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36TH ANNUAL MEETING OF THE ARBEITSGEMEINSCHAFT DERMATOLOGISCHE FORSCHUNG (ADF)

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ABSTRACT

The ADF (Arbeitsgemeinschaft Dermatologische Forschung), or the German Society for Dermatological Research, is an organization that presents every year at this meeting relevant studies with impact in the field of investigative dermatology. This year the meeting took place in Heidelberg on March 5-7. Interesting presentations related to skin inflammation, scleroderma, melanoma, and a new strategy for gene therapy based on spliceosome-mediated RNA trans-splicing of cutaneous genetic diseases are highlighted in this report.

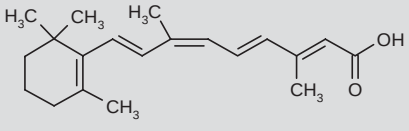
Cutaneous allergic inflammation was the focus of several studies presented at the ADF, in particular related to defective innate immune responses, skin barrier function and alitretinoin. A.S. Büchau from the Ludwig-Maximilians Universität in Munich identified Bcl-3 as a transcriptional modulator of Th2 cytokines that induced downregulation of antimicrobial peptide expression in atopic dermatitis keratinocytes. IL-4, IL-10 and IL-13 can downregulate antimicrobial peptide expression in atopic dermatitis, and this phenomenon may account for the propensity for skin infections observed in these patients. RNA interference with small interfering RNA (siRNA) silencing of Bcl-3 reversed the downregulatory effect of IL-4 and the upregulatory effect of TNF-α on human beta-defensin 3 expression. Bcl-3 is considered an important modulator of cutaneous innate immune responses and a possible therapeutic target for atopic dermatitis (1).

J. Jin from the Department of Dermatology, Medical University of Vienna, investigated the knockdown of filaggrin in an organotypic skin model. Human primary keratinocytes were transfected with siRNA specific for filaggrin and seeded onto fibroblast–collagen suspensions to generate a multilayered epidermis. Filaggrin knockdown in this model did not affect the expression and solubility of other differentiation-associated proteins, and morphological alterations of the epidermis were restricted to the granular layers, without influencing stratum corneum formation (2).

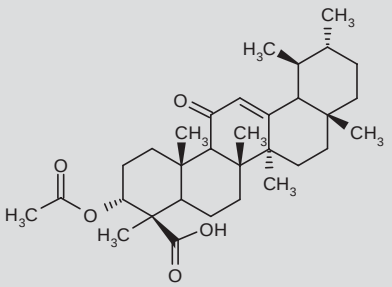
A novel function for keratinocytes in allergen uptake was described by C. Blume from the ZAUM - Center for Allergy and Environment, Division of Environmental Dermatology and Allergy (Munich). In the skin, keratinocytes act as a physical, chemical and immunological barrier. The uptake of allergens and nonallergenic proteins by keratinocytes increases constantly over time. Inflammatory conditions stimulated by interferon gamma lead to enhanced uptake of proteins. The greater uptake of allergens by keratinocytes in inflammatory states suggests a higher susceptibility of inflamed skin for allergen uptake, and possibly a higher risk for sensitization under conditions of natural exposure such as chronic atopic eczema (3).

Alitretinoin (9-*cis*-retinoic acid) is an agonist for RAR and RXR nuclear retinoid receptors that was recently approved by the EMEA for the oral treatment of chronic hand eczema in adult patients who are unresponsive to potent topical corticosteroids. B. Homey from the Department of Dermatology, Heinrich-Heine-Universität, Düsseldorf, presented data on its mechanism of action. Alitretinoin downregulates keratinocyte-derived chemokine (CCL27, CXCL9, CXCL10, CXCL11 and CCL20) production, impairs the mixed leukocyte reaction, suppresses the induction of the very early activation antigen CD69 on the surface of activated T, B and dendritic cells, and decreases the expression of co-stimulatory molecules such as CD80 and CD86 on the surface of antigen-presenting cells (4). These effects of alitretinoin on immunological mechanisms involved in chronic hand eczema may be related to its mechanism of action.

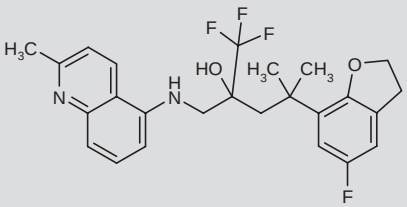
H. Wang from the Universität Ulm studied, using the murine CD18 hypomorphic psoriasis model, the selective inhibition of nuclear factor NF-kappa-B in macrophages by s.c. or i.p. injection of the liposome-encapsulated naturally occurring NF-kappa-B inhibitor **acetyl-11-keto-β-boswelic acid** (AkβBA), which was purified to chemical homogeneity from gum resins of *Boswellia* species. AkβBA-liposomes specifically inhibited the phosphorylation of IkB-alpha at Ser32 in F4/80 macrophages and also inhibited the NF-kappa-B activation-dependent genes *TNF*, *MCP1*, *EGFR* and *IL20* 24 h after injection. Improvements in the psoriasis-like lesions in this model were evident (5).



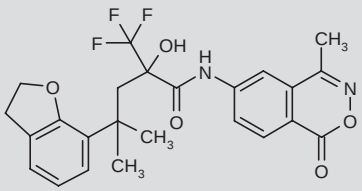
Alitretinoin



Acetyl-11-keto-β-boswelic acid (AkβBA)



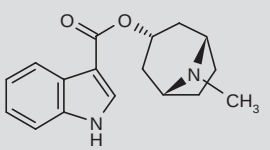
ZK-245186



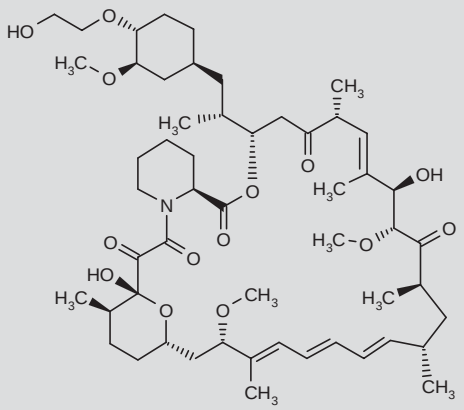
ZK-216348

Data on two low-molecular-weight, nonsteroidal, selective glucocorticoid receptor agonist (SEGRA) compounds from Bayer Schering Pharma (**ZK-245186** and **ZK-216348**) were presented at the meeting. H. Schäcke discussed ZK-245186, which is currently in clinical trials. In vivo, using irritant contact dermatitis and T-cell-mediated contact allergy models in mice and rats, ZK-245186 showed similar anti-inflammatory efficacy after topical application compared to the classical glucocorticoids mometasone furoate and methylprednisolone aceponate. ZK-245186, however, exhibited a superior safety profile with a lower risk for induction of diabetes and thymus atrophy. After long-term topical application, skin atrophy was reduced and lesser effects on the growth of the animals were observed (6). S. Schmittel described ZK-216348, a potent inhibitor of acute inflammation but less harmful to naïve T cells and therefore with reduced nonspecific immunosuppressive effects compared to glucocorticoids (7).

To date, there are no effective treatments for scleroderma. At the meeting four different new therapeutic approaches were presented related to serotonin 5-HT_3 receptor antagonism, the renin–angiotensin system, protease-activated receptors and everolimus. **Tropisetron** is a 5-HT_3 receptor antagonist with antiemetic activity that has also been associated with improvement of symptoms in patients with progressive systemic sclerosis. A. Kokot from the Westfälische Wilhelms-Universität Münster showed that tropisetron inhibited TGF-β1-induced collagen synthesis in human dermal fibroblasts in vitro, but did not affect SMAD 3 phosphorylation, nuclear translocation of SMAD 2/3 or SMAD 3/4-dependent promoter activity. Since the expression of 5-HT_3 receptors was undetectable in human dermal fibroblasts, it was suggested that tropisetron presumably acts in a 5-HT_3 receptor-independent manner (8).



Tropisetron



Everolimus

F. Santi from the Center for Cardiovascular Research, Charité-Universitätsmedizin Berlin, investigated the relevance of the renin–angiotensin system in a mouse model of scleroderma in which female C3/H mice were treated with bleomycin. The fibrotic reaction was ameliorated by an AT_2 receptor agonist and candesartan treatment, which also reduced the expression of precollagen I and TGF-β, as estimated by Western blot (9).

F. Cevikbas from the Westfälische Wilhelms-Universität Münster evaluated the role of protease-activated receptors (PARs) in murine skin fibrosis induced by bleomycin. In wild-type mice a significant increase in dermal thickness, high amounts of collagen accumulation and thickening of vessel walls were observed compared to controls. Furthermore, the sclerotic changes in wild-type mice were characterized by a significant loss of hair follicles. PAR-deficient mice were protected against skin fibrosis. These results suggest that PAR-1 and PAR-2 and their ligands exert profibrotic effects in murine skin. Thus, targeting PAR-1 and PAR-2 signaling in skin fibrosis may be a novel therapeutic approach in scleroderma (10).

M. Böhm from the Westfälisch Wilhelms-Universität Münster presented results that supported a role for **everolimus** in scleroderma. Everolimus is a rapamycin derivative shown to concentration-

dependently suppress basal and basic fibroblast growth factor (bFGF)-induced metabolic activity and proliferation of human dermal fibroblasts in vitro. In a mouse model of bleomycin-induced scleroderma, everolimus suppressed dermal collagen synthesis and fibrosis (11).

A number of abstracts presented at the meeting were related to new biomarkers and exploratory therapies for melanoma. I. Okamoto from the University of Vienna found that functional microsatellite polymorphism of the antiapoptotic heme oxygenase 1 (HO-1) could be a negative prognostic marker for melanoma progression (12).

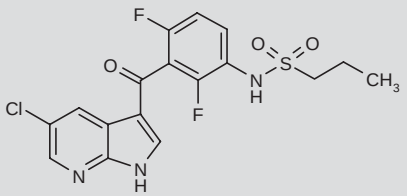
A. Mauerer from the Universität Regensburg identified novel genes associated with malignant melanoma using gene expression profiling of lesions at different stages of melanoma. Decreased expression levels of *FRZB*, an antagonist of Wnt-induced cytosolic accumulation of β-catenin, and of *TLE1* were found. In addition, high levels of expression of two genes that belong to the serine protease inhibitor family, *SERPINB3* and *SERPINB4*, and of *GDF15*, a downstream target of p53, were detected in primary melanoma tissue sections (13).

S. Ugurel from the Universität Würzburg identified serum amyloid A as a serological biomarker in early-stage melanoma patients by proteomic profiling of 569 melanoma patients (14).

An interesting approach to identify targets involved in melanoma progression was presented by J. Schultz from the Department of Dermatology and Venereology (Universität Rostock). He used a genome-wide lentiviral RNA interference (RNAi) loss-of-function screen in metastatic melanoma cells for more than 100,000 different short hairpin (sh) RNAs. The shRNAs of functional relevance for melanoma cell growth and survival were identified and genomically integrated shRNAs were extracted from melanoma cells. Using this method, key signaling pathways/molecules for melanoma cell growth and survival were identified, such as B-Raf kinase, phosphoinositide 3-kinase and integrin-linked kinase, as well as others such as mitogen-activated protein kinase kinase kinase 1 (MEKK 1), Janus kinase 1 (JAK1), cAMP-dependent protein kinase catalytic subunit beta (PKA C-beta) and protein kinase PKC-eta (15).

Novel exploratory strategies for melanoma treatment were also presented. T. Johnson from Heidelberg University studied the activity of a single-chain fragment variable (scFv) antigen fusion protein for the antigen uptake receptor DEC-205, which is expressed exclusively on dendritic cells and fuses to the melanoma antigen gp100. When injected in C57BL/6 mice, scFv-gp100 positively stained dendritic cells in the proper axillary lymph node. Furthermore, the induction of gp100-specific $CD8^+$ T cells examined via ELISPOT interferon gamma assays indicated the superior antigen targeting and presentation of the scFv-gp100 fusion protein via dendritic cells. When tested in a transplantable melanoma tumor model, dose-dependent suppression of tumor growth by scFv-gp100 was found, which indicates that scFv is a viable method of targeting various tumor antigens to dendritic cells in order to elicit a specific immune response to cells that overexpress endogenous antigens (16).

N.K. Haass from the Centenary Institute of Cancer Medicine and Cell Biology (University of Sydney) presented a novel kinase inhibitor with potent and specific activity against melanoma. About 66% of melanomas harbor an activating Raf mutation ($B\text{-}Raf^{V600E}$) that constitutively activates the mitogen-activated protein kinase (MAPK; Ras/Raf/MEK/ERK) pathway in melanoma. Specific inhibition of $B\text{-}Raf^{V600E}$ with **PLX-4720** blocks proliferation exclusively in melanoma cells harboring the $B\text{-}Raf^{V600E}$ mutation and leads to antiproliferative activity in vitro and tumor regression in vivo. The antimelanoma activity of PLX-4720 was also tested in a novel three-dimensional spheroid model, a three-dimensional angiogenesis model and an in vivo model (17).



PLX-4720

NF-kappa-B is required for the induction and maintenance of epithelial–mesenchymal transition, a central process governing both morphogenesis and carcinoma progression in multicellular organisms. M. Huber from the Research Institute of Molecular Pathology in Vienna studied a small-molecule inhibitor highly selective for IKK2, named BI-605700. This molecule significantly limited tumor growth and markedly reduced the metastatic potential of 4T1 cells in vivo after injection into mouse mammary glands. Targeting IKK2 may represent an attractive opportunity for developing novel therapeutics to counteract tumor progression in a broad range of tumors, possibly including metastatic melanomas (18).

Spliceosome-mediated RNA *trans*-splicing (SMaRT) constitutes a new gene therapy approach for the correction of large genes using endogenous splicing machinery to recombine a target cellular pre-mRNA and a pre-*trans*-splicing molecule, or PTM by *trans*-splicing, or replacing the disease-causing parts of a gene with their wild-type copy. Current gene therapy efforts are presently focused on the transfer of wild-type cDNA into affected cells, which is still associated with a number of technical challenges. At the meeting, several abstracts related to the SMaRT approach for genetic skin diseases were presented by investigators at the Laboratory for Molecular Therapy, Department of Dermatology (University Hospital Salzburg). The long-term goal of these studies is to provide an ex vivo gene therapy in which skin grafts taken from patients are transfected with specific PTMs and are then retransplanted to the patients.

V. Wally presented SMaRT for a mutation in the *KRT14* gene responsible for different types of the blistering skin disease epidermolysis bullosa simplex (EBS), which is mostly inherited in an autosomal dominant manner. A screening method based on fluorescence molecules to identify highly functional PTMs, differing in their binding domain specific for intron 7 of the *KRT14* gene, was presented. Identified isolated PTMs showed a high *trans*-splicing efficiency in HaCat cells, revealing specific *trans*-splicing into exon 8 of the endogenous *KRT14* gene (19).

E.M. Murauer focused on *COL7A1,* a gene responsible for functional defects in type VII collagen that lead to the inherited blistering skin

disorder dystrophic epidermolysis bullosa (DEB). Primary and immortalized keratinocytes from a recessive DEB patient carrying two heterozygous nonsense mutations in *COL7A1* exons 14 and 104 that provoke collagen VII deficiency were retrovirally transduced with a 3'-PTM encoding *COL7A1* wild-type exons 65-118. The retroviral transduction of the cells resulted in correction of the 3'-portion of the *COL7A1* transcript via *trans*-splicing (20).

Finally, U. Koller developed a high-throughput screen for finding the most specific and efficient PTMs to optimize gene correction for the plectin (*PLEC1*) gene in patients suffering from EBS (21).

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