Histochem Cell Biol (2009) 132:1–10
DOI 10.1007/s00418-009-0586-0

REVIEW

Basement membranes in skin: unique matrix structures with diverse functions?

Dirk Breitkreutz · Nicolae Mirancea · Roswitha Nischt

Accepted: 6 March 2009 / Published online: 31 March 2009

Abstract The view of extracellular matrix (ECM) has evolved from a merely scaffolding and space filling tissue element to an interface actively controlling cellular activities and tissue functions. A highly specialized form of ECM is the basement membrane (BM), an ubiquitous sheet-like polymeric structure composed of a set of distinct glycoproteins and proteoglycans. In this review we are largely focusing on function and assembly of BM in skin (1) at the dermo-epidermal interface and (2) in the resident microvasculature. The role of the non-polymeric components perlecan and particularly nidogen is exemplified by reviewing experiments based on genetic approaches and adequate experimental skin models in vivo and in vitro. While in mice total deficiency of one of these components is eventually developmentally lethal, the severity of the defects varies drastically between tissues and also the skin models recapitulating BM formation in vitro. There is accumulating evidence that this relies on the mechanical properties, the molecular composition of the BM, the adjacent ECM or connective tissue, the dynamics of molecular assembly, and 'minor' tissue-specific modifier or adapter components. Though the role of nidogen or perlecan is still remaining a controversial issue, the statements 'being essential for BM/or not' should be consequently referred to the developmental, tissue, and functional (e.g., repair) context.

Keywords Dermo-epidermal junction · Basement membrane · Molecular composition · Molecular assembly · Nidogen function · Organotypic co-culture · Micro-vasculature · Ultrastructure

D. Breitkreutz (✉)
DGZ (B 050) Rm 145, German Cancer Research Center (DKFZ),
Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
e-mail: dirkbtz@gmx.de

N. Mirancea
Institute of Biology, Romanian Academy,
Bucharest, Romania

R. Nischt
Department of Dermatology, University of Cologne,
Cologne, Germany

Introduction

The skin is protecting the organism against physical, chemical, and microbial impacts of the environment. Serving multiple other functions, it represents our second largest organ only surpassed by the vascular system. The skin consists of two principal compartments, the epidermis and dermis, which communicate in various ways and at different levels to establish, maintain, and restore homeostasis. The vital barrier function of skin is provided by its upper compartment, the epidermis which represents a stratifying, keratinizing epithelium (Fuchs and Raghavan 2002). Thus, special lipids and tight junctions between cells in upper layers prevent penetration or loss of water (Niessen 2007) and cornified envelopes composed of a highly cross-linked protein coat warrant chemical resistance (Eckert et al. 2005; Huber et al. 2005; Sevilla et al. 2007). The mechanical strength within the epidermis relies on a dense meshwork of intracellular keratin filaments interconnected between neighboring cells by epithelia-specific junctions, the desmosomes. In contrast, the tensile strength and elasticity in the dermis underneath is determined by extracellular matrix (ECM) composed of type I and III collagen fibrils, microfibrils, and elastic fibers, embedded in 'ground substance' mainly composed of proteoglycans. Both skin compartments cooperate in the formation of a highly specialized

matrix structure, the basement membrane (BM), which physically separates the two compartments providing a stabilizing as well as dynamic interface.

Being ubiquitous in the body, BMs underlie all epithelia and endothelia, envelop nerves, muscle fibers, distinct cell compartments, and whole organs. Besides structural properties, the dermo-epidermal BM has gate-keeping functions strictly controlling the traffic of cells and bioactive molecules in both directions. On the other hand, the BM is able to bind a variety of cytokines and growth factors, thus representing a reservoir for the controlled release during physiological remodeling or repair processes (Iozzo 2005). In pathologic situations like inflammation or cancer the enhanced release of factors due to BM destruction contributes to the activating stroma reaction (Müller and Fusenig 2004). Crucial are the altered interactions of epithelial cells in wounds or invading tumors with different, newly accessible ECM molecules and/or proteolytic fragments in the neighboring stroma. The epidermal cell matrix interactions are mainly mediated by integrins, a large family of hetero-dimeric transmembrane proteins (Watt 2002). Generally, integrins are associated with the actin-microfilaments, allowing both cell adhesion and migration. Only integrin $\alpha6\beta4$ is connected to keratins, at least when integrated into hemidesmosomes, the firm epidermal adhesion points to the BM (Litjens et al. 2006; Wilhelmsen et al. 2006). In the quiescent state integrins display a polar distribution, with integrins $\alpha2\beta1$, $\alpha3\beta1$ decorating the entire basal cell surface and $\alpha6\beta4$ mainly the ventral site. Contrarily, in wounds or tumors the patterns expand, reflecting a drastic reduction in cell and tissue polarity (Tennenbaum et al. 1993; Tomakidi et al. 1999).

Molecular structure of basement membranes

Determining their common structure, BMs contain four main components, i.e., at least one member of the protein sub-/families laminin, type IV collagen and nidogen, and the proteoglycan perlecan (Timpl and Brown 1996), while differential expression of those isoforms gives rise to tissue-specific functional diversity (Miner and Yurchenco 2004; Aumailley et al. 2005; Iozzo 2005; LeBleu et al. 2007). Being responsible for overall structure, laminin and collagen IV molecules form independent networks. The self-assembly of laminin occurs via the 'sticky' N-terminal globular domains of the α, β, and γ chains by non-covalent (reversible) bonds. The main cell binding site is located at the C-terminal globular end of the long arm of the α chain (Miner 2008). Contrarily, collagen IV molecules are covalently cross-linked by disulfide and chemically resistant bonds at their non-collagenous C- and globular N-terminus, forming a 'chicken wire'-like meshwork. Accordingly, the mechanical stability of the BM largely depends on this collagen IV scaffold (Pöschl et al. 2004), while the initial development of a BM in vivo requires the presence of laminin (Smyth et al. 1999; Miner et al. 2004). The components perlecan and nidogen do not form polymers but are integrated by their multiple binding sites into the laminin/collagen IV scaffold (Fox et al. 1991; Battaglia et al. 1992; Aumailley et al. 1993). The stringency of molecular requirements seems to differ in organ development, complete deficiency being lethal for perlecan at midgestation of mouse embryos (Arikawa-Hirasawa et al. 1999; Costell et al. 1999) and for nidogen at birth (Bader et al. 2005). While perlecan implements a strong negative charge in the BM due to its three heparan sulfate side chains, nidogen has been classically considered as a cross-linker mainly between BM-laminin and collagen IV with pronounced high affinity to the laminin $\gamma1$ chain (Aumailley et al. 1993; Pöschl et al. 1994). Regarding the BM ultrastructure (Fig. 1) seen by conventional transmission electron microscopy (EM; standard fixation), these polymers form the body of the *lamina densa* below the translucent *lamina lucida*, which has been confirmed by immune EM applying epitope-specific antibodies (McMillan et al. 2003). It should be noted that the *lamina lucida* virtually disappears using cryo-preservation/fixation for EM sample processing, reflecting a flexible or pliable submolecular structure making this interface less rigid. In the hemidesmosomes, which anchor the epidermis to the BM, intracellular plaque proteins link the keratin filaments to the transmembrane proteins integrin $\alpha6\beta4$ and collagen XVII (synonym BP180, BPAG2; Franzke et al. 2005). The extracellular part of $\alpha6\beta4$ binds laminin-332 (laminin-5; Aumailley et al. 2005), which forms with the extracellular collagen XVII domain the anchoring filaments, the bridge to the *lamina densa* (spanning the *lamina lucida*). Herein laminin-332 gives the most prominent example for adapting molecular ECM functions by sequential proteolytic processing (Fig. 1e). Initially, the genuine laminin-332 molecule favors keratinocyte migration, the cleavage of distinct modules will then contribute to strong and stable adhesion, while further truncation promotes again cell motility (for comprehensive review: Miner and Yurchenco 2004; Schneider et al. 2007; Sugawara et al. 2008). To the dermis underneath, the BM is connected by anchoring fibrils (loop structures of collagen VII) interwoven with lattices formed by collagen I and III fibrils (Villone et al. 2008). These adhesion complexes are crucial for the overall structural and functional integrity of the skin (McMillan et al. 2003). Thus, inherited or acquired defects in many of those molecules of the dermo-epidermal BM zone result in devastating or lethal blistering diseases (Bruckner-Tuderman 1999; Aumailley et al. 2006; Chaudhari and Marinkovich 2007).

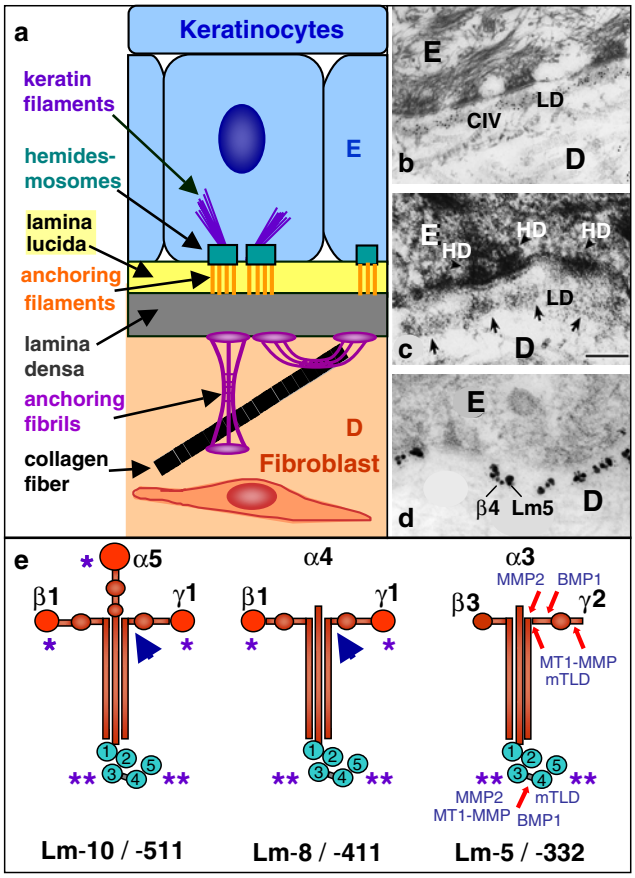

Fig. 1 Ultrastructural elements (**a**, **c**) of the basement membrane (BM) zone and molecular associations (**b**, **d**). The cartoon (**a**) depicts the anchorage between epidermis (E) and dermis (D) corresponding to the ultrastructure of the collagen–epidermal interface (**c**) of a 3D co-culture of keratinocytes with fibroblasts, resembling skin. Immune-EM demonstrates the co-alignment of collagen IV with the *lamina densa* (**b**) and co-localization of integrin $\alpha 6\beta 4$ with laminin-332 (**d**; small/large gold particles). Representatives of main subtypes of laminin in adult skin (**e**): the main adult BM-type laminin-511, the vascular laminin-411, and laminin-332 present in anchoring filaments. Like laminin-511, most isoforms carry three N-terminal self-assembly sites (*asterisk*) required for polymerization, while some like laminin-411 have only two. As an exception, laminin-332 has none of those 'sticky' sites. Common to all are the C-terminal cell binding sites (*double asterisk*); *large arrows* point to the $\gamma 1$ nidogen-binding domain. Further typical for laminin-332 is extensive proteolytic processing; major cleavage sites (*small arrows*) and some of the involved enzymes are indicated (see also: Miner and Yurchenco 2004; Sugawara et al. 2008)

Attempts for epidermal reconstruction using defined cell populations

The two main cell types of skin, epidermal keratinocytes and dermal fibroblasts have been analyzed extensively in vitro for studying skin physiology, repair, and tumorigenesis. But the major drawbacks of those approaches are that (1) both cell types behave very differently on the two-dimensional (2D) surface of a culture dish and (2) they intensively communicate in vivo which regulates growth and phenotype. Fibroblasts are in vivo completely embedded in ECM (collagen, fibronectin) which keeps them apart and gives rise to their spindle-shape morphology. Although keratinocytes form coherent cell layers in vitro showing some degree of differentiation as in epidermis, the tissue polarity is severely disturbed. In particular, under 2D culture conditions the direction of nutrient supply is drastically changed from the cell attachment site (facing BM and dermis) to the epithelial surface (supposed to provide a water barrier in vivo). Nevertheless, still a variety of physiological responses, including growth control, cell migration or adhesion, and differentiation processes, have been studied in such 2D cultures. Thus, analysis of keratins, as suitable indicators for the epithelial cell state, revealed that the keratin pair K5/14 (expressed in basal cells) was maintained in vitro, whereas K1/10 (early differentiation marker) was to a large extent substituted by the wound-type K6/16. A major breakthrough was the development of the human keratinocyte line HaCaT (Boukamp et al. 1988), offering an alternative, consistent cell source. HaCaT cells regularly respond to modulators of differentiation such as retinoid and Ca^{2+} levels or high cell density (Ryle et al. 1989; Hohl et al. 1991; Breitkreutz et al. 1993). Reduction of retinoids, for example, induced K1/10 whereas elevation favored the mucosa-type K4/13. However, benign and malignant HaCaT variants, generated by implementing a mutated Ha *ras*-gene, did not reveal specific changes in keratin profiles in vitro which could refer to their tumorigenic properties in vivo (Ryle et al. 1989; Breitkreutz et al. 1993).

Development of regular epidermal architecture in cell transplants

The full potential of cells for epidermal morphogenesis was first demonstrated in transplantation models, providing an in vivo like microenvironment. Initially, keratinocytes from newborn mice, later from human skin or HaCaT cells were grafted on the back of immune compatible or nude mice which elicit no xenograft rejection (Boukamp et al. 1988; Breitkreutz et al. 1997, 1998; also references therein). In all those cases epidermal tissue architecture was restored, corresponding to normalized differentiation and keratin patterns with a distinct single basal (K5/14 positive) and overlying multiple differentiated layers (K1/10 positive). The stroma at the graft site also induced epidermal reconstruction by outer root sheath cells of hair follicles (Limat et al. 1995, 1996). Consistently, the consolidation of epidermal architecture correlated to completion of the dermo-epidermal junction, including distinct BM and adhesion structures (Breitkreutz et al. 1997, 1998), resembling late stages of wound repair. Accordingly, individual BM

components were deposited in a defined sequence, as visualized by immunofluorescence. First, seen 1 day after grafting, the non-polymerizing laminin-332 accumulated at the junctional zone, followed by nidogen and then (around days 5–7) more slowly by BM-laminin (laminin-511) and collagen IV. Whereas all integrins were irregularly distributed early on, β1-integrins were reoriented at that late stage decorating the entire basal cell surface and $\alpha6\beta4$ mainly the ventral part, coinciding with assembly of other hemidesmosomal components. Concomitantly with BM formation, proliferation decreased and became restricted to basal cells (about 5% being BrdU labeled indicating DNA replication) like in normal epidermis, which implies that BM contributes to growth regulation. Detailed analysis by EM revealed a gradual formation of the BM zone with specific epidermal BM anchoring structures. However, the delay of ultrastructural BM development in comparison to deposition of individual BM components as shown by immunostaining strongly implicates that certain threshold levels are necessary for proceeding BM assembly.

To develop an experimental tumor model with human cells, benign and malignant HaCaT-ras variants were also grafted onto nude mice, a more sensitive tumorigenicity assay than subcutaneous cell injection (Tomakidi et al. 1999, 2003). Initially, malignant cells formed polarized, stratifying epithelia exhibiting epidermal markers (e.g., K5/14, K1/10) and to some extent BM structures. Thus, the influence of normal host connective tissue was first dominating. This changed dramatically with the mounting tumor stroma reaction, apparently induced by tumor cells and related to their grade of malignancy. A hallmark of this event is the inflammatory cell infiltrate and increased, persisting angiogenesis (Müller and Fusenig 2004). Epithelial polarity was largely lost, demarcated by irregular clusters of proliferating or differentiating cells, though respective markers like K1/10, filaggrin, or loricrin were still detectable. Finally, non-epidermal 'simple' keratins K8/K18 and the mesenchymal marker vimentin appeared at the front of invading cells (Tomakidi et al. 2003) as reported for human squamous cell carcinomas. Concomitantly, we observed a strong increase and broad distribution of proliferating cells beyond basal cell alignments, reflected by strongly expanding integrin β1 and $\alpha6\beta4$ patterns (Tomakidi et al. 1999). Similar changes have been observed by our Israeli collaborators (Tennenbaum et al. 1993) in their mouse model of two-stage carcinogenesis (references in Breitkreutz et al. 2007). Also BM structures vanished, whereas laminin-332 persisted aberrantly decorating lateral cell surfaces and demarcating deep epithelial clefts, which preceded tumor septa formation. Most BM structures and anchoring components were still seen by immune EM, but were displaced or dissociated (Mirancea et al. 2002).

Generating artificial skin in vitro as functional model

While conventional cultures proved insufficient to study physiological and pathological processes relevant for compound tissues such as skin, the situation in cell grafts on mouse was still very complex. To provide a simpler, but well defined experimental system, skin-organotypic co-cultures were established containing the basic elements of skin (Smola et al. 1998; Stark et al. 1999). For this purpose keratinocytes were grown in multiwell filter inserts on top of collagen I gels with dispersed living fibroblasts. Getting in contact with media only underneath and being exposed to the gas phase in the incubator, epithelial polarity is implemented in this 3D system (Fig. 1b–d) like in vivo in skin or keratinocyte grafts. This also allowed to supplement various factors and regulators of growth and differentiation or to combine genetically altered mouse and human cells with deleted, silenced, or inducible expression of selected genes (Sadagurski et al. 2006; Sher et al. 2006; Nischt et al. 2007). Normal epidermal morphology and differentiation were achieved as indicated by the location of keratins (K5/14, K1/10) and intermediate or late markers like epidermal transglutaminase, filaggrin, and loricrin (Limat et al. 1996; Stark et al. 1999).

Perlecan and nidogen exert different functions at the dermo-epidermal interface

The variant tissue defects of perlecan (Arikawa-Hirasawa et al. 1999; Costell et al. 1999) and nidogen (Bader et al. 2005) deficient mice raised the question for specific functions in skin. While most BM molecules are made by both cell types, laminin-332 originates from keratinocytes and nidogen from fibroblasts (Fleischmajer et al. 1995; Marionnet et al. 2006; Nischt et al. 2007). Ablation of 'dermal' perlecan in 3D co-culture using deficient fibroblasts (perlecan-null embryos; Costell et al. 1999) had no consequences in combination with regular HaCaT cells, neither for BM deposition nor epithelial morphology. However, when perlecan deficient HaCaT cells (silenced by stable antisense RNA expression) were grown with wild-type fibroblasts, which express perlecan, we observed a marked delay of epithelial growth, though eventually a regular structure (thickness and full differentiation) was achieved. But also in this case BM deposition was unaffected at least by light microscopy. In conclusion, perlecan does not seem to play a major role for BM assembly, though it appears to be crucial for functional BM properties. In accordance with a balanced growth control perlecan prevented apoptosis of HaCaT cells in that system (Sher et al. 2006).

In contrast to perlecan, lack of nidogen or interference with the laminin–nidogen interaction had a diametrically

opposed effect. As outlined below, in the 3D model the function of nidogen was indispensable for deposition and assembly of the other BM components whereas epithelial growth and differentiation proceeded normally.

Nidogen plays an active role for BM assembly in vitro

Besides the predominating nidogen, which is nidogen-1, nidogen-2 was discovered as second mammalian isoform (Kohfeldt et al. 1998; Salmivirta et al. 2002). They are both ubiquitous BM proteins, though nidogen-2 shows more restricted expression patterns throughout development and some tissue specificity in adulthood. Both isoforms interact in vitro with many other BM molecules, in particular laminin and collagen IV, qualifying these components as integrating elements for BM assembly (Fox et al. 1991; Aumailley et al. 1993; Kohfeldt et al. 1998; Salmivirta et al. 2002). For this, cross-bridging of the laminin γ1 chain and collagen IV by nidogen-1 has been proposed as initial step. For functional proof, we inhibited nidogen-binding to laminin in 3D co-cultures with a laminin γ1 fragment (γ1III3-5 module) spanning the binding site located within the γ1III4 module (Mayer et al. 1993a; Pöschl et al. 1994). Repeated treatment abolished the deposition of nidogen but also laminin and perlecan at the matrix interface, while some other components of the BM zone were only moderately affected (Breitkreutz et al. 2004). Thus, laminin-332, collagen IV, and integrin α6ß4 still showed distinct regular staining as shown by immunofluorescence. The dynamics of this process became apparent by the reversibility of both BM blockade and assembly. So, already assembled structures disappeared again by late treatment with the γ1 fragment, while BM formation was resumed when treatment was halted at an advanced stage. Epidermal morphology and differentiation remained largely normal (K1/K10 and other, 'late' markers). According to ultrastructure, the γ1 fragment completely abolished not only BM formation (no *lamina densa* visible) but also the formation of hemidesmosomal adhesion complexes leading to dissociation of keratin filaments from the ventral cell aspect. Alternatively to BM adhesion, basal cells adhered now directly to 'dermal' collagen fibrils. Despite a marked reduction several BM and hemidesmosomal components were still seen by immune EM, though largely dispersed. Interestingly, in protein extracts of separated dermal and epidermal parts of co-cultures we still detected the major BM components nidogen-1, collagen IV, laminin-511 (laminin-10), and laminin-332 at similar levels and without detectable aberrant processing (Breitkreutz et al. 2004; see also nidogen-null below). In conclusion, the major defect observed in this co-culture system was the lack of BM assembly, triggering a devastating chain reaction for the entire adhesion apparatus.

A direct role of nidogen could be demonstrated employing fibroblasts from knockout mice lacking both nidogen-1 and -2 in the 3D co-culture system (Nischt et al. 2007). In the complete absence of nidogens, no BM deposition or structure was detectable, while the effects on individual BM components were comparable to the blocking experiments described before (Fig. 2a–c). Thus, immunoblot analysis disclosed in the absence of nidogen neither a marked decrease of the other BM components collagen IV, laminin-511 or the 'anchoring' laminin-332 nor any abnormal processing (Fig. 3). Accordingly, though mostly not visible by immunostaining, all major components were detected by immune EM, but scattered over a broader area. Furthermore, the use of fibroblasts from respective mouse genotypes (hetero- and homozygous nidogen deletions) revealed some dose effects. Supplementing depleted 3D co-cultures with either recombinant nidogen-1 or nidogen-2 restored the BM zone as shown by immunofluorescence and EM, indicating a regular ultrastructure (Fig. 2d–i). This confirmed that in this skin model nidogen-2 is equivalent to nidogen-1 in promoting the formation of an ultrastructurally normal appearing BM zone (Nischt et al. 2007).

Microvascular defects due to BM anomalies

Genetic ablation of nidogen-1 (Murshed et al. 2000) or nidogen-2 (Schymeinsky et al. 2002) alone did not cause obvious BM alterations. However, in nidogen-1 null mice a redistribution and increase of nidogen-2 is observed suggesting that nidogen-2 can compensate the loss of nidogen-1 for BM formation (Murshed et al. 2000; Miosge et al. 2002). However, nidogen-1 null mice show certain developmental and neurological defects indicating only partial redundancy (Dong et al. 2002; Köhling et al. 2006). Mice lacking both nidogens die perinatally from lung and heart anomalies, directly related to BM defects, while in some other tissues BMs appeared largely unaffected (Bader et al. 2005). This demonstrates in vivo that BMs can per se develop in the absence of nidogens. But this also indicates tissue-specific requirements of nidogens for BM formation. Toward this end we have scrutinized the dermo-epidermal junction and the microvasculature in skin from nidogen deficient embryos (E18.5; Mokkapati et al. 2008). Overall skin morphology and ultrastructure were largely normal with only mildly disturbed dermo-epidermal BM (occasional microblisters) and few damaged basal cells in the epidermis. The striking differences between dermo-epidermal BM formation in vivo and in vitro in 3D co-cultures implies tissue-related requirements for additional components or special chemical and mechanical properties of the connective tissue (like the dermal ECM). However, this discrepancy can

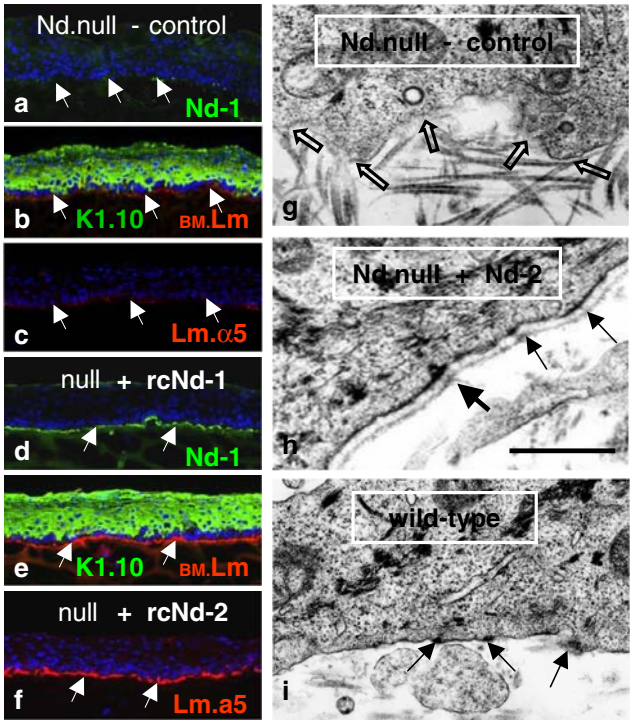

Fig. 2 Influence of nidogen on BM formation in 3D co-culture. In co-cultures with nidogen-null fibroblasts (**a–c**) no BM components (all in *red*) like nidogen-1 (**a**), BM-laminin (**b**) counterstained for K1/10, or the laminin α5 chain (**c**) (in laminin-511) are seen at the interface (*arrows*). Recombinant nidogen-1 added to the medium (**d**, **e**) accumulates at the interface (**d**) and restores the BM (**e**) indicated by BM-laminin (*red*; antibodies recognizing several isoforms); recombinant nidogen-2 has a comparable effect as shown here for the laminin α5 chain (**f**). Stained in *green* suprabasal keratin K1/10; *blue* nuclear staining, indirect immunofluorescence (IIF). Ultrastructural elements of the BM zone: in the absence of nidogens (**g**) no *lamina densa* is detectable by electron microscopy. Since hemidesmosomes (HDs) are absent as well, keratin filaments (KF) are ventrally detached and keratinocytes adhere directly to collagen fibrils (*open arrows*). Similar to nidogen-1 (IIF) addition of nidogen-2 restores the BM zone (**h**), showing a continuous *lamina densa*, mature and immature HDs (thick, *thin arrows*) and KF close to HDs. External nidogen supplementation seems to accelerate the assembly process, since in 3D co-cultures with wild-type fibroblast (**i**) still only marginal BM or HD structures (*thin arrows*) were detectable at that stage (same day). Bar = 1 μm (modified from Nischt et al. 2007)

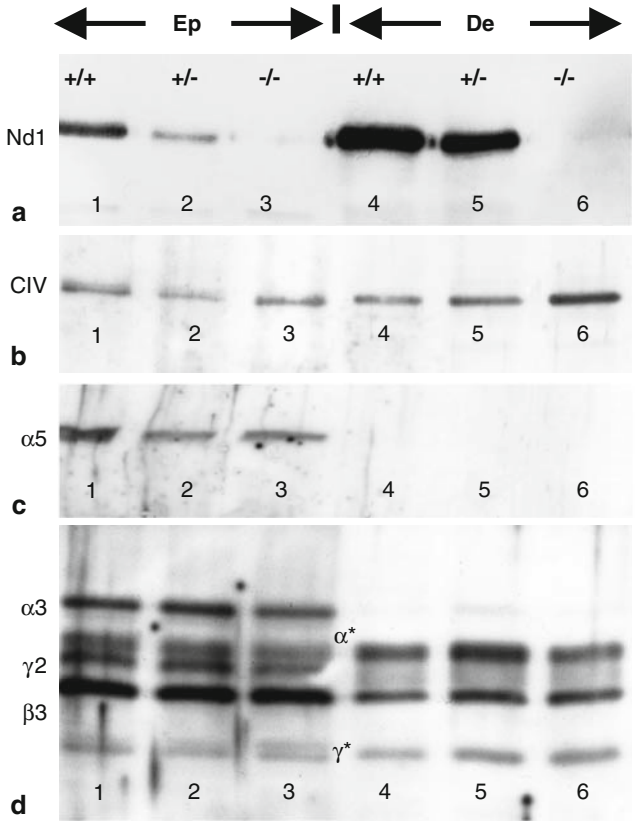

Fig. 3 Persisting patterns of BM proteins. Western blot analysis of the separated 'epidermal' (Ep) and 'dermal' (De) parts are shown from 3D co-cultures with wild-type (+/+), nidogen-1(+/−), and nidogen double null (−/−) fibroblasts. The results indicate some dose effects for nidogen-1 (**a**) with higher concentrations in dermal parts of positive samples (site of nidogen synthesis). Collagen IV (**b**; CIV) is more evenly distributed, the slight rise in nidogen-null may reflect lower collagen IV cross-linking. No differences are seen for the laminin α5 chain (**c**; laminin-511), being restricted to the Ep-part, and laminin-332 (**d**), here revealing in all samples from the dermal part complete α3/γ2 processing (compare Fig. 1). Collectively, these data clearly show that immunofluorescence staining (Fig. 2) does not necessarily match the actual protein levels in the tissue (modified from Nischt et al. 2007)

also possibly be explained by the conditions used in the 3D co-culture. The relatively large volumes of media used may reduce the probability that other BM molecules reach critical concentrations high enough to induce their polymerization or integration into a BM ('molecular crowding', for discussion: Lareu et al. 2007). This might be indeed the case as nidogens appear to catalyze and/or stabilize the development of the BM in the 3D co-culture system (Nischt et al. 2007).

On the contrary, in small vessels BMs were virtually missing in the absence of nidogens, showing by immunofluorescence an irregular, patchy distribution or marked reduction of collagen IV, perlecan and particularly laminin-411 (laminin-8) as illustrated in Fig. 4. Ultrastructural analysis revealed in small blood vessels thin leaky walls, completely lacking a distinct BM (Fig. 5a; Mokkapati et al. 2008). Collectively, our results indicate that in skin the laminin composition (laminin-511, being absent in capillaries at E18.5, versus laminin-411 with only two short arms unable to form networks by self-polymerization) of the various BMs determines the nidogen requirement for their assembly or stabilization. Nevertheless, modulation by other 'minor' components, for example additional, 'associated' collagen types like collagens XV and XVIII (Ortega and Werb 2002) must be considered, too. Finally, small vessels devoid of BMs causing leakiness and extravasation of blood cells are also common in tumors.

Another, apparently restrictive regulator of BM assembly, the 'extracellular matrix protein 1' (ECM1) we became

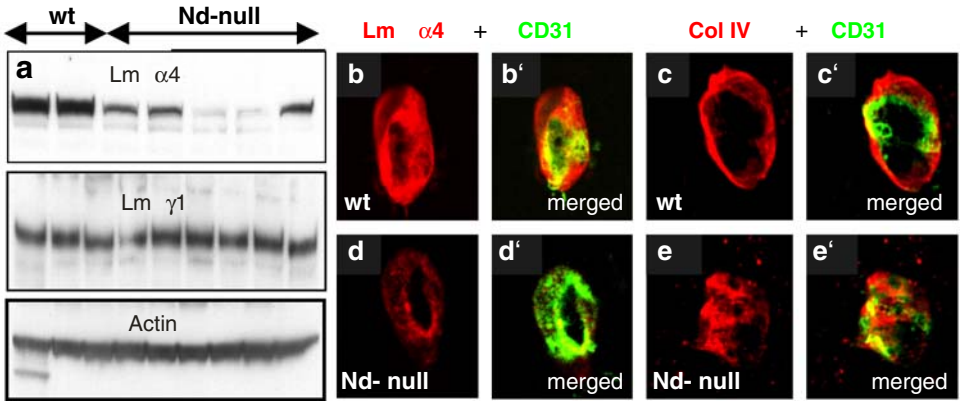

Fig. 4 Nidogens are essential for functional microvessels. The western blots of skin protein extracts (**a**) demonstrate the dramatic reduction of the laminin α4 chain (of laminin-411) in the preparations from nidogen double null mice, while there are no differences in the laminin γ1 content compared to the wild-type samples (actin shown as loading control). Indirect immunofluorescence (**b–e**) confirms that, in comparison to the wild-type (**b**, **c**), only very little laminin α4 (*red*; **d** versus **b**) is present in microvessels of nidogen deficient mice. Whereas the collagen IV (*red*) staining is comparable (**e** versus **c**), in deficient mice collagen IV appears rather patchy and is not very well aligned with the endothelial CD31 (PECAM; in *green*) shown in merged pictures (**b′–e′**) (modified from Mokkapati et al. 2008)

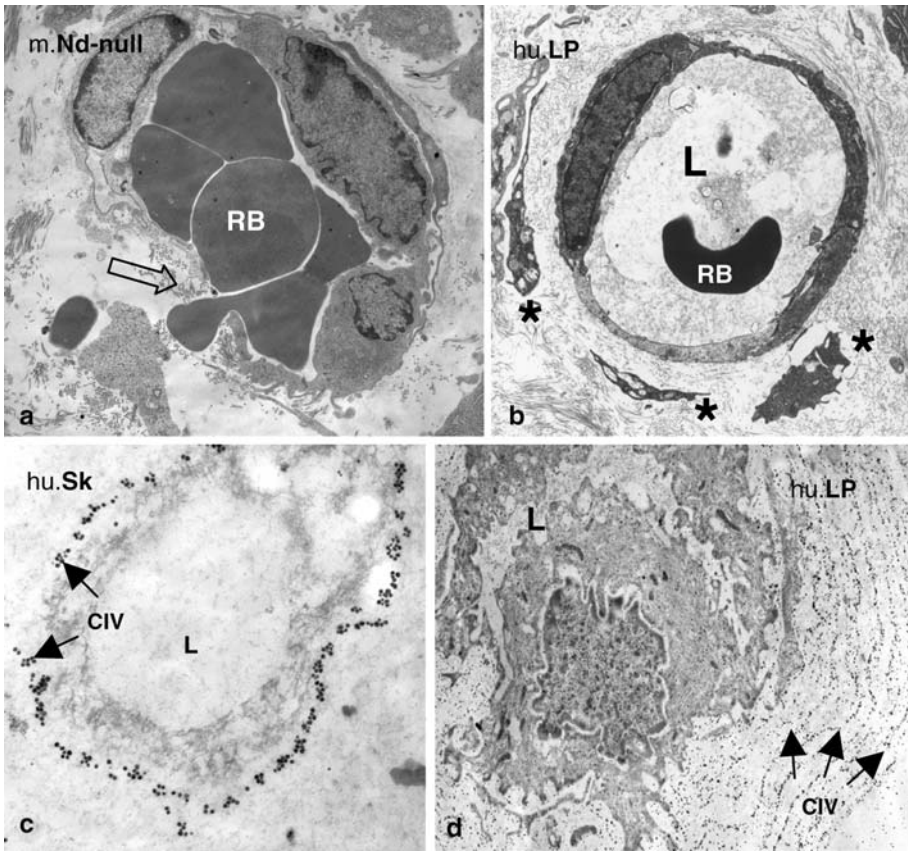

Fig. 5 Ultrastructural vascular anomalies caused by BM defects. In the nidogen double null mice no BM structures were visible around small blood vessels by electron microscopy, resulting in leaky vessel walls and extravasations of red blood cells (**a**; RB). Contrarily, lipoid proteinosis patients (LP; ECM1 mutations) have multiple BMs around small vessels (**b**) causing dissociation of pericytes (*asterisk*) from the outer vessel surface. This is mirrored by altered collagen IV (CIV) deposition, which normally occurs in a narrow distinct zone (**c**) but in the disease (**d**) reveals onion shell-like patterns (both immune electron microscopy, gold-label); frequent collapse of vascular lumina (L) is also a common complication in LP (modified from Mirancea et al. 2006; Mokkapati et al. 2008)

aware of when analyzing skin biopsies of lipoid proteinosis (LP) patients. In this inherited disorder dysfunction or lack of ECM1 causes excessive BM deposition. This again primarily affects small vessels, where enormously sized, multiple BMs compromise vascular function (Fig. 5b–d; Mirancea et al. 2006). The junctional BM revealed some duplications (perhaps due to repeated BM remodeling like in certain skin tumors) which only locally disturbs dermo-epidermal adhesion complexes in the skin of LP patients. As shown by EM, adhesion structures were markedly displaced, though remaining partially associated like laminin-332 and collagen VII (Mirancea et al. 2007). Regarding functional consequences, an involvement of both molecules has been suggested in invasive carcinomas, being maintained

in contrast to the turnover of other BM components (Ortiz-Urda et al. 2005; Marinkovich 2007). However, patients with recessive dystrophic epidermolysis bullosa who lack expression of collagen VII, also develop skin tumors indicating that collagen VII is not an absolute requirement for squamous cell carcinoma development (Rodeck and Uitto 2007).

Conclusions and perspective

The current state of the art allows no definitive answer on the function of nidogens in general or the two isoforms specifically. Nevertheless, the data from the in vitro skin model clearly show that both nidogens can induce or dramatically accelerate the formation of BMs and epidermal adhesion structures, possibly functioning also as stabilizers. This would suggest a particularly important role in situations of fast tissue and BM remodeling.

Thus, studies on interactions of distinct nidogen and laminin isoforms should be continued combining genetic and 3D co-culture approaches to define isoform specific roles of these proteins and their interactions for skin physiology and pathology. Other players like ECM1 or 'minor' collagens shall be included as well, which may create a nucleus for assembly or some BM micro-heterogeneity. The latter could be potentially correlated to the postulated stem cell niche in the epidermis outside of the hair follicle (Kaur 2006; Ito and Cotsarelis 2008; for general considerations: Morrison and Spradling 2008). Crucial for following this concept in the 3D co-cultures are recent improvements of the 'dermal' part in this model, approaching physiological features and stability of genuine dermis (Stark et al. 2004; Muffler et al. 2008). To bridge the discrepancies between the in vitro and in vivo models, a promising approach would be to create mice with skin-specific constitutive or inducible ablation of both nidogens. Though not an easy but very time consuming task, this would actually allow to test skin and epidermal barrier functions in otherwise normal adult animals. In this context, it should be kept in mind as well, that weakening of BM structures of tissues and the vascular system is a hallmark of invasiveness in cancer. Since nidogens exhibit a rather high susceptibility against proteases like matrix metalloproteases (Mayer et al. 1993b, 1995), they are presumably preferential early targets in tissue barrier destruction.

Acknowledgments The work was in part supported by the *Deutsche Forschungsgemeinschaft* through the *Sonderforschungsbereich* (SFB) 589 at the University of Cologne, the grants NI-304/11-1 (Roswitha Nischt), BR-530/8-1 (Dirk Breitkreutz), and industrial grants (*Sanofi-Aventis*); furthermore we like to thank all students and colleagues involved or contributing to these studies at any stage, also by many fruitful discussions. We apologize to the many scientists whose paper we were unable to cite owing to space constraints.

References

Arikawa-Hirasawa E, Watanabe H, Takami J, Hassell JR, Yamada Y (1999) Perlecan is essential for cartilage and cephalic development. Nat Genet 23:354–358

Aumailley M, Battaglia C, Mayer U, Nischt R, Timpl R, Fox JW (1993) Nidogen mediates the formation of ternary complexes of basement membrane components. Kidney Int 43:7–12

Aumailley M, Bruckner-Tuderman L, Carter WG, Deutzmann R, Edgar D, Ekblom P, Engel J, Engvall E, Hohenester E, Jones JC, Kleinman HK, Marinkovich MP, Martin GR, Mayer U, Meneguzzi G, Miner JH, Miyazaki K, Patarroyo M, Paulsson M, Quaranta V, Sanes JR, Sasaki T, Sekiguchi K, Sorokin LM, Talts JF, Tryggvason K, Uitto J, Virtanen I, von der Mark K, Wewer UM, Yamada Y, Yurchenco PD (2005) A simplified laminin nomenclature. Matrix Biol 24:326–332

Aumailley M, Has C, Tunggal L, Bruckner-Tudermann L (2006) Molecular basis of inherited skin-blistering disorders, and therapeutic implications. Expert Rev Mol Med 8:1–21

Bader B, Smyth N, Nedbal S, Miosge N, Baranowsky A, Mokkapati S, Murshed M, Nischt R (2005) Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice. Mol Cell Biol 25:6846–6856

Battaglia C, Mayer U, Aumailley M, Timpl R (1992) Basement-membrane heparan sulfate proteoglycan binds to laminin by its heparan sulfate side chains and to nidogen by sites in the protein core. Eur J Biochem 208:359–366

Boukamp P, Dzarlieva-Petrusevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE (1988) Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol 106:761–771

Breitkreutz D, Stark HJ, Plein P, Baur M, Fusenig NE (1993) Differential modulation of epidermal keratinization in immortalized (HaCaT) and tumorigenic human skin keratinocytes (HaCaT-ras) by retinoic acid and extracellular Ca^{2+}. Differentiation 54:201–217

Breitkreutz D, Stark HJ, Mirancea N, Tomakidi P, Steinbauer H, Fusenig NE (1997) Integrin and basement membrane normalization in mouse grafts of human keratinocytes—implications for epidermal homeostasis. Differentiation 61:195–209

Breitkreutz D, Schoop VM, Mirancea N, Baur M, Stark HJ, Fusenig NE (1998) Epidermal differentiation and basement membrane formation by HaCaT cells in surface transplants. Eur J Cell Biol 75:273–286

Breitkreutz D, Mirancea N, Schmidt C, Beck R, Werner U, Stark HJ, Gerl M, Fusenig NE (2004) Inhibition of basement membrane formation by a nidogen-binding laminin γ1-chain fragment in human skin-organotypic cocultures. J Cell Sci 117:2611–2622

Breitkreutz D, Braimann-Wiksman L, Daum N, Denning MF, Tennenbaum T (2007) Protein kinase C family—on the crossroads of cell signaling in skin. J Cancer Res Clin Oncol 133:793–808

Bruckner-Tuderman L (1999) Hereditary skin diseases of anchoring fibrils. J Dermatol Sci 20:122–133

Chaudhari P, Marinkovich MP (2007) What's new in blistering disorders. Curr Allergy Asthma Rep 7:222–263

Costell M, Gustafsson E, Aszódi A, Mörgelin M, Bloch W, Hunziker E, Addicks K, Timpl R, Fässler R (1999) Perlecan maintains the integrity of cartilage and some basement membranes. J Cell Biol 147:354–358

Dong L, Chen Y, Lewis M, Hsieh JC, Reing J, Chaillet JR, Howell CY, Melhem M, Inoue S, Kuszak JR, DeGeest K, Chung AE (2002) Neurological defects and selective disruption of basement

membranes in mice lacking entactin-1/nidogen-1. Lab Invest 82:1617–1630
Eckert RL, Sturniolo MT, Broome AM, Ruse M, Rorke EA (2005) Transglutaminase function in epidermis. J Invest Dermatol 124:481–492
Fleischmajer R, Schechter A, Bruns M, Perlish JS, Macdonald ED, Pan TC, Timpl R, Chu ML (1995) Skin fibroblasts are the only source of nidogen during early basal lamina formation. J Invest Dermatol 105:597–601
Fox JW, Mayer U, Nischt R, Aumailley M, Reinhardt D, Wiedemann H, Mann K, Timpl R, Krieg T, Engel J (1991) Recombinant nidogen consists of three globular domains and mediates binding of laminin to collagen IV. EMBO J 10:3137–3146
Franzke CW, Bruckner P, Bruckner-Tuderman L (2005) Collagenous transmembrane proteins: recent insights into biology and pathology. J Biol Chem 280:4005–4008
Fuchs E, Raghavan S (2002) Getting under the skin of epidermal morphogenesis. Nat Rev Genet 3:199–209
Hohl D, Lichti U, Breitkreutz D, Steinert PM, Roop DR (1991) Transcription of the human loricrin gene in vitro is induced by calcium and cell density and repressed by retinoic acid. J Invest Dermatol 96:414–418
Huber M, Siegenthaler G, Mirancea N, Marenholz I, Nizetic D, Breitkreutz D, Mischke D, Hohl D (2005) Isolation and characterization of human repetin, a member of the fused gene family of the epidermal differentiation complex. J Invest Dermatol 124:998–1007
Iozzo RV (2005) Basement membrane proteoglycans: from cellular to ceiling. Nat Rev Mol Cell Biol 6:646–656
Ito M, Cotsarelis G (2008) Is the hair follicle necessary for normal wound healing? J Invest Dermatol 128:1059–1061
Kaur P (2006) Interfollicular epidermal stem cells: identification, challenges, potential. J Invest Dermatol 126:1450–1458
Kohfeldt E, Sasaki T, Göhring W, Timpl R (1998) Nidogen-2: a new basement membrane protein with diverse binding properties. J Mol Biol 282:99–109
Köhling R, Nischt R, Vasudevan A, Ho M, Weiergräber M, Schneider T, Smyth N (2006) Nidogen and nidogen-associated basement membrane proteins and neuronal plasticity. Neurodegener Dis 3:56–61
Lareu RR, Arsianti I, Subramhanya HK, Yanxian P, Raghunath M (2007) In vitro enhancement of collagen matrix formation and crosslinking for applications in tissue engineering: a preliminary study. Tissue Eng 13:385–391
LeBleu VS, Macdonald B, Kalluri R (2007) Structure and function of basement membranes. Exp Biol Med 232:1121–1129
Limat A, Breitkreutz D, Thiekoetter G, Klein CE, Braathen LR, Hunziker T, Fusenig NE (1995) Formation of a regular neoepidermis by cultured human outer root sheath cells grafted on nude mice. Transplantation 59:1032–1038
Limat A, Stockhammer E, Breitkreutz D, Schaffner T, Egelrud T, Salomon D, Fusenig NE, Braathen LR, Hunziker T (1996) Endogenously regulated site-specific differentiation of human palmar skin keratinocytes in organotypic cocultures and in nude mouse transplants. Eur J Cell Biol 69:245–258
Litjens SH, de Pereda JM, Sonnenberg A (2006) Current insight into the formation and breakdown of hemidesmosomes. Trends Cell Biol 16:376–383
Marinkovich MP (2007) Tumor microenvironment: laminin-322 in squamous cell carcinoma. Nat Rev Cancer 7:370–380
Marionnet C, Pierrard C, Vioux-Chagnoleau C, Sok J, Asselineau D, Bernerd F (2006) Interactions between fibroblasts and keratinocytes in morphogenesis of dermal epidermal junction in a model of reconstructed skin. J Invest Dermatol 126:971–979
Mayer U, Nischt R, Pöschl E, Mann K, Fukuda K, Gerl M, Yamada Y, Timpl R (1993a) A single EGF-like motif of laminins is responsible for high affinity nidogen binding. EMBO J 12:1879–1885
Mayer U, Mann K, Timpl R, Murphy G (1993b) Sites of nidogen cleavage by proteases involved in tissue homeostasis and remodeling. Eur J Biochem 217:877–884
Mayer U, Zimmermann K, Mann K, Reinhardt D, Timpl R, Nischt R (1995) Binding properties and protease stability of recombinant human nidogen. Eur J Biochem 227:681–686
McMillan JR, Akiyama M, Shimizu H (2003) Epidermal basement membrane zone components: ultrastructural distribution and molecular interactions. J Dermatol Sci 31:169–177
Miner JH (2008) Laminins and their role in development. Microsc Res Tech 71:349–356
Miner JH, Yurchenco PD (2004) Laminin function in tissue morphogenesis. Annu Rev Cell Dev Biol 20:255–284
Miner JH, Li C, Mudd JL, Go G, Sutherland AE (2004) Compositional and structural requirements for laminin and basement membranes during mouse embryo implantation and gastrulation. Development 131:2247–2256
Miosge N, Sasaki T, Timpl R (2002) Evidence for nidogen-2 compensation for nidogen-1 deficiency in transgenic mice. Matrix Biol 21:611–621
Mirancea N, Schmidt C, Daum N, Tomakidi P, Stark HJ, Fusenig NE, Breitkreutz D (2002) Basement membrane defects in xeno-grafts of malignant human cells. In: Witz IP (ed) Proceedings of the 2nd international conference tumor microenvironment. Monduzzi, Bologna, pp 55–58
Mirancea N, Hausser I, Beck R, Metze D, Fusenig NE, Breitkreutz D (2006) Vascular anomalies in lipoid proteinosis (hyalinosis cutis et mucosae): basement membrane components and ultrastructure. J Dermatol Sci 42:231–239
Mirancea N, Hausser I, Metze D, Stark HJ, Boukamp P, Breitkreutz D (2007) Junctional basement membrane anomalies of skin and mucosa in lipoid proteinosis (hyalinosis cutis et mucosae): components and ultrastructure. J Dermatol Sci 45:175–185
Mokkapati S, Baranowsky A, Mirancea N, Smyth N, Breitkreutz D, Nischt R (2008) Basement membranes are differently affected by lack of nidogen 1 and 2. J Invest Dermatol 128:2259–2267
Morrison SJ, Spradling AC (2008) Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132:598–611
Muffler S, Stark HJ, Amoros M, Falkowska-Hansen B, Boehnke K, Bühring HJ, Marme A, Bickenbach JR, Boukamp P (2008) A stable niche supports long-term maintenance of human epidermal stem cells in organotypic cultures. Stem Cells 26:2506–2515
Müller MM, Fusenig NE (2004) Friends or foes—bipolar effects of the tumour stroma in cancer. Nat Rev Cancer 42:735–744
Murshed M, Smyth N, Miosge N, Karolat J, Krieg T, Paulsson M, Nischt R (2000) The absence of nidogen-1 does not affect murine basement membrane formation. Mol Cell Biol 20:7007–7012
Niessen CM (2007) Tight junctions/adherens junctions: basic structure and function. J Invest Dermatol 127:2525–2532
Nischt R, Schmidt C, Mirancea N, Baranowsky A, Mokkapati S, Smyth N, Woenne EC, Stark HJ, Boukamp P, Breitkreutz D (2007) Lack of nidogen-1 and -2 prevents basement membrane assembly in skin-organotypic co-culture. J Invest Dermatol 127:545–554
Ortega N, Werb Z (2002) New functional roles for non-collagenous domains of basement membrane collagens. J Cell Sci 115:4201–4214
Ortiz-Urda S, Garcia J, Green CL, Chen L, Lin Q, Veitch DP, Sakai LY, Lee H, Marinkovich MP, Khavari PA (2005) Type VII collagen is required for RAS-driven human epidermal carcinogensis. Science 307:1773–1776
Pöschl E, Fox JW, Block D, Mayer U, Timpl R (1994) Two non-contiguous regions contribute to nidogen binding to a single EGF-like motif of the laminin gamma1 chain. EMBO J 13:3741–3747
Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U (2004) Collagen IV is essential for basement

membrane stability but dispensable for initiation of its assembly during early development. Development 131:1619–1621

Rodeck U, Uitto J (2007) Recessive dystrophic epidermolysis bullosa-associated squamous-cell carcinoma: an enigmatic entity with complex pathogenesis. J Invest Dermatol 127:2295–2296

Ryle CM, Breitkreutz D, Stark HJ, Leigh IM, Steinert PM, Roop D, Fusenig NE (1989) Density-dependent modulation of synthesis of keratins l and 10 in the human keratinocyte line HaCaT and in ras-transfected tumorigenic clones. Differentiation 40:42–54

Sadagurski M, Yakar S, Weingarten G, Holzenberger M, Rhodes CJ, Breitkreutz D, Leroith D, Wertheimer E (2006) Insulin-like growth factor 1 receptor signaling regulates skin development and inhibits skin keratinocyte differentiation. Mol Cell Biol 7:2675–2687

Salmivirta K, Talts J, Olsson M, Sasaki T, Timpl R, Ekblom P (2002) Binding of mouse nidogen-2 to basement membrane components and cells in embryonic and adult tissues suggest complementary functions of the two nidogens. Exp Cell Res 279:188–201

Schneider H, Mühle C, Pacho F (2007) Biological function of laminin-5 and pathogenic impact of its deficiency. Eur J Cell Biol 86:701–717

Schymeinsky J, Nedbal S, Miosge N, Pöschl E, Rao C, Beier DR, Skarnes WC, Timpl R, Bader BL (2002) Gene structure and functional analysis of the mouse nidogen-2 gene: nidogen-2 is not essential for basement membrane formation in mice. Mol Cell Biol 22:6820–6830

Sevilla LM, Nachat R, Groot KR, Klement JF, Uitto J, Djian P, Määttä A, Watt FM (2007) Mice deficient in involucrin, envoplakin, and periplakin have a defective epidermal barrier. J Cell Biol 179:1599–1612

Sher I, Zisman-Rozen S, Eliahu L, Whitelock JM, Maas-Szabowski N, Yamada Y, Breitkreutz D, Fusenig NE, Arikawa-Hirasawa E, Iozzo RV, Bergman R, Ron D (2006) Targeting perlecan in human keratinocytes reveals novel roles for perlecan in epidermis formation. J Biol Chem 281:5178–5187

Smola H, Stark HJ, Thiekötter G, Mirancea N, Krieg T, Fusenig NE (1998) Dynamics of Basement membrane formation by keratinocyte-fibroblast interactions in organotypic skin cultures. Exp Cell Res 239:399–410

Smyth N, Vatansever HS, Murray P, Meyer M, Frie C, Paulsson M, Edgar D (1999) Absence of basement membrane after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation. J Cell Biol 144:151–160

Stark HJ, Baur M, Breitkreutz D, Mirancea N, Fusenig NE (1999) Organotypic keratinocyte cocultures in defined medium with regular epidermal morphogenesis and differentiation. J Invest Dermatol 112:681–691

Stark HJ, Willhauck MJ, Mirancea N, Boehnke K, Nord I, Breitkreutz D, Pavesio A, Boukamp P, Fusenig NE (2004) Authentic fibroblast matrix in dermal equivalents normalizes epidermal histogenesis and dermo-epidermal junction in organotypic co-culture. Eur J Cell Biol 83:631–645

Sugawara K, Tsuruta D, Ishii M, Jones JC, Kobayashi H (2008) Laminin-332 and laminin-511 in skin. Exp Dermatol 17:473–480

Tennenbaum T, Weiner AK, Belanger AJ, Glick AB, Hennings H, Yuspa SH (1993) The suprabasal expression of alpha 6 beta 4 integrin is associated with a high risk for malignant progression in mouse skin carcinogenesis. Cancer Res 53:4803–4810

Timpl R, Brown JC (1996) Supramolecular assembly of basement membranes. Bioessays 18:123–132

Tomakidi P, Mirancea N, Fusenig NE, Herold-Mende C, Bosch FX, Breitkreutz D (1999) Defects of basement membrane and hemidesmosome structure correlate with malignant phenotype and stromal interactions in HaCaT-ras transplants. Differentiation 64:263–275

Tomakidi P, Stark HJ, Herold-Mende C, Bosch FX, Steinbauer H, Fusenig NE, Breitkreutz D (2003) Discriminating expression of differentiation markers evolving in transplants of benign and malignant human skin keratinocytes through stromal interactions. J Pathol 200:298–307

Villone D, Fritsch A, Koch M, Bruckner-Tuderman L, Hansen U, Bruckner P (2008) Supramolecular interactions in the dermo-epidermal junction zone: anchoring fibril-collagen VII tightly binds to banded collagen fibrils. J Biol Chem 283:24506–24513

Watt FM (2002) Role of integrins in regulating epidermal adhesion, growth, and differentiation. EMBO J 21:3919–3926

Wilhelmsen K, Litjens SH, Sonnenberg A (2006) Multiple functions of the integrin alpha6beta4 in epidermal homeostasis and tumorigenesis. Mol Cell Biol 26:2877–2886