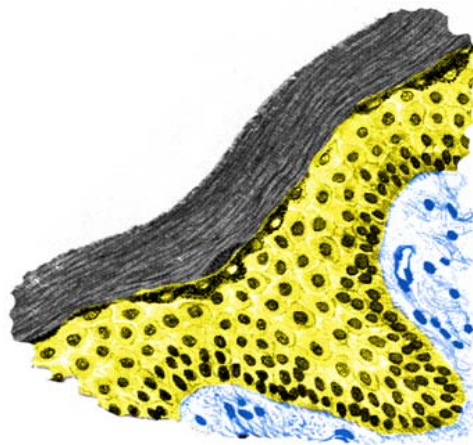


Symposium

“Keratinocytes – Proliferation and Differentiation in the Epidermis”

Lectures



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Role of keratinocytes and cutaneous dendritic cells for the induction of antigen-specific immune responses in the skin using genetic immunization strategies

Thomas Tüting

Laboratory of Experimental Dermatology, Department of Dermatology, University of Bonn

The skin is the largest immune organ of the body and therefore represents an ideal target for vaccine strategies. Various mechanisms of innate as well as adaptive immunity provide protection against diverse pathogens. Antigen-specific skin-homing CD8⁺ cytotoxic T lymphocytes (CTL) are particularly important for the defense of cutaneous viral infections and for immune-mediated destruction of skin tumors. However, effector functions of CD8⁺ CTL in the skin must be tightly regulated because the epidermis is continuously exposed to many potentially immunogenic proteins derived from microbes, animals and plants. Inappropriate activation of CD8⁺ CTL against harmless foreign or self antigens may lead to allergic contact dermatitis or autoimmune skin disease. The experimental development of cutaneous DNA vaccination has contributed significantly to the understanding how CD8⁺ T cell responses are induced in the skin. We have intensively investigated direct in vivo transfection of the epidermis with the "gene gun". This gene transfer method promotes antigen expression predominantly in keratinocytes and leads to activation of dendritic cells, which migrate to the draining lymph nodes and stimulate cellular as well as humoral immunity. In direct comparison we have employed intracutaneous injection of cultured, antigen-transduced DC derived from bone marrow precursors which results in strong stimulation of cellular immunity in the absence of a significant humoral immune response. Our experimental system provides an ideal opportunity to further investigate the mechanisms governing the induction and regulation of cytotoxic cellular immunity in the skin which may ultimately lead to improved cutaneous genetic immunization strategies.

First insights into mechanisms leading to the migration of LC precursors into the skin.

Thomas Bieber

Dept. of Dermatology, University of Bonn, Germany.

Precursors of dendritic cells (DC) are supposed to be myeloid cells transported via the blood stream, which migrate into tissues, where they differentiate to DC, most probably following locally released chemotactic signals.

To study immigration of human Langerhans cell (LC) precursor cells into the skin, we established a three-compartmental skin model consisting of an endothelial cell layer, a dermal matrix and an epidermal sheet of keratinocytes. We tested the individual components of the skin model for their influence on phenotype and function (transendothelial migration, pinocytosis and activation of T cells) of LC precursors, which are paradigmatic DC from the epithelial layer in the skin. LC precursors were generated *in vitro* from monocytes (monocyte-derived DC, MoDC) or from CD34^{pos} stem cells (CD34^{pos} cell-derived DC, CD34DC). Four DC precursor subpopulations were characterized by their differential expression of the monocytic marker CD14 and the DC marker CD1a. The expression of chemokine receptors CCR1, CCR2, CCR6 and CX3CR1 was monitored at distinct time points during differentiation and allowed discrimination of monocytes from peripheral blood and CD14^{pos}/CD1a^{neg} CD34DC. Both precursor subtypes expressed the alpha integrins LFA-1, Mac-1, CR4, VLA-4, VLA-5 and the beta 2 integrin CD18. CD34DC and MoDC were negative for VLA-3, whereas MoDC, but not CD34DC expressed VLA-6. Most importantly, in transwell assays, migration of MoDC and CD34DC was differentially enhanced by supernatants from endothelial cells, from fibroblasts and from keratinocytes. CD14^{pos}/CD1a^{neg} cells migrated strongest towards most chemoattractants. These results suggest that endothelial cells, fibroblasts and keratinocytes secrete chemoattractants for LC precursors, which direct them stepwise to the dermal and epidermal compartments, respectively. Thereby, the LC pool in the epidermis, which may be depleted under circumstances like UV exposition or after inflammation, is replenished. In order to further precise the behaviour and differentiation of these distinct subpopulations, CD34DC subpopulations at day 7 of culture were sorted according to their expression of CD14 and CD1a and then recultured in the presence or absence of GM-CSF or TGF- β 1. Thereby, both cytokines induced the CD14^{neg}/CD1a^{neg} DC and the CD14^{pos} population to become CD14^{pos}/CD1a^{pos} cells. We propose that the CD14^{pos} subpopulation is related to the monocytic precursors transported in the blood stream. The CD14^{pos}/CD1a^{pos} subpopulation could mimic cells that had already immigrated into the dermal tissue. CD1a^{pos}/CD14^{neg} cells, which are partly Langerin/CD207 positive, reflect epidermal Langerhans cells.

TGF-beta / activin signaling in epidermal homeostasis

Manfred Blessing

Biotechnologisch-Biomedizinisches Zentrum, Universität Leipzig

Members of the Transforming Growth Factor-beta (TGF-beta) superfamily of signalling molecules mediate inductive tissue interactions during development as well as regulation of cellular functions in adult organs especially during regeneration. Intracellular signal transduction occurs *via* the Smad pathway. Signals from BMP and GDF receptors are transmitted by Smad1, Smad5 and Smad8 while signals from TGF-beta and activin receptors are transmitted by Smad2 and Smad3. Smad6 and Smad7 are inhibitors of the Smad signalling pathway with a specificity for the TGF-beta/activin pathway in the case of Smad7 and a preference for the BMP pathway in the case of Smad8. In order to discriminate between TGF-beta and activin in keratinocytes *in vivo*, we specifically interrupted TGF-beta signalling by epidermal expression of a dominant negative type II receptor and TGF-beta/activin signalling by epidermal expression of Smad7. Interruption of TGF-beta signalling alone in keratinocytes did not alter development or maintenance of skin in unchallenged transgenics. However, upon tumor initiation, skin tumorigenesis was increased and upon wounding, reepithelialization was accelerated in these animals. By contrast, interruption of both TGF-beta and activin signaling in keratinocytes resulted in lethality within a few weeks after birth. Upon conditional interruption of both TGF-beta and activin signalling in keratinocytes of adults, severe abnormalities of skin and hair was noted. Notably, a strong hyperproliferation of keratinocytes as well as hyperkeratosis in conjunction with a severe decrease of Smad3 levels could be demonstrated. Thus, TGF-beta signaling alone is not essential for the maintenance of epidermal homeostasis in unchallenged skin, whereas activin signalling is crucial for the regulation of epidermal cell proliferation and differentiation in adult skin.

The multi-step process of skin carcinogenesis: genetic analysis and functional consequences

Petra Boukamp

German Cancer Research Center (DKFZ), Heidelberg

Skin cancer is dependent on the accumulation of genetic changes. These can either be induced as point mutations (oncogene activation - tumor suppressor gene inactivation) of specific genes or by gross aberrations (aberrant karyotype) such as loss or gain of chromosomal parts or translocation chromosomes. While in skin cancer point mutations are thought to be UV-B-dependent (p53 tumor suppressor gene, Ha-ras oncogene), mechanism causing chromosomal changes are still poorly understood and may in part be induced by length-dependent and -independent telomere-related mechanisms. To unravel the genetic changes involved in skin carcinogenesis we first compared keratoacanthomas (KAs), a benign skin tumor, and the malignant skin squamous cell carcinomas (SCC) by comparative genomic hybridisation (CGH). This genetic screening approach allowed us to identify distinct CGH profiles for KAs and SCCs and subsequently demonstrated specific changes related to the different stages of skin carcinogenesis. These changes were further analysed for defined genes and verified by their respective expression analyses. Finally, we performed functional analyses of a very specific lesion, namely gain of 11q13, a frequent though early (still compatible with spontaneous regression of the KAs) event. We now show that gain of 11q13 was correlated with amplification of the cyclin D1 gene locus and up-regulation of cyclin D1 protein in many tumors. Surprisingly, the phenotype of cyclin D1 up-regulation in HaCaT skin keratinocytes was not, as expected from a cell cycle regulatory gene, a prominent increase in proliferation but a reduced and altered differentiation and interaction with its environment thus, supporting the various interactions of the changes occurring during and required for the multi-step process of skin cancer development and progression.

Significance of cathepsins B, L, and V for migration and proliferation of keratinocytes.

Klaudia Brix

International University Bremen

Regeneration from epidermal wounding comprises migration of keratinocytes for rapid wound closure and proliferation for re-epithelialisation. We used an *in vitro*-model of scratch-wounded HaCaT keratinocytes to elucidate cellular responses and the significance of cathepsins during such regeneration processes. Within the first hour after wounding, cathepsin B-containing vesicles of HaCaT keratinocytes scattered towards the cell periphery and the protease was secreted into the extracellular space. Cathepsin B associated with surfaces of migrating HaCaT cells by binding to the scavenger receptor LRP1. Inhibition of the proteolytic activity of cathepsin B resulted in impaired HaCaT cell migration, indicating cathepsin B's function as an extracellular matrix-remodelling enzyme. Cathepsin L was also detected within vesicles of HaCaT cells, but its localization did not change after scratch-wounding. Furthermore, its expression was unaltered during regeneration, demonstrating that cathepsin L is less important for regeneration during wound healing. In clear contrast, expression of the closely related cysteine peptidase cathepsin V was up-regulated after about 6 hours, shortly before the scratch-wounded HaCaT keratinocytes started to proliferate. Astonishingly, cathepsin V was not detected within vesicles, but it was localized to circumscribed subnuclear structures. The distinct localization patterns of all three cathepsins were verified in normal human skin keratinocytes. We conclude that cathepsin B serves extracellular functions in that it contributes to matrix remodelling to enable keratinocyte migration, whereas cathepsins L and V are more important intracellularly. The unexpected and novel finding of cathepsin V within nuclei of HaCaT cells may thus point to its tasks in regulation of keratinocyte proliferation.

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Molecular analysis of the tumor suppressor function of Cyld

Reinhard Fässler

MPI Biochemie, Martinsried

Mutations in the *CYLD* gene cause tumors of hair follicle keratinocytes. The *CYLD* gene encodes a deubiquitinase that removes lysine-63-linked ubiquitin chains from TRAF-2 and inhibits p65/p50 NF- κ B activation. To analyze *Cyld* function *in vivo* we generated mice lacking *Cyld* and found that they are highly susceptible to chemically-induced skin tumors. *Cyld*^{-/-} tumors and TPA- or UV light-treated keratinocytes are hyperproliferative and have elevated cyclin D1 levels. The cyclin D1 elevation was not caused by increased p65/p50 action, but rather by increased nuclear activity of Bcl-3-associated NF- κ B p50 and p52. In *Cyld*^{+/+} keratinocytes, TPA or UV light triggers the translocation of *Cyld* from the cytoplasm to the perinuclear region where *Cyld* binds and deubiquitinates Bcl-3 thereby preventing nuclear accumulation of Bcl-3 and p50/Bcl-3- or p52/Bcl-3-dependent proliferation. These data indicate that depending on the external signals *Cyld* can negatively regulate different NF- κ B pathways; inactivation of TRAF-2 controls survival and inflammation, while inhibition of Bcl-3 controls proliferation and tumor growth.

The Tight Junction Systems of Stratified Epithelia and Carcinomas Derived Therefrom

Werner W. Franke

German Cancer Research Center (DKFZ), Heidelberg

In recent years we have noticed that – in contrast to common textbook dogmata – multi-stratified epithelia also contain tight junction (TJ) proteins and form extended *zonula occludens* systems, usually located in the uppermost living cell layer of the specific tissue such as the epidermal *stratum granulosum*, including hair follicle cell layers. We report compositional principles and structural variants of TJs in diverse mammalian stratified epithelia as well as in special tissue elements derived therefrom such as the Hassall bodies of thymus. In addition, we show that many diverse squamous cell carcinomas contain regions characterized by circumference borderlines represented by TJ-like structures, and we follow the advent of TJ proteins and structures in the formation of stratified epithelial cell layers in vitro. Moreover, we present compositionally TJ-related novel structures in various subapical layers such as the *stratum spinosum* of epidermis, and in stratified cell layers formed in dense cell cultures. In particular we describe the "lamellated" and the "sandwich" junctions as well as – for the first time – *puncta occludentia* abundant in interdesmosomal regions, and we show the immunolocalization of the TJ-system completing tetraspan membrane protein, tricellulin, in stratified growth forms of certain epithelial cells. Possible functions in normal epithelia as well as function-oriented and therapy-relevant phenomena in carcinomas are discussed.

Epidermal Tissue Homeostasis in Skin Equivalents

Norbert E. Fusenig,

German Cancer Research Center (DKFZ), Heidelberg

Epidermal tissue homeostasis has been defined as the maintenance of tissue integrity by sustaining a constant epidermal cell pool balancing the rate of mitosis and the rate of cell loss by desquamation and apoptosis. In a broader sense, epidermal tissue homeostasis is understood as the maintenance of normal tissue structure and function. As far as human skin is concerned, the lack of appropriate experimental models has largely prevented a better understanding of the regulatory mechanisms maintaining and controlling epidermal tissue homeostasis. Following an initial phase of keratinocyte research directed towards their isolation, cultivation and functional characterization, it soon became apparent that normal epidermal differentiation only occurred in a tissue context and was dependent on the presence of dermal tissue or isolated fibroblasts. Despite the striking success of the feeder layer technique to grow human keratinocytes normal keratinisation was only occurring in 3D tissue models. Such organotypic cocultures with keratinocytes growing on collagen gels populated with fibroblasts were suitable models to reproduce major features of epidermal regeneration to an organized epithelium. In these in vitro skin equivalents basic mechanisms of epithelial-stromal interactions in controlling keratinocyte proliferation could be elucidated, whereas regulatory mechanisms controlling tissue homeostasis remained largely obscure. This was among others due to the lack of longevity in the conventional organotypic cocultures which tended to shrink and degenerate after two weeks. Recently we succeeded in establishing an optimized skin equivalent model by growing keratinocytes on a 3D scaffold in which preseeded fibroblasts synthesized an authentic matrix. Under such coculture conditions epidermal regeneration occurs faster than on collagen gels including formation of a basement membrane. Moreover, epidermal cell proliferation and tissue organization was more regular and sensitive differentiation markers as well as keratinocyte proliferation normalized with culture times up to 12 weeks. Whereas authentic dermal equivalents control keratinocyte proliferation and differentiation, fibroblast proliferation and production of ECM components on the other hand is regulated by the epithelial compartment. Thus, this advanced model of skin equivalents exhibits features of epidermal homeostasis demonstrating mutual epidermal-dermal control mechanisms and thus will be suited to study molecular mechanisms controlling tissue homeostasis.

Regulation of repair and defence functions in epidermal keratinocytes

Ingo Haase

University of Cologne, Dermatology

Epidermal keratinocytes cover the surface of the organism and build a protective shield that is essential for life, the epidermis. If this first line of defence is broken, mechanisms are activated in keratinocytes that both stimulate its quickest possible repair and send signals of danger to cells of the innate immune system in order to activate the immune defence machinery. We are investigating such repair and defence mechanisms in epidermal keratinocytes and are particularly interested in the signalling pathways that control them. We believe that aberrant activation of these pathways could be involved in the pathogenesis of hyperproliferative- inflammatory and tumourous skin disease. Using dominant negative and constitutively active mutants of the small GTP binding protein Rac1 we are analysing its functions in the maintenance and repair of the epidermis as well as in tumour formation. We can show that inhibition of Rac function by expression of N17Rac1 as a transgene under the control of the keratin 14 promoter in the basal epidermal layer of mice results in a delay of wound re- epithelialization in vivo. This defect is most likely due to disturbed lamellipodia dynamics which result in impaired keratinocyte migration. In addition, repression of Rac function in basal epidermal keratinocytes inhibited their proliferation without having effects on epidermal differentiation. Both mechanisms can account for the observed delay in wound healing. In order to further characterise the role of keratinocytes in immune defence we are analysing functions of components of the NF κ B signalling pathway in the epidermis. Using mice with conditionally targeted genes we can show that modulation of NF κ B signalling in epidermal keratinocytes leads to the development of an inflammatory- hyperproliferative skin disease by the recruitment of immune cells into the skin. Inhibition of NF κ B signalling by targeted, epidermis specific deletion of I κ B kinase 2 (IKK2) results in an inflammatory reaction that is independent of $\alpha\beta$ T cells and granulocytes but depends on the presence of macrophages in the dermis and on the presence of TNF receptor I. We are currently working to understand relevant mechanisms of interaction between keratinocytes and other cell types participating in this innate immune response.

The biological role of the amyloid precursor protein family and of its soluble N-terminal form in the epidermis

Volker Herzog,

University of Bonn, Institute of Cell Biology

In recent years we focussed our research on the biological role of the amyloid precursor protein (APP) family in the epidermis where APP and the amyloid precursor-like protein APLP2 are strongly expressed in keratinocytes of the basal layer but also in melanocytes. We have shown that full length APP is a marker protein for melanocytes and melanoma cells and that APP facilitates keratinocyte adhesion to the extracellular matrix. A major biological role, however, is related to sAPP α , the soluble N-terminal form of APP and of APLP2. We have shown that sAPP α facilitates cell adhesion similar to full length APP and strongly stimulates keratinocyte proliferation and migration and the exocytic release of melanin from melanocytes. Pharmacologically induced absence of sAPP α (1) and genetically based deficiency of APP and APLP2 (2) support these initial observations: 1) The release of sAPP α can almost completely be blocked by inhibition of α -secretase by the use of hydroxamic acid-based zinc metalloproteinase inhibitors resulting in strongly reduced proliferation and migratory velocity. In hyperproliferate keratinocytes from human psoriatic skin this inhibition results in normalized growth. 2) Mice genetically deficient in APP and APLP2 die shortly after birth but do not display a specific epidermal phenotype. Keratinocytes in APP/APLP2-deficient mice show reduced proliferation in vivo and, additionally, reduced cell substrate adhesion and migration in vitro, which could be completely rescued by exogenously added recombinant sAPP α or by co-culture with fibroblasts. We consider these data as growing evidence for the notion that sAPP α represents an essential epidermal growth factor fostering keratinocyte proliferation, migration and adhesion as well as regulating melanocyte function.

Clinical and experimental models for elucidating the roles of gap junctions and connexins in the epidermis

Malcolm B. Hodgins

University of Glasgow, Dermatology

Keratinocytes in the epidermis, and in other stratified epithelia, are connected by an extensive network of gap junctions. During embryonic development of skin, it appears that there is a degree of separation between gap junctional “communication compartments” within the epidermis and the developing appendages. Although the significance of such compartmentation remains unclear, its structural basis probably arises from the overlapping patterns of expression of the multiple connexin proteins that constitute gap junctions within the epidermis and appendages. Some clues to the functions of individual connexins in the epidermis are provided by inherited disorders in which connexins (e.g. connexins 26, 30, 31) are mutated. This will be illustrated by our clinical and experimental studies of families carrying rare mutations of connexin 26 and by our studies of transgenic mice that express these mutants in the epidermis.

Epidermal barrier development and sphingolipid metabolism

Walter Holleran

VA Medical Center, San Francisco

Ceramides (Cer) are not only critical for epidermal barrier function, but also can inhibit proliferation and induce apoptosis in keratinocytes. In contrast, other metabolic products of Cer, including glucosylceramides and sphingosine-1-phosphate, can exert either pro-mitogenic or anti-apoptotic effects, suggesting that the relative levels of these and other sphingolipid metabolites must be tightly regulated in the epidermis. Thus, we investigated the mechanisms that mammalian epidermis employs to counteract the potentially detrimental effect(s) of inappropriate elevations of key sphingolipid metabolites. First, we demonstrate that up-regulation of GlcCer synthesis represents a protective mechanism(s) against Cer-induced stress in cultured human keratinocytes. Second, we reveal the involvement of *de novo* Cer synthesis in UVB-induced keratinocyte apoptosis. This Cer-dependent cell death pathway appears to operate independently of the classical caspase-3 activation pathway. Finally, we investigate the role of ceramidase (CDase) activity in the keratinocyte response to UVB-induced, Cer-dependent cell death. Although human keratinocytes/epidermis express all five known CDase isoforms, only acidic- and alkaline-CDase isoforms increase significantly with differentiation, and both of these activities are involved in the protection of keratinocytes from UVB-induced apoptosis. Together, these results reveal that Cer is an effector of epidermal oxidative stress responses, and keratinocytes deploy metabolic protective mechanisms against Cer induced-apoptosis in response to a key physiologic oxidative stressor, UVB. Given that apoptosis is implicated in numerous cutaneous diseases, including eczematous dermatitis, lichen planus, atopic dermatitis, and lupus erythematosus, it will be important to determine the contributions of sphingolipid metabolic products to the progression of these distinct cutaneous disorders.

A novel role of epidermal keratins in melanosome transport and distribution

Thomas M. Magin

University Bonn, Institut für Physiologische Chemie

Skin melanosomes are lysosome-related organelles synthesized in melanocytes and present a paradigm for the mechanisms involved in vesicle transport and positioning. Melanosome transport in melanocytes is mediated by Rab27 GTPases which regulate melanosome interaction with kinesin and myosin V motors through distinct Rab effectors. While melanosome transport in melanocytes is relatively well understood due to diseases including Griscelli and Hermansky-Pudlak syndromes, mechanisms responsible for their uptake into keratinocytes and intra-keratinocyte distribution are largely unknown. We have identified loss-of-function mutations in the keratin 5 gene in patients suffering from Dowling Degos disease, a keratinocyte pigmentation disorder characterized by epithelial downgrowth and disorganized distribution of melanosomes. Our data suggest a crucial role for keratins in the organization of cell adhesion, melanosome uptake, organelle transport and nuclear anchorage.

Lipid Binding and Transfer Proteins in the Epidermis

Natascha Remmel, Silvia Locatelli Hoops, Konrad Sandhoff

University of Bonn, Kekulé-Institut für Org. Chemie und Biochemie

The epidermal permeability is maintained by extracellular lipid membranes within the interstices of the stratum corneum. Ceramides, the major components of these multilayered membranes, derive in large part from hydrolysis of glucosylceramides mediated by stratum corneum β -glucocerebrosidase. Prosaposin (pSap) is a large precursor protein that is proteolytically cleaved to form four distinct saposins, which stimulate enzymatic hydrolysis of sphingolipids. Deficiency of pSap in the epidermis of knock out mice results in an accumulation of glucosylceramides and in a striking abnormality in the lamellar membrane maturation within the interstices of the stratum corneum: Extruded lamellar body contents retain a spherical pattern in the interstices, suggesting a defect in lipid binding and mobilization activity. Using liposomes and surface plasmon resonance spectroscopy we tested the lipid binding and mobilization capacity of recombinant and human saposins A and B. Both, saposins A and B, were able to bind lipids and mobilized them at acid pH values. Anionic lipids and low cholesterol levels stimulate their lipid mobilization capabilities. Variant saposins, A and B, missing either a glycosylation site or a disulfide bridge were ineffective.

The pathogenic impact of laminin-5 deficiency

Holm Schneider

University of Erlangen-Nuernberg, Nikolaus Fiebiger Centre of Molecular Medicine

The basement membrane glycoprotein laminin-5 is a key component of the anchoring complex connecting keratinocytes to the underlying dermis. It is secreted by keratinocytes as a cross-shaped heterotrimer of alpha3, beta3 and gamma2 chains and serves as a ligand of various transmembrane receptors, thereby regulating keratinocyte adhesion, motility and proliferation. In intact skin, laminin-5 provides essential links to both the hemidesmosomal alpha6beta4 integrin and the collagen type VII molecules which form the anchoring fibrils inserting into the dermis. If the basement membrane is injured, laminin-5 production is increased, generating leading keratinocytes that migrate via beta1 integrins. Laminin-5 then serves as a scaffold for cell migration, initiates the formation of hemidesmosomes and accelerates basement membrane assembly at the dermal-epidermal junction. The major contributions of laminin-5 to the resistance of the epidermis against frictional stress, but also for the regeneration of the epidermal basement membrane and the repair of damaged skin, are reflected by the phenotype of Herlitz junctional epidermolysis bullosa, which is caused by absence of functional laminin-5. This lethal disease becomes manifest in widespread blistering of skin and mucous membranes, impaired wound healing and chronic erosions. Here, we discuss current understanding of the pathogenic impact of laminin-5 deficiency and its implications on molecular approaches to the treatment of junctional epidermolysis bullosa.

Chemotactic movement of dendritic cells within the skin

Michael Sixt

MPI of Biochemistry, Martinsried

The current paradigm of cell migration involves receptor mediated adhesion at the leading front of the cell followed by contraction of the cell body and subsequent release of the trailing edge. Integrins are considered to be essential for migration as they are the main receptors that connect the cytoskeleton of the migrating cell with proteins of the extracellular matrix or cellular counter-receptors. Antibody blocking studies, gene targeting approaches and the study of human diseases have definitely shown that integrins are essential for immune cell trafficking. However, integrin ligand binding and the signaling pathways that are subsequently triggered can affect a number of cellular functions (differentiation, polarization, activation, survival, adhesion, retention, positioning) other than migration and it is currently unclear how essential integrins are for the directed migration of leukocytes in the three dimensional space of the interstitium or the parenchyma of secondary lymphatic organs. We use dendritic cell migration within the skin as a model system to study chemokine mediated leukocyte migration through the interstitium and challenge the concept that integrins are essential for this process.

Epithelial or mesenchymal – ROCK determines the mode of migration by modulating substrate adhesion

Robert Torka

University of Bonn, Institute of Cell Biology

Two different strategies of cell migration, the mesenchymal and the epithelial mode, are currently being discussed. The ability of human mammary epithelia cells (HUMEC) to switch from the epithelial to the mesenchymal mode to become highly invasive tumour cells (MDA-MB231) makes them a suitable model system. Here we show by employing high resolution 2D-migration assays that MDA-MB231 cells exhibited a significantly reduced migration velocity as compared to HUMECs. This reduced migration was apparently due to significant differences in actin organisation and a reduced turnover of cell-substrate contacts, which resulted from a 2.5-fold higher activity of the Rho-kinase ROCK and were mediated by its downstream effectors myosin light chain kinase and cofilin. Thus, inhibition of ROCK activity caused a marked increase in 2D migration efficiency by decreasing the adhesion potential of MDA-MB231 cells. Vice versa, migration of MDA-MB231 cells was significantly reduced on extracellular matrix of normal human mammary epithelia cells (HUMEC-MATRIX) in a ROCK-dependant mechanism. In conclusion, our results show the epithelial mode of migration is limited by lamellipodia persistence due to the formation of focal contacts at the leading edge, whereas the mesenchymal mode depends on the efficient turnover of cell-substrate adhesion regulated by ROCK.

Consequences of targeted mutation or ablation of connexins expressed in mouse epidermis

Klaus Willecke

University of Bonn, Institute of Genetics

Several human genodermatoses are caused by defective connexin genes and are in most cases autosomal dominantly inherited. One of them is Erythrokeratoderma variabilis (EKV) whose symptoms vary from transient fast moving erythema to persistent brown hypokeratoses. We have introduced the human EKV causing mutation, connexin31F137L, into the mouse connexin31 gene, in order to generate a mouse model for EKV. First characterization of this mouse will be reported (see abstract by Schnichels et al., this meeting). In addition, we have generated Cx30.3 and Cx31.1 deficient mice each of which express a lacZ reporter gene instead of the corresponding connexin coding DNAs. Although both genes are expressed in the suprabasal layers of the epidermis, no skin abnormalities have been noticed so far in homozygously defective animals. Instead, we found that the lacZ reporter genes in these mice are expressed in the olfactory epithelium and vomeronasal organ, which may reveal a new function of connexins (see abstract by Zheng-Fischhöfer et al., this meeting).

