follicles and regulates their growth during hair follicle morphogenesis and cycling and the cutaneous responses to psychoemotional stress. Fluctuations in NGF expression are mirrored by changes in nerve fiber density and neuro–immune interactions in hair follicles and interfollicular neural networks. Our recent findings document that, in organ-cultured human hair follicles, NGF signaling inhibits hair growth (anagen) by the induction of apoptosis-driven hair follicle regression (catagen). Interestingly, this process appears to depend on stimulation of the pan-low-affinity neurotrophin receptor p75, which has recently been identified as a high affinity receptor for the NGF prohormone, proNGF. Moreover, hair follicle regression by neurotrophin signaling may be induced by key stress mediators such as substance P and potentially involves neurogenic inflammation in analogy to

the murine model and subsequent collapse of the hair follicle immune privilege. Thus mouse and human hair follicle epithelia are both a prominent peripheral source and a key target for neurotrophins and provide a highly instructive model for dissecting not only general growth-modulatory activities of NTs in epithelial biology, but also neuro–immune interactions under physiological and pathological conditions.

Bidirectional modification of IL-6 expression by angiotensin II in human dermal fibroblasts

U. M. Steckelings[1], F. Rompe[1], J. Reinemund[1], M. Artuc[2] and TH. Unger[1]

[1]Center for Cardiovascular Research, Institut für Pharmakologie und Toxikologie und, Charité – Universitätsmedizin Berlin, Berlin, Germany;

[2]Department of Dermatology und Allergy, Charité – Universitätsmedizin Berlin, Berlin, Germany

Recently, we demonstrated the presence of a complete rennin–angiotensin system in human skin and the up-regulation of angiotensin AT1- and AT2-receptors in cutaneous wounds. However, the characterisation of cutaneous actions of angiotensin II (Ang II) under physiological or pathophysiological conditions is only at the very beginning. It is well established for non-cutaneous tissues that the AT1-receptor (AT1-R) mediates pro-inflammatory actions. The role of the AT2-receptor (AT2-R) is far less understood. In order to look for a putative role of Ang II in cutaneous inflammation, we chose interleukin 6 (Il-6) as a representative inflammation marker. Il-6 expression was studied in response to Ang II with or without co-stimulation by TNFalpha, and it was distinguished between AT1- or AT2-receptor-mediated responses, respectively.

Human primary dermal fibroblasts (passage 3–5) were isolated from female thoracic skin derived in the course of cosmetic breast surgery. Fibroblasts were stimulated with Ang II (10^{-7} M) for 24 h and co-incubated with irbesartan (10–5 M; AT1-receptor antagonist) or PD 123319 (10–5 M; AT2-receptor antagonist), respectively. The same experiment was performed on a second set of cells, additionally treated with TNFalpha (10 ng/ml) in order to stimulate Il-6 expression and to examine the effect of Ang II on elevated Il-6 levels. Moreover, AT2-R mediated effects were determined by incubation of cells with the novel AT2-R agonist Compound 21. Il-6 mRNA was detected by Real-Time-PCR.

Ang II stimulated Il-6 expression fivefold. This stimulation could be inhibited by irbesartan, but not by PD 123319. TNFalpha caused a marked increase in Il-6 expression, which could be inhibited by AT2-receptor stimulation (by Ang II in the presence of irbesartan or by Compound 21). The effect of Compound 21 was abrogated by PD 123319. Intracellular signalling leading to the induction of IL-6 expression via the AT1-R seemed to involve the CYP-dependent arachidonic acid metabolite 20-hydroxyeicosatetraenoic acid (20-HETE). A CYP-dependent metabolite involved in the AT2-R mediated effects on IL-6 expression could not be defined.

Thus, in human dermal fibroblasts, Il-6 expression is stimulated by TNFalpha and to a lesser extent by Ang II via the AT1-receptor. In contrast, Ang II via the AT2-receptor diminishes TNFalpha-induced Il-6 expression. Consequently, Ang II may be involved in cutaneous inflammation by a dual mechanism: it acts pro-inflammatory via the AT1-receptor and anti-inflammatory via the AT2-receptor.

Role of proteinase-activated receptors (PAR_s) during cutaneous inflammation and the immune response

M. Steinhoff

Department of Dermatology and Boltzmann Institute for Immunobiology of the Skin, University of Muenster, Germany

Proteinase-activated receptors constitute a new subfamily of G protein-coupled receptors with seven transmembrane domains which are activated by various exogenous or endogenous serine proteinases such as thrombin, cathepsin G, trypsins, tryptase, clotting factors (VV, Xa), bacterial proteases or mite antigens. In addition, PAR_1 can be activated by MMP-1. Recent knowledge indicate that proteases are not merely enzymes which cleave proteins in the extracellular space, but are signaling molecules involved in many processes by activating specific receptors in an autocrine, paracrine and endocrine fashion. MMP-1 activates PAR_1 and is thereby involved in the migration and invasive potential of melanoma cells. PAR_2 is a receptor for mast cell tryptase or house dust mite allergens which is released during inflammation and allergic reactions. In the skin, PAR_2 is diversly expressed by keratinocytes, endothelial cells and occasionally on sensory nerves of human skin in various disease states. Moreover, immunocompetent cells such as mast cells and neutrophils express functional PAR_2 thereby contributing to inflammation and host defense. Own data revealed that PAR_2 contributes to neurogenic inflammation by releasing neuropeptides from sensory nerves resulting in edema, plasma extravasation and infiltration of neutrophils. Thus, mast cells may communicate with sensory nerves in inflammatory tissues by activating PAR_2 via tryptase. Vice versa, PAR_2 activation of cutaneous mast cells leads to a release of histamine suggesting a bidirectional pathway of mast cell–nerve communication. Moreover, PAR_2 agonists up-regulate expression of certain cell adhesion molecules and cytokines such as interleukin-6 and interleukin-8 on dermal microvascular endothelial cells or regulate neutrophil migration or integrin expression – a role of PAR_2 in leukocyte/endothelial interactions. These effects may be partly mediated by NF-κB, an important transcription factor during inflammation and immune response. PAR_2 stimulation results in the activation of NF-κB on microvascular endothelial cells and keratinocytes thereby regulating ICAM-1 expression. We also demonstrate evidence for a diverse expression of PAR_2 in various skin diseases and highlight the recent knowledge about the important role of PAR_2 during inflammation and the immune response. Together, PAR_2-modulating agents may be new tools for the treatment of inflammatory and allergic diseases in the skin.

The skin as a mirror of the ageing process in the human organism – results of the ageing research in the German National Genome Research Network 2

CH. C. Zouboulis

Departments of Dermatology and Immunology, Dessau Medical Center, Dessau, and Laboratory for Biogerontology, Dermato-Pharmacology and Dermato-Endocrinology, Institute of Clinical Pharmacology and Toxicology, Charité Universitaetsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany

Intrinsic human skin ageing is influenced by the individual genetic predisposition and reflects degradation processes of the body. Hormones are decisively involved in intrinsic ageing with reduced secretion of pituitary, adrenal glands, and gonads, which leads to characteristic body and skin phenotypes. A number of advances were recently made in understanding skin ageing mechanisms and major molecular changes, especiallly of the extracellular matrix, were identified. Gene expression patterns compatible with mitotic misregulation and alterations in intracellular transport and metabolism were identified in fibroblasts of ageing humans and humans with progeria. Age-associated changes of extracellular matrix of the skin correlate

well with changes been detected in the extracellular matrix of other organs of the human body. Within the National Genome Research Network 2 (NGFN-2) in Germany, the explorative project 'Genetic etiology of human longevity' targets the identification of age-related molecular pathways. For this purpose, skin models of ageing are used. Expression profiling employing cDNA microarrays from known and novel genes and RT-PCR are employed for gene detection and confirmation. Among the potential candidate genes several interesting target genes have been identified. The evaluation of ageing-associated genes in skin models will facilitate the understanding of global molecular ageing mechanisms in the future.

Vitamin D, clusterin and melanoma growth

B. Shannan, M. Seifert, W. Tilgen and J. Reichrath
Department of Dermatology, The Saarland University Hospital, Homburg, Germany

The secretory glycoprotein clusterin (CLU) was shown to be involved in the regulation of various cell functions including cell growth and apoptosis. The nuclear (nCLU) and the secretory (sCLU) are two isoforms of CLU that are obtained by alternate splicing. While the pro-apoptotic nCLU was shown to be involved in the regulation of cell-cycle progression and apoptosis, sCLU has been shown to have cytoprotective properties. We have analyzed the expression of CLU (mRNA and protein) in various melanoma cell lines that have previously been characterized as sensitive (SK-MEL-28 and MeWo) or resistant (SK-MEL-5, SK-MEL-25) to antiproliferative effects of vitamin D analogues. Vitamin D-resistant melanoma cell lines were shown to reveal a functional defect in vitamin D receptor-mediated gene transcription. CLU mRNA and protein were present in all melanoma cells analyzed, with sCLU being the most dominant isoform in both vitamin D-sensitive and resistant cells. 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] treatment of vitamin D-sensitive, but not of resistant, cells resulted in a time-dependent up-regulation of CLU mRNA levels, as measured using conventional PCR. Treatment with $1,25(OH)_2D_3$ did neither in vitamin D-sensitive nor in resistant cells alter the CLU splice variant pattern. Our findings indicate that; CLU is expressed in melanoma cells. sCLU represents the most dominant isoform both in vitamin D-sensitive and resistant melanoma cells. CLU mRNA expression is increased after $1,25(OH)_2D_3$ treatment in vitamin D-sensitive but not in resistant melanoma cells. In conclusion, CLU may be of importance for the growth characteristics of melanoma cells. Our findings indicate (at least in melanoma cells) that CLU may be involved in signalling pathways that mediate the antiproliferative effects of $1,25(OH)_2D_3$.

Role of serotonergic system in inflammation

B. L. Fiebich
Department of Psychiatry and Psychotherapy, University of Freiburg Medical School, Freiburg, Germany

Serotonin (5-hydroxytryptamin, 5-HT) is a peptide originally thought to be mainly produced in the nervous system and functioning as a neurotransmitter. There is increasing evidence that serotonin has an important role in cellular immune response since serotonin is also produced in peripheral cells and due to the fact that more 5-HT receptors are expressed in immune cells than in neuronal cells. However, the role of serotonin in inflammation is not well understood. There is evidence from both human and animal research that 5-hydroxytryptamin (5-HT) 3 receptor antagonists, particularly tropisetron, exert analgesic and anti-inflammatory activity, and we were therefore interested to elucidate the underlying mechanisms of these effects. We studied the anti-inflammatory effects of tropisetron and ondansetron in various peripheral cells involved in inflammation. We were able to identify the two major splice variants of the 5-HT3 receptors, the short A-isoform and the long B-isoform in T-cells and monocytes. We furthermore found tropisetron to be an inhibitor of LPS-stimulated tumor necrosis factor (TNF) α and interleukin-(IL-)1β secretion in monocytes and of IL-2 expression in T-cells. Besides the 5-HT3 receptor, 5-HT7 seems to be involved in immune regulation, since we found this receptor to mediate serotonin-induced IL-6 release in astrocytes. Overall, our data suggest that the serotonergic system plays an import role in peripheral immune function.

Hormone-related hair disorders – from clinical needs to new therapeutic perspectives

U. Blume-Peytavi, N. Mandt, J. Lademann and A. Vogt
Clinical Research Center for Hair and Skin Physiology, Department of Dermatology and Allergy, Charité – Universitätsmedizin Berlin, Germany

The hair follicle and its appendages are important target structures for hormones. Plasma levels, local hormone metabolism and differential receptor expression regulate hair growth and hair cycling as well as sebaceous gland function. Even minor imbalances in this system, e.g. abnormal expression of hair follicle-associated isoenzymes, may cause clinical symptoms such as hair loss, seborrhoea or hirsutism.

Conventional therapeutic strategies, which aim to control peripheral hyperandrogenism, include systemic administration of androgen inhibitors (e.g. oral contraceptives, GnRH analogues), peripheral androgen blockers (CPA, CMA, glutamide, finasteride) as well as topical application of estrogens. As our knowledge about hormone systems and their complex interactions increases, new molecules are gaining importance: rosiglitazone and metformin are compounds which increase the sensitivity to insulin, topical application of minoxidil has been shown to regulate perifollicular blood flow and vascularization, and anti-inflammatory compounds can as well be beneficial in the treatment of androgenetic alopecia.

Because such therapies are, still, rather unspecific, current research focuses on the development of individualized strategies which allow to specifically treat the various manifestations caused by hormone-related disorders. From our perspective, the aims of future drug development are as follows.

In the first place, the identification of new targets, possibly beyond sex steroids in the narrow sense is highly important. Components of the POMC-system, for example, have been shown to regulate cell differentiation and also hair cycling.

And secondly, to develop drug delivery systems, which allow to specifically directing active compounds to target structures. Such improved drug delivery may allow to reduce side-effects and to increase the efficacy of current therapies. Studies by our group and others suggest that microparticles, due to their ability to aggregate in the hair follicle openings, are promising drug delivery systems. In fact, we recently reported that the penetration depths of transcutaneously applied microparticles into the follicular duct depends on the size of the particles and on the dimension of the hair follicle, suggesting that particle-based drug delivery systems allow to selectively target hair follicle compartments such as the entry level of the sebaceous duct or the bulge region.

25-hydroxyvitamin D_3 1alpha-hydroxylase splice variants in human skin

M. Seifert, W. Tilgen and J. Reichrath
Klinik für Dermatologie, Venerologie und Allergologie, Universitätskliniken des Saarlandes, Homburg/Saar, Germany

1,25-Dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], the biologically active metabolite of vitamin D, has been shown to regulate

the growth of various cell types, including human keratinocytes. There are two principal enzymes involved in the formation of circulating $1,25(OH)_2D_3$ from vitamin D, the hepatic microsomal or mitochondrial vitamin D 25-hydroxylase (25-OHase) and the renal mitochondrial enzyme 1α-hydroxylase (1α-OHase) for vitamin D and $25(OH)D_3$, respectively. 25-hydroxyvitamin D_3−1α-hydroxylase (1α-hydroxylase) catalyses the synthesis of the active form of vitamin D, 1,25-dihydroxyvitamin D_3, in the kidney. Recently, extrarenal activity of 1α-OHase has been reported in various cell types including macrophages, keratinocytes, prostate and colon cancer cells. Local production of calcitriol has been postulated to play an autocrine or paracrine role in vitamin D-mediated growth control. Previously, we reported mRNA splice variants of the gene encoding the *P*450 cytochrome 25-hydroxyvitamin D_3-1-hydroxylase in human melanoma cell lines. As already described for other cytochrome *P*450 genes, alternative splicing can play a role in regulating the enzyme level and may cause tissue-specific variations in healthy cells. Using nested touchdown reverse transcription-PCR (RT-PCR) and Western blot analysis, we identified CYP27B1 splice variants in cultured normal human melanocytes and keratinocytes (HaCaT) after treatment with UV-B (10–50 mJ/cm^2). Using real-time RT-PCR, we quantified the expression of CYP27B1. We identified several splice variants and a clear influence of UV-B treatment on the expression pattern of CYP27B1 in HaCaT cells.