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REVIEW

Basement membranes and human disease

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Abstract In 1990, the role of basement membranes in human disease was established by the identification of *COL4A5* mutations in Alport's syndrome. Since then, the number of diseases caused by mutations in basement membrane components has steadily increased as has our understanding of the roles of basement membranes in organ development and function. However, many questions remain as to the molecular and cellular consequences of these mutations and the way in which they lead to the observed disease phenotypes. Despite this, exciting progress has recently been made with potential treatment options for some of these so far incurable diseases.

Keywords Basement membrane · Laminin · Collagen · Nidogen

Abbreviations

BM	basement membrane
DDR1	discoidin domain receptor I
DDSH	dyssegmental dysplasia Silverman-Handmaker type
DEJ	dermal-epidermal junction
EB	epidermolysis bullosa
ER	endoplasmic reticulum
GBM	glomerular basement membrane
NMJ	neuromuscular junction
SJS	Schwartz-Jampel syndrome
VEGF	vascular endothelial growth factor

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Introduction

Basement membranes (BM) are specialized extracellular matrices that provide tissue structure and influence cell behaviour. They are present throughout the body and form compartments within tissues by separating endothelial and epithelial cells from underlying mesenchyme. In general, BMs are composed of collagens, perlecan, nidogens and laminins but individual BMs differ in their composition leading to an abundance of diverse interacting partners and added complexity. Consequently, BMs are crucial to life and mutations in their components lead to a wide variety of clinical phenotypes affecting different organs. In this review, we will cover, after a short overview of the main BM components, the current knowledge of diseases caused by abnormalities of these molecules in the eye, kidney, vasculature, skin and neuro-muscular junction.

Laminins

Laminin is, besides collagen type IV, the most abundant BM component and is present in all BMs. A laminin network links the BM to the cell surface through interactions with cell surface receptors that enables it to influence cell signalling and behaviour, in addition to

providing a structural role. Laminins are heterotrimeric glycoproteins consisting of an α, β and γ chain; the 5 α, 3 β and 3 γ chains are encoded by the genes *LAMA1-5*, *LAMB1-3* and *LAMC1-3* (Durbeej 2009). The chains can assemble into at least 15 laminin proteins (Miner 2008) named in the new nomenclature according to their composition, such that LM-111 consists of the α 1, β 1 and γ 1 chains (Aumailley et al. 2005). As some chains are components of multiple laminins, e.g. laminin α5 chain is a component of LM-511, LM-521 and LM-523, characterizing the role of individual laminins is difficult as mutations may affect multiple laminins.

Generally, laminins are cruciform-shaped proteins (Fig. 1), although rod- or Y-shaped laminin molecules occur (Durbeej 2009). The N-terminal end of the chains contains globular domains separated by laminin epidermal-growth-factor-like repeats (LEa, LEb, LEc). All chains contain laminin N-terminal (LN) domains, which are important for laminin network formation. The α chain also contains the L4a and L4b globular domains, whereas the β and γ chains contain the LF and L4 domains, respectively. The central coiled-coil region is flanked by the C-terminal end of the α chain, which forms the globular LG domain consisting of five LG domains that mediate cell adhesion through binding receptors including integrins, dystroglycan, syndecan and heparin (Miner 2008). These interactions can be modulated by the proteolytical cleavage of LG domains 3–5.

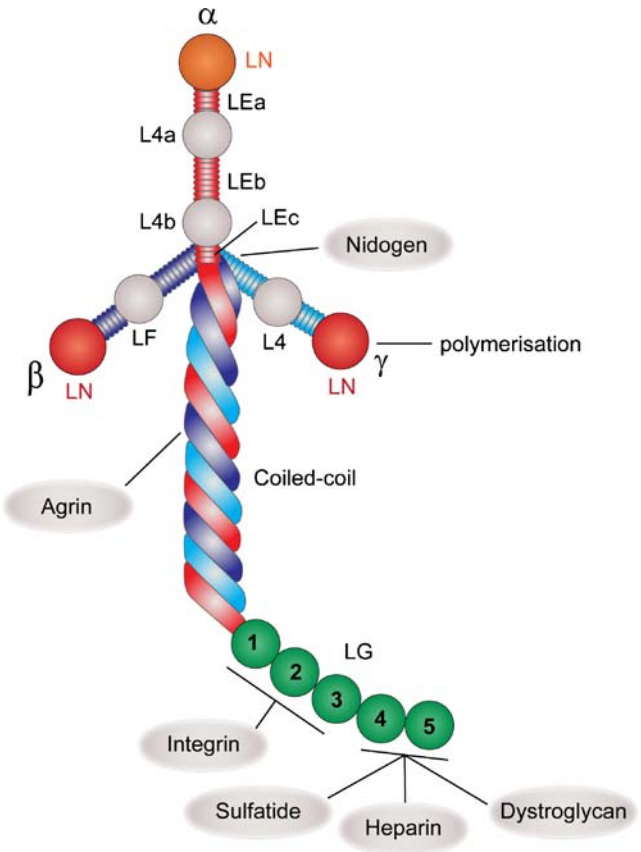

Fig. 1 Representation of the general cruciform shape of laminin proteins. Rod-shaped and Y-shaped laminin occur because of the incorporation of truncated α3 and α4 and of γ2 chains, respectively. The globular domains at the N-terminal end of the chains are separated by laminin epidermal-growth-factor-like repeats (LEa, LEb, LEc). Laminin N-terminal (LN) domains, which are important for laminin network formation, are present in all chains. The α chain contains the L4a and L4b globular domains. The β and γ chains contain the LF and L4 domains, respectively. The C-terminal end of the α chain forms the globular LG domain consisting of five LG (numbered 1–5)

Because laminin, collagen type IV, nidogen and perlecan are key BM components, one can imagine that aberrations in any of these components prohibit BM formation. The absence of BM formation in mice deficient for laminin γ1 or β1 (Miner et al. 2004; Smyth et al. 1999) shows indeed that laminin is required for initial BM formation and early development. In contrast, collagen type IV (Poschl et al. 2004), nidogen (Bader et al. 2005) and perlecan (Arikawa-Hirasawa et al. 1999) are required for BM maintenance rather than initial formation. Laminin's role in development is not limited to early stages as both the interaction between nidogen and laminin γ1, and laminin α5 are required for kidney formation (Miner and Li 2000; Willem et al. 2002). In addition, laminin α5 plays a role in the development of placenta, brain, limb and lung (Miner 2008) and elegant rescue experiments have begun to address the way in which laminin α5 functions. These data have shown that, during development, its role is mediated through the LG1-2 domains, whereas the LG3-5 domains function in the glomerular filtration barrier (Kikkawa and Miner 2006).

One of the central concepts observed following laminin deficiency is the induced expression of other laminins. The effectiveness of this for organ function depends on the redundancy of the various laminin molecules as illustrated by the successful compensatory expression in notochord development of laminin α4 following laminin α1 deficiency in zebrafish (Pollard et al. 2006). However, this compensation may not always restore organ function, as laminin β1 expression in the glomerular basement membrane (GBM) of *Lamb2*$^{-/-}$ mice does not rescue filtration barrier function, despite producing a structurally intact BM (Noakes et al. 1995b).

BMs underlying stratified epithelia, such as skin, contain laminins with α3 and α5 chains (Aumailley et al. 2006), which form LM-332 (previously known as laminin 5) and LM-311 (laminin 6), which together with the ectodomain of collagen XVII constitutes the anchoring filaments (Nonaka et al. 2000; Rousselle et al. 1991). LM-511 (laminin 10), which is more ubiquitous than the preceding laminins, is also present at the dermal-epidermal junction (DEJ). The N-termini of LM-332 binds to collagen VII, a major component of the anchoring fibrils thereby connecting

anchoring filaments with fibrils. In contrast to other laminin molecules, LM-332 may rely on the association with LM-331 to form oligomers, as it can not self-polymerize (Aumailley et al. 2006). In addition, nidogen 1 and fibulin-1 and -2 can bind the recombinant N-terminal region of the γ2 chain of LM-332 (Sasaki et al. 2001). These interactions may be important for the integration of LM-332 into the extracellular matrix before maturation of the γ2 chain (Gagnoux-Palacios et al. 2001), as a substantial portion of the N-terminus of the γ2 chain is cleaved in human adult skin (Tunggal et al. 2002).

Collagens

Collagens are the major structural component of the extracellular matrix but are also important for tissue architecture organization and cellular processes such as adhesion and migration. The interactions of collagens with extracellular matrix proteins and cell surface receptors are important during processes such as development, growth, tissue remodelling and disease pathologies. Currently, 28 different types of collagen are known, which can be grouped into subfamilies such as fibrillar collagens, fibril-associated collagens with interrupted triple helices, transmembrane collagens and network-forming collagens (Myllyharju and Kivirikko 2004; see also Gordon and Hahn 2009, in this issue). All collagen proteins contain one or more collagenous domains consisting of GLY-X-Y amino acid repeats and many human mutations affect this glycine residue, which is crucial for the formation of the triple-helical structure of collagen (Myllyharju and Kivirikko 2004).

Collagen type IV

Collagen type IV is the most abundant structural BM component and vertebrates express six α(IV) chains, encoded by the genes *COL4A1-COL4A6*, which are genomically organized into pairs in a head-to-head fashion (*COL4A1* with *COL4A2*, *COL4A3* with *COL4A4*, and *COl4A5* with *COL4A6*). In each alpha chain, the central collagen domain is flanked by an N-terminal 7S domain and a C-terminal non-collageneous domain (NC1; Fig. 2a; Khoshnoodi et al. 2008).

In the endoplasmic reticulum (ER), three alpha chains fold to form a heterotrimeric molecule, called a protomer, and despite the 56 possible combinations, only three different protomers, α1.α1.α2(IV), α3.α4.α5(IV) and α5.α5.α6(IV), have been described. Protomer formation occurs through the interaction of three NC1 domains and protomer composition is determined by the relative affinity of NC1 domains for the different alpha chains and the presence of recognition sites on the NC1 domains (Khoshnoodi et al. 2006). Following establishment of the NC1 trimer, triple-helix formation occurs in a zipper-like fashion and protomer secretion occurs. Once secreted, a complex set of interactions takes place between protomers forming a collagen type IV network in the shape of a lattice. Two protomers interact to form a dimer with a NC1 hexamer and four protomers interact through interactions between residues of the 7S domains. Finally, triple-helical domains can also interact with each other (Khoshnoodi et al. 2008).

The collagen type IV networks are differentially expressed, with α1.α1.α2(IV) being the most widely expressed throughout embryonic development. The onset of α3.α4.α5(IV) and α5.α5.α6(IV) expression occurs later in development and in some renal and testicular BMs α3.α4.α5(IV) replaces α1.α1.α2(IV) (Harvey et al. 2006; Kalluri et al. 1997).

Despite being a predominant component of all BMs, collagen type IV is not required for BM formation but is necessary for BM maintenance (Poschl et al. 2004). The important structural role of collagen type IV is illustrated by the clinical consequences of collagen IV mutations (see below) and by the defective placental structure and haemorrhaging, attributable to BM defects, in α1.α1.α2 (IV)-deficient mice (Poschl et al. 2004).

In addition, collagen type IV mediates cell adhesion to the BM through binding to integrin and non-integrin receptors. Collagen type IV interacts mainly with β1 integrin receptors (Leitinger and Hohenester 2007), in particular $\alpha_2\beta_1$ and $\alpha_1\beta_1$ integrin, through sequences in the collagen and NC1 domains (Khoshnoodi et al. 2008; Fig. 2a). Integrin binding is important during angiogenesis as the NC1-derived fragments of α1(IV) (arresten), and α3 (IV) (tumstatin) rely on $\alpha_1\beta_1$ and $\alpha_v\beta_3$ integrin interactions, respectively, for their anti-angiogenic capabilities (Mundel and Kalluri 2007). Finally, during angiogenesis, exposure of cryptic integrin-binding sites may occur because of the proteolytic degradation or denaturation of collagen type IV, and antibodies against these sites may inhibit angiogenesis (Xu et al. 2001).

In addition to integrin receptors, collagen type IV binds DDR1 (discoidin domain receptor I), a tyrosine kinase receptor that becomes phosphorylated following collagen binding (Leitinger and Hohenester 2007). The physiological importance of the interaction is underlined by the various renal phenotypes including proteinuria and GBM thickening in DDR1-deficient animals (Gross et al. 2004), similar to phenotypes relevant to collagen type IV pathology (see below).

Collagen types XVIII and XV and endostatin

Collagen type XVIII belongs to the multiplexin (multiple triple-helix domains with interruptions) subfamily of collagen proteins and is a heparan sulphate proteoglycan consisting of a protein core with negatively charged

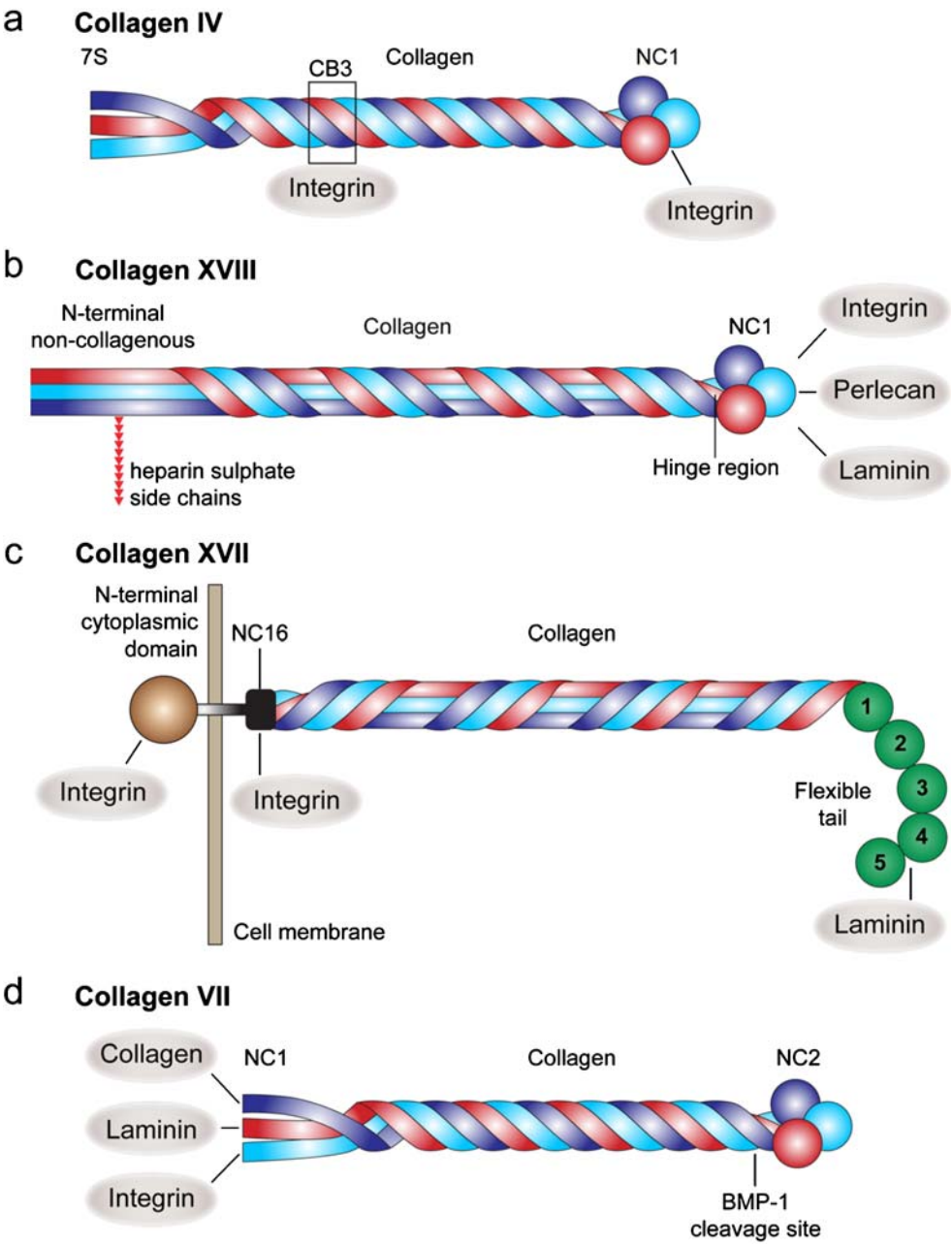

Fig. 2 Schematic diagram of basement membrane (BM) collagens. **a** Collagen type IV protomer structure consists of a central collagen domain flanked by the N-terminal 7S domain and C-terminal NC1 (non-collageneous 1) domain. Interactions with integrin receptors occur through sequences in the GB3 domain and NC1 domain. **b** The central collagen domain of collagen type XVIII contains interruptions in the Gly-X-Y repeat and is flanked by the N-terminal non-collagenous domain and a C-terminal NC1 domain harbouring the endostatin fragment. The N-terminal domain contains the heparan sulphate side chains. **c** The intracellular domain of collagen type XVII contains four tandemly arranged 24-amino-acid repeats and four membrane-proximal cysteine residues. The ectodomain contains interrupted collagenous domains, the NC16a domain which harbours the ADAMS cleavage site, and a flexible tail. **d** Collagen type VII has a central collagen domain flanked by the N-terminal NC1 domain and the NC2 domain, which is proteolytically cleaved

heparan sulphate side chains. Besides perlecan and agrin, it is the third main heparan sulphate proteoglycan of the BM and is structurally similar to the chondroitin sulphate proteoglycan collagen type XV (Iozzo 2005).

Collagen type XVIII is expressed in BMs underlying epithelial and endothelial cells and has three isoforms, with different N-terminal fragments, that exhibit specific expression patterns (Marneros and Olsen 2005). Collagen type XVIII displays a polarized position in BMs, as its C-terminal domain is embedded in the lamina densa, where it interacts with perlecan and laminins, whereas in most BMs, the N-terminal domain extends into the matrix below the lamina densa of the BM (Elamaa et al. 2005).

The central domain of collagen type XVIII consists of collagenous repeats separated by non-collagenous regions. This central domain is flanked by an N-terminal domain with thrombospondin-1-like and frizzled motifs, which can inhibit Wnt signalling (Quelard et al. 2008), and a C-terminal trimerization domain, which harbours the fragment endostatin, also present in collagen type XV (Sasaki et al. 2000). The release of endostatin, through cleavage of the hinge that separates the C-terminal domain from the triple-helical domain, occurs in vivo as endostatin can be detected in plasma and tissue extracts (Iozzo 2005). Endostatin has been heavily investigated because of its anti-angiogenic activity (Iozzo 2005), although the absence of a difference

in tumour growth in endostatin-deficient mice challenged with fibrosarcoma and melanoma (Fukai et al. 2002) has questioned the anti-angiogenic role of endogenous endostatin. However, evidence showing increased vascular outgrowth in aortic explant cultures (Li and Olsen 2004) and increased levels of corneal neovascularization (Marneros et al. 2007) in *Col18a1*$^{-/-}$ mice do support an anti-angiogenic role for endogenous endostatin relying at least in part on binding heparan sulphate proteoglycans such as perlecan and $\alpha_v\beta_5$-integrin (Iozzo 2005).

In contrast to the C-terminal end, the function of the N-terminal domain is much less characterized but analysis of the inner limiting membrane in eyes suggests it may function in anchoring the BM to underlying structures (Fukai et al. 2002).

Despite their structural similarity, collagen type XVIII and XV have separate biological roles, as *Col15a1*$^{-/-}$ animals display muscle defects (Eklund et al. 2001) compared with the eye phenotype of *Col18*$^{-/-}$ mice (see below).

Epithelial-specific collagen types VII and XVII

Collagen types VII and XVII and their ligand LM-332 occur mainly in BMs below stratified squamous epithelia in skin and mucous membranes of the orifices, where they function as components of the hemidesmosome-anchoring filament–anchoring fibril adhesion complex, which attaches the epithelium to the underlying dermal mesenchyme.

Collagen type XVII belongs to the family of transmembrane collagens and is a homotrimer of α1(XVII) chains (Fig. 2c; Franzke et al. 2005). The intracellular domain of collagen type XVII co-localizes and interacts with the intracellular tail of integrin β4, BP230 and plectin (Hopkinson and Jones 2000; Koster et al. 2003) in the cytoplasmic plaque of hemidesmosomes. The ectodomain of human collagen type XVII contains collagenous domains (Col1-15) interspaced by non-collagenous (NC1 to NC16) segments and its tertiary structure is longitudinal with a C-terminal flexible tail (Franzke et al. 2005; Fig. 2c). Sheddases of the ADAMS (a disintegrin and metalloproteinase) family release the ectodomain from the cell surface by cleavage within the NC16 domain (Franzke et al. 2005), which can also interact with the extracellular stalk of integrin α6 (Hopkinson and Jones 2000), at least in vitro. The ectodomain of collagen type XVII co-localizes with LM-332 in the anchoring filaments, and its C-terminus reaches the lamina densa (Nonaka et al. 2000) and binds to LM-332 (Tasanen et al. 2004), suggesting that the C-terminal end of collagen type XVII is important for the spatial orientation of LM-332 and its localization in the collagen type IV-containing lamina densa.

Collagen type VII is the largest collagen and the main structural component of the anchoring fibrils that attach the BM to the papillary dermis in the skin (Aumailley et al. 2006). It is synthesized as a proα1(VII) polypeptide chain that contains an amino-terminal globular domain named NC-1, a central triple-helical domain and a small C-terminal globular domain termed NC-2 (Fig. 2d). The pro-collagen type VII molecule is a homotrimer of proα1(VII)-chains and each pro-collagen molecule forms an anti-parallel dimer, with a short C-terminal overlap and disulphide-bonding (Chen et al. 2001). Finally, dimers aggregate laterally to form the anchoring fibrils. During the biosynthesis and the supra-molecular aggregation of the fibrils, pro-collagen type VII is processed to mature collagen via proteolytic processing of the NC-2 domain by bone morphogenic protein-1 (BMP-1) (Rattenholl et al. 2002). Both keratinocytes and fibroblasts can synthesize collagen type VII, and interactions between collagen VII and LM-332 (Chen et al. 1997; Rousselle et al. 1997 or banded collagen fibrils (Villone et al. 2008) in the skin stabilize the DEJ.

Perlecan

Perlecan (HSPG2) is a large heparan sulphate proteoglycan (see also Schäfer and Schäfer 2009 in this issue) that is present in BMs. It can also be found at the cell surface and in tissues such as cartilage and connective tissue stroma (Iozzo 2005; Schäfer and Schäfer 2009). Numerous binding partners have been identified including other BM components (nidogen and collagen type IV), integrins and growth factors (Fig. 3), which may underlie some of the functions of perlecan such as cell and growth factor signalling, direction of neurite outgrowth, ligand internalization signal and BM maintenance (Schäfer and Schäfer 2009).

Perlecan consists of five domains (Fig. 3). Domain I is the attachment site for the heparan sulphate chains, whereas domain II is homologous to the low-density-lipid receptor ligand-binding domain. Domain III has laminin-like domain modules and epidermal growth factor (EGF)-like repeats, whereas domain IV is important for BM integration by binding other BM components (Hopf et al. 1999). The C-terminal domain V contains the fragment endorepellin (Iozzo 2005).

Analysis of perlecan functions has been greatly aided by the generation of the perlecan knock-out mouse model, which develops a lethal multi-organ phenotype affecting the heart, brain and cartilage development (Costell et al. 1999) underscoring the importance of perlecan for development. A viable mouse model (Hspg2$^{\Delta 3/\Delta 3}$) harbouring a deletion of exon 3, which greatly reduces the amount of heparan sulphate side chains (Tran et al. 2004), has enabled the elucidation of the relative contributions of the core protein versus the side chains to perlecan function.

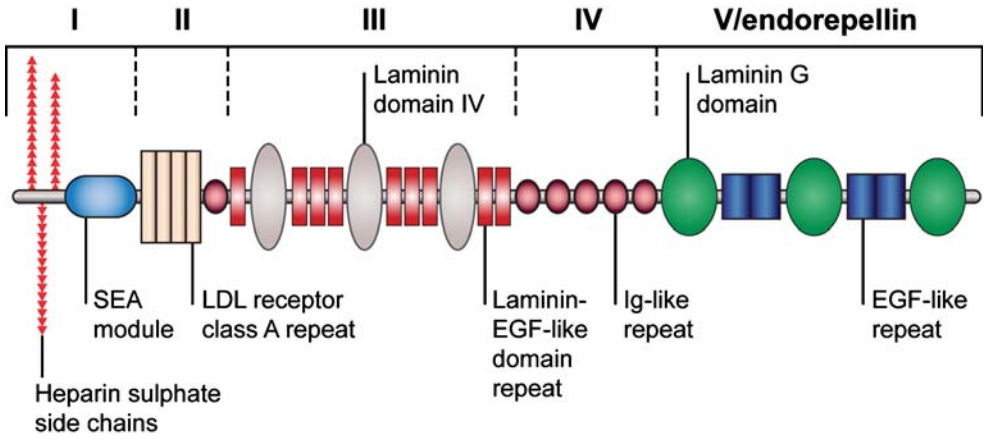

Fig. 3 Representation of perlecan structure. Perlecan domain I is the attachment site for the heparan sulphate chains and contains a *SEA* (sperm protein, enterokinase, agrin) module and three closely spaced SGD (Ser-Gly-Asp) tripeptide sequences. Domain II is homologous to the low-density-lipid (*LDL*) receptor ligand-binding domain, whereas domain III has three laminin-like domain modules and eight epidermal growth factor (*EGF*)-like repeats. Domain IV contains N-CAM-like Ig repeats and binds other BM components. Domain V consists of laminin-like globular domains separated by four EGF-like repeats

Similarly to the C-terminal fragments of collagens type IV (tumstatin, arresten), XV and XVIII (endostatin), endorepellin possesses anti-angiogenic activity (see also Pihlajaniemi and Soininen 2009 in this issue), both in vitro and in vivo, which is mediated through the interaction with $\alpha_2\beta_1$ integrin (Iozzo 2005; Woodall et al. 2008). In contrast to endorepellin, perlecan itself seems to support tumour and developmental angiogenesis, as it promotes tumour growth (Iozzo 2005), and perlecan knockdown in zebrafish affects the sprouting of intersegmental vessels (Zoeller et al. 2008). This activity seems to be mediated through the binding of fibroblast growth factors by its heparan sulphate side chains or core protein (Aviezer et al. 1994; Mongiat et al. 2000; Sharma et al. 1998; Zhou et al. 2004).

Nidogen

All BMs contain nidogen and vertebrates express two nidogens, nidogen 1 and nidogen 2, which are sulphated glycoproteins. Although in some BMs, e.g. kidney and blood vessels, both proteins co-localize, the expression of nidogen 2 is generally more restricted than that of nidogen 1 (Ho et al. 2008).

Nidogens consist of three globular domains (G1-3) connected by rod-like or thin segment domains (Ho et al. 2008), which can interact with BM components and integrin receptors (Fig. 4). The ability to bind perlecan, laminin and collagen type IV (Gerl et al. 1991; Mayer et al. 1993) originally led to the hypothesis that nidogen acts as an essential bridge between BM components (Ho et al. 2008). However, this has been challenged by the results that a loss of function mutation of nidogen in *Caenorhabditis elegans* does not affect BM assembly (Kang and Kramer 2000; Kim and Wadsworth 2000) and that *Nid1*$^{-/-}$ mice have a normal BM structure and no pathological features (Murshed et al. 2000). The presence of cardiac, kidney, lung and limb defects in perinatal lethal *Nid1*$^{-/-}$*Nid2*$^{-/-}$ mice associated with BM defects (Bader et al. 2005; Bose et al. 2006) has shown that nidogen 2 compensates for the absence of nidogen 1 (Bader et al. 2005).

At least some of the functions of nidogen are mediated through its interacting partners. Deletion of the nidogen-binding site in laminin γ1 (formerly known as γ1III4, but γ1LEb3 in the new nomenclature) has demonstrated that nidogen functions mediated through laminin are important for lung and kidney development, as both γ1LEb3 and *Nid1*$^{-/-}$ *Nid2*$^{-/-}$ mice exhibit delayed lung development (Bader et al. 2005; Willem et al. 2002) and renal agenesis has been observed in γ1LEb3 mice (Willem et al. 2002). In limb development, nidogen might function through perlecan, as limb defects in *Nid1*$^{-/-}$*Nid2*$^{-/-}$ mice are associated with aberrant FGF8 signalling (Bose et al. 2006) and perlecan plays a role in growth factor signalling. The observation of defective BMs in these models shows that nidogen 1 and the laminin nidogen interaction are not required for BM formation but are necessary for the maintenance of particular BMs but not for other BMs (Kang and Kramer 2000; Kim and Wadsworth 2000; Mokkapati et al. 2008). The necessity of nidogen for BM function might depend on the composition of individual BMs and/or compensation by other BM proteins. Detailed investigation of animal models should enable the elucidation of this important question and may identify putative disease processes caused by nidogen abnormalities as, thus far, no nidogen mutations have been described in human disease.

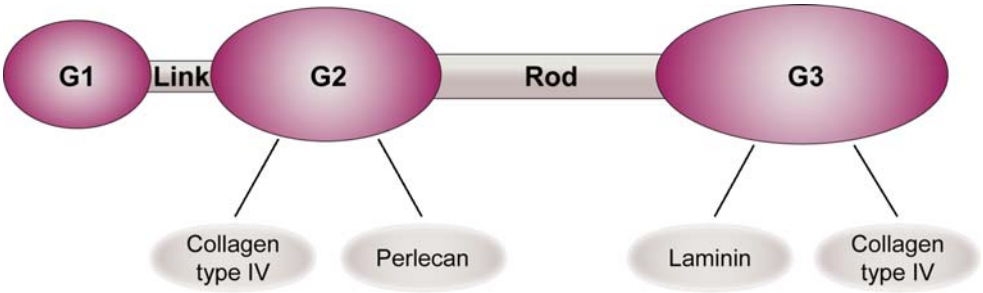

Fig. 4 Nidogen consists of globular domains, important for interaction with other BM molecules, separated by rod-like domains

Human diseases caused by mutations in BM components

Mutations in BM proteins result in altered phenotypes in a few organs but the molecular disease mechanisms are still not fully understood. The restricted number of affected tissues is perhaps surprising considering the wide expression patterns of BM proteins. Future research will probably disclose phenotypic functional variations that depend on the exact location of the mutation within the BM molecule, e.g. at ligand-binding sites, and on the role of genetic modifiers in the various organs and diseases. The current knowledge of BM diseases and their mouse models are summarized below (Tables 1 and 2).

Kidney

The kidney contains three BMs; a tubular BM, a glomerular BM (GBM) and the BM of Bowman's capsule, in addition to the vascular BMs. The GBM is an integral part of the filtration apparatus, as it separates the glomerular endothelial cells from the podocytes (specialized epithelial cells) and their foot processes. In addition to structural support, the GBM plays an important functional role in the filtration process by forming an albumin barrier (Jarad et al. 2006).

Alport's syndrome, thin basement nephropathy and diffuse leiomyomatosis

Alport's syndrome affects mainly the kidney, with haematuria, interstitial nephritis and progressive renal failure as major symptoms, although patients can also develop sensorineural deafness, lenticonus of the anterior capsule (a protrusion of the lens) and dot fleck reflection (retinopathy; Gubler 2008; Hudson et al. 2003). Kidneys display progressive thickening of the GBM, resulting in splitting and multi-lamellations, and areas of BM thinning leading to a basket-weave appearance.

Alport's syndrome is caused by *COL4A5*, *COL4A3* or *COL4A4* mutations. *COL4A3* or *COL4A4* mutations only impact on the α3.α4.α5(IV) network but *COL4A5* mutations affect both the α3.α4.α5(IV) and α5.α5.α6(IV) networks (Gubler 2008). Inheritance can be X-linked (*COL4A5* mutations, 85% of all cases), autosomal recessive (*COL4A3* or *COL4A4* mutations, 10%–15%) or autosomal dominant (*COL4A3* or *COL4A4* mutations, 0%-5%; Hudson et al. 2003). Deletion, insertion, splice site and missense mutations have been described and the majority of missense mutations affect the glycine residues of the Gly-X-Y repeat. Patients with the autosomal recessive disease carry compound heterozygous or homozygous *COL4A3* or *COL4A4* mutations (Gubler 2008; Hudson et al. 2003).

The age of onset and phenotypic severity of Alport's syndrome is variable and depends on the inheritance pattern, and type and location of the mutation. In general, early onset end-stage renal disease is observed in autosomal recessive Alport's syndrome, whereas the autosomal dominant form may not progress to end-stage renal disease (Hudson et al. 2003). In addition, insertion and deletion *COL4A5* mutations result in a juvenile age of onset, whereas the position of missense mutations affects the age of onset and disease severity (Gubler 2008). Finally, X-chromosome inactivation greatly influences severity in female carriers of X-linked Alport's syndrome and lends the disease a wide range of phenotypes (Gubler 2008). The main causative mechanism in Alport's syndrome is widely regarded as being the absence of the α3.α4.α5(IV) network in the adult mature GBM, as observed by a lack of immunostaining (Gubler 2008). This can also be found in female carriers of *COL4A5* mutations and in female *Col4a5*$^{-/-}$ mice, which have a characteristic mosaic expression pattern attributable to X-chromosome inactivation.

In contrast to the α1.α1.α2(IV) network in the fetal GBM, the α3.α4.α5(IV) network of the mature GBM has a higher degree of crosslinking and resistance to proteolysis (Kalluri et al. 1997), a characteristic that may be required to cope with the increased stress on the adult kidney. This hypothesis is supported by the presence of structural abnormalities in mature GBM in X-linked Alport's syndrome containing α1.α1.α2(IV) and by filtration defects in *Col4a3*$^{-/-}$ mice in areas of the GBM that are structurally normal (Abrahamson et al. 2007).

Analysis of *Col4a3*$^{-/-}$ mice has revealed that genetic modifiers can influence phenotype severity by inducing the expression of α5.α5.α6(IV) in the GBM, thereby resulting in a delayed age of onset of renal failure (Kang et al. 2006).

Table 1 Basement membrane (BM) protein targets in inherited human diseases

Organ	BM component	Affected gene(s)	Disease
Eye	Collagen type IV	*COL4A3*	Alport's syndrome
		COL4A4	Cataract
		COL4A5	Axenfeld-Rieger anomaly
	Collagen type XVIII	-	Knobloch syndrome
Kidney	Collagen type IV	*COL4A1*	Hereditary angiopathy with nephropathy, aneurysm and cramps (HANAC) syndrome
		COL4A3 *COL4A4*	Alport's syndrome, thin basement membrane nephropathy
		COL4A5	
	Laminin β2	*LAMB2*	Pierson syndrome
Vasculature	Collagen type IV	*COL4A1*	Hemorrhagic stroke
			Porencephaly
			HANAC syndrome
Muscle	Laminin α2	*LAMA2*	Merosin congenital muscular dystrophy
	Perlecan	*HSPG2*	Schwartz-Jampel syndrome
			Dystrophic dysplasia Silverman Handmaker
Skin	Collagen type XVII	*COL17A1*	Junctional epidermolysis bullosa
	Laminin 332	*LAMA3* *LAMB3*	Junctional epidermolysis bullosa
		LAMC2	
		LAMA3	Laryngo-oculo-cutaneous syndrome
	Collagen type VII	*COL7A1*	Dystrophic epidermolysis bullosa

Moreover, although the actual contribution to the phenotype of altered laminin α5 and α1 expression in the GBM of *Col4a3*$^{-/-}$ mice (Abrahamson et al. 2007) remains to be determined, the above data suggest that clinical severity and disease progression in Alport's syndrome is subject to multiple modifiers. This knowledge may shed light on potential treatment options.

Carriers in families with autosomal Alport's syndrome may present with haematuria and BM thinning (Hudson et al. 2003). These symptoms also occur in individuals diagnosed with "thin basement membrane nephropathy (TBMN)" (also known as benign familial haematuria). The clinical similarity may not be surprising as 40% of TBMN cases are caused by *COL4A3* or *COL4A4* mutations (Gubler 2008). Traditionally, TBMN has been considered to be a benign disorder as it often does not progress to renal failure. However, longitudinal analysis of patients has challenged this assumption, since 30% develop chronic renal failure and 20% end-stage renal disease (Voskarides et al. 2007), compared with 24% of autosomal dominant Alport's syndrome patients that develop end-stage renal disease (Marcocci et al. 2009). In addition, heterozygous *Col4a3*$^{+/-}$ mice, which exhibit TBMN, develop chronic renal failure and have a reduced life expectancy (Beirowski et al. 2006). Consequently, these data question the separate entity of TBMN and suggest it could be considered a mild form of Alport´s syndrome (Hudson et al. 2003).

X-linked Alport's syndrome may occur combined with diffuse leiomyomatosis, a disorder characterized by benign smooth muscle tumours leading to oesophageal dysfunction and genital leiomyomas (Hudson et al. 2003). Diffuse leiomyomatosis is caused by deletions affecting both *COL4A5* and the first two exons of *COL4A6* attributable to deletion breakpoints in intron 2 of *COL4A6*. Although the deletions abrogate both α3.α4.α5(IV) and α5.α5.α6 (IV), dominant negative effects are also possible as deletions extending beyond exon 3 of *COL4A6* do not cause diffuse leiomyomatosis (Heidet et al. 1995), indicating that COL4A6 deficiency is not responsible for tumour formation. A further possibility is that the deletions may alter the expression of other genes through the removal of regulatory elements such as silencers/enhancers or non-coding RNAs that might increase cell proliferation (Hammond 2006).

COL4A1 mutations, HANAC syndrome and polycystic kidney disease

The role of α1.α1.α2(IV) in human disease was unknown until the recent analysis of ENU (N-ethyl-N-nitrosourea, a powerful mutagen that results in single-base-pair mutations) mouse models with *Col4a1* mutations enabling the identification of patients with *COL4A1* mutations. In mice, *Col4a1* missense mutations cause a mild glomerulopathy

Table 2 Phenotypes of mouse models of BM components mentioned in text

BM component	Affected gene	Phenotype	Reference
Laminin	*Lama2*	Merosin congenital muscular dystrophy	Patton et al. 2008; Xu et al. 1994a, 1994b
	Lama5	Tooth and lung development, glomerulogenesis polycystic kidney disease	Miner and Li 2000; Shannon et al. 2006
	Lamb1	Early embryonic lethal, lack of BM formation	Miner et al. 2004
	Lamb2	Neuromuscular junction (NMJ) defect, congenital nephrosis, failure to thrive, retinal defects	Libby et al. 1999; Miner et al. 2006; Noakes et al. 1995a, 1995b; Patton et al. 1998
	Lamc1	Early embryonic lethal, lack of BM formation, renal agenesis, delayed lung development	Smyth et al. 1999; Willem et al. 2002
Collagen type IV	*Col4a1*	Anterior segment dysgenesis, glomerulopathy, porencephaly, haemorrhagic stroke	Favor et al. 2007; Gould et al. 2005, 2006, 2007; Poschl et al. 2004; Van Agtmael et al. 2005
	Col4a2	Anterior segment dysgenesis, haemorrhaging, porencephaly	Favor et al. 2007
	Col4a3	Proteinuria, kidney failure, Alport's syndrome, thin basement nephropathy	Abrahamson et al. 2007; Beirowski et al. 2006; Kang et al. 2006
	Col4a5	X-linked Alport's syndrome	Rheault et al. 2004
Collagen type VII	*Col7a1*	Recessive dystrophic epidermolysis bullosa	Fritsch et al. 2008; Heinonen et al. 1999
Collagen type XV	*Col15a1*	Cardiac and muscle phenotype	Eklund et al. 2001
Collagen type XVII	*Col17a1*	Non-Herlitz epidermolysis bullosa, growth retardation	Nishie et al. 2007
Collagen type XVIII	*Col18*	Knobloch syndrome, hydrocephalus, renal filtration defect	Fukai et al. 2002; Hurskainen et al. 2005; Marneros et al. 2004; Utriainen et al. 2004
Perlecan	*HSPG2*	Developmental cardiac and cephalic defects, skeletal dysplasia, exencephaly, vascular defect, Schwartz-Jampel syndrome, dyssegmental dysplasia Silverman-Handmaker type	Costell et al. 1999; Goldberg et al. 2009; Morita et al. 2005; Rodgers et al. 2007; Rossi et al. 2003; Stum et al. 2008; Tran et al. 2004
Nidogen	*Nid1*	No phenotype	Murshed et al. 2000
	Nid 1 Nid2	NMJ maturation, syndactyly, cardiac phenotypes, delayed lung development, BM defects in vasculature	Bader et al. 2005; Bose et al. 2006; Mokkapati et al. 2008

with extensive BM defects in Bowman's capsule (Fig. 5; Van Agtmael et al. 2005), mild BM abnormalities in the GBM and proteinuria (Favor et al. 2007; Gould et al. 2006). Some of these phenotypes have also been observed in HANAC syndrome (hereditary angiopathy with nephropathy, aneurysm and cramps) caused by *COL4A1* missense mutations. The patients exhibit a normal GBM but defects in the BM of Bowman's capsule, tubules and arterioles (Plaisier et al. 2007). Clinically, HANAC patients develop kidney phenotypes including haematuria, reduced glomerular filtration rate (although no kidney failure is observed) and cystic kidney disease, in addition to eye and vascular pathology (see below). Some phenotypes may develop with age, as cystic kidney disease has been observed only in older patients (Plaisier et al. 2007).

Currently, all mutations identified in HANAC syndrome are located in exons 24 or 25 of *COL4A1* (Plaisier et al. 2007). Other *COL4A1* mutations have been described in families with vascular and eye disease (Breedveld et al. 2006; de Vries et al. 2009; Gould et al. 2005, 2006; Shah et al. 2009; Sibon et al. 2007; Vahedi et al. 2007b; van der Knaap et al. 2006; see also below), although whether these patients have renal phenotypes is not known. Analysis of patients and ENU mouse models with *COL4A1* mutations has revealed that the mutations result in a spectrum of phenotypes determined by the individual mutation (Van Agtmael et al. 2005), its location within the gene and genetic and environmental modifiers (Gould et al. 2006, 2007). Mutations affecting the Y residue of the G-X-Y repeat or residues in the NC1 domain result in milder phenotypes than mutations affecting glycine residues (Favor et al. 2007; Van Agtmael et al. 2005). This difference has also been observed at the BM level suggesting that BM defects underlie the phenotypes (Van Agtmael et al. 2005). In this case, the mutation may result in focal absence of $\alpha1.\alpha1.\alpha2$(IV) and/or affect interactions with collagen type IV receptors, such as integrins and DDR1, or other BM components. However, the presence of swollen ER vesicles (Gould et al. 2005; Van Agtmael et al. 2005) and increased expression of chaperones and intracellular retention of mutant protein (Gould et al. 2005, 2007) suggest that ER stress may also play a role in disease

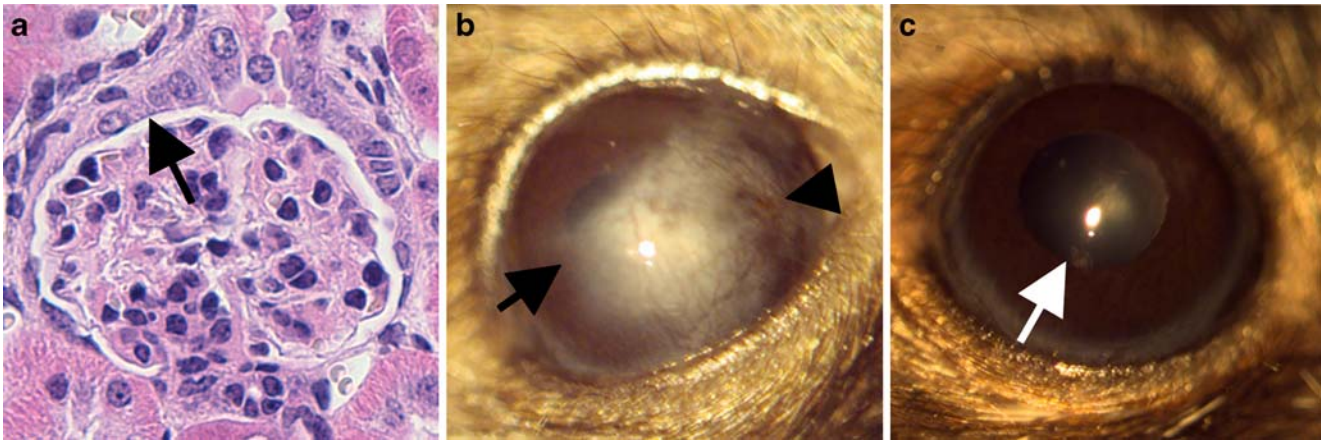

Fig. 5 **a** Col4a1 mutations lead to defects in the Bowman's capsule consisting of hypertrophy of the parietal epithelium (*arrow*). **b** Anterior segment dysgenesis caused by Col4a1 mutation includes corneal opacity (*arrow*) and neovascularization (*arrowhead*). **c** Buphthalmos and iris hypoplasia (*arrow*) can be observed in some animals

pathogenesis. At present, the contributions of ER stress and BM defects to phenotypes remain to be determined. Either way, the mutations act in a dominant fashion as the absence of a phenotype in *Col4a1/Col4a2*$^{+/-}$ (Poschl et al. 2004) mice excludes haploinsufficiency as an underlying mechanism. Moreover, the phenotypes are influenced by genetic modifiers, as the anterior segment dysgenesis (see below) is dependent on genetic background in mice (Gould et al. 2007).

The observations of polycystic kidney disease in HANAC syndrome and in the hypomorphic mouse model of laminin alpha 5 (*Lama5*$^{neo/neo}$; Shannon et al. 2006) have placed emphasis on the role of the BM in cystic kidney disease. However, the mechanisms underlying cyst formation remain elusive and may be different in the two models. Whereas HANAC syndrome patients have altered tubular BMs, suggesting a structural defect (Plaisier et al. 2007), *Lama5*$^{neo/neo}$ mice do not display overt BM abnormalities, leading to the hypthesis that laminin α5 plays a role in tubular epithelium homeostasis (Shannon et al. 2006). Further analysis of these models may reveal novel insights into the pathogenesis of cystic kidney disease.

LAMB2 gene mutations, Pierson syndrome and the GBM as a filtration barrier

Pierson syndrome is an autosomal recessive congenital disorder characterized by microcoria (extremely narrow non-responsive pupils), additional ocular abnormalities and nephrotic syndrome leading to kidney failure before or immediately after birth (Gubler 2008). It is caused by *LAMB2* gene mutations, which result in the absence or severe reduction of laminin β2 in tissues (Zenker et al. 2004). The different missense and nonsense mutations result in great clinical variability, possibly attributable to variations in expression levels, as not all patients with missense mutations have ocular defects (Choi et al. 2008; Hasselbacher et al. 2006).

Because of the better management of renal problems, the life expectancy of patients with Pierson syndrome has increased and revealed the manifestation of a neuromuscular phenotype in some individuals (Maselli et al. 2009; Wuhl et al. 2007). These defects include the invasion of Schwann cells into the synaptic space and structural defects leading to muscle weakness and fatigability (Maselli et al. 2009), symptoms also observed in *Lamb2*$^{-/-}$ mice (Patton et al. 1998). The mice also exhibit congenital nephrosis (Noakes et al. 1995b), retinal defects (Libby et al. 1999) and failure to thrive. Elegant transgenic rescue experiments in *Lamb2*$^{-/-}$ mice have shown that the restoration of renal and synaptic *Lamb2* expression is required for complete recovery and that the NMJ defects cause a failure to thrive (Miner et al. 2006). The congenital nephrosis is characterized by the effacement of podocyte foot processes reflecting the important structural role of laminin β2 in the GBM for anchoring the foot processes (Noakes et al. 1995b). Importantly, proteinuria and GBM permeability preceded podocyte defects in *Lamb2*$^{-/-}$ animals showing that the GBM is an active filtration barrier, in addition to the slit diaphragm present between foot processes (Jarad et al. 2006).

Proteoglycans and kidney function

The presence of mild glomerular, tubular and filtration defects in *Col18a1*$^{-/-}$ mice (Utriainen et al. 2004) and renal abnormalities in Knobloch syndrome (see below; Williams et al. 2008) indicate the importance of collagen type XVIII, a proteoglycan, in kidney function and urine filtration.

The kidney filtration barrier provides both a size- and charge-selective barrier and the GBM and its charge are

considered to be important in this context. The negative charge of the GBM is provided by side chains of the heparin sulphate proteoglycans (Kanwar et al. 1980), perlecan, collagen type XVIII and agrin. Charge barrier dysfunction may contribute to glomerular diseases (Myers et al. 1982) as a loss of heparan sulphate has been observed in diseases such as diabetic nephropathy (Tamsma et al. 1994). This hypothesis is supported by the presence of proteinuria in mice following enzymatic removal of heparan sulphate side chains (Comper and Glasgow 1995) and in HSPG2$^{\Delta 3/\Delta 3}$ mice following administration of super physiological levels of bovine serum albumin (Morita et al. 2005). However, the role of heparan sulphate side chains remains elusive as HSPG2$^{\Delta 3/\Delta 3}$ mice have normal basal kidney function and no pathology under unchallenged conditions (Rossi et al. 2003). In addition, agrin deficiency, which affects the anionic charge of the BM, does not affect the filtration of a negatively charged tracer (Harvey et al. 2007) and HSPG2$^{\Delta 3/\Delta 3}$*Agrn*$^{del/del}$ mice, deficient for both the perlecan heparin sulphate side chain and agrin, do not present with proteinuria (Goldberg et al. 2009). Therefore, the results cast doubt on the role of the GBM in charge selection and suggest a role for the endothelial glycocalyx (Harvey et al. 2007).

Eye

Knobloch syndrome

Knobloch syndrome is an autosomal recessive disease characterized by high myopia (short sightedness), vitreoretinal degeneration, retinal detachment, macular abnormalities and occipital encephalocele. It can be caused by *COL18A1* mutations resulting in truncation or loss of protein because of nonsense-mediated decay (Sertie et al. 2000; Suzuki et al. 2002) but is genetically heterogeneous as additional loci have been identified (Khaliq et al. 2007; Menzel et al. 2004). This genetic heterogeneity combined with genetic modifiers (Utriainen et al. 2004) may underlie the highly variable clinical presentation of Knobloch syndrome.

Because of the rarity of the syndrome and the subsequent lack of human histopathology, most of our knowledge of Knobloch syndrome and its pathways is based on analysis of *Col18a1*$^{-/-}$ mice.

The persistence of the hyaloid vasculature, which is present in the vitreous body during development but regresses subsequently, is a feature of Knobloch syndrome (Duh et al. 2004) and of *Col18a1*$^{-/-}$ mice in which it is associated with defective growth of the retinal vasculature (Fukai et al. 2002). The hyaloid vasculature abnormalities may be attributable to changes in the inner limiting membrane leading to abnormal retinal attachment (Fukai et al. 2002; Hurskainen et al. 2005) and/or a loss of frizzled domain function as observed due to a loss of function of frizzled 5 (Liu and Nathans 2008) and frizzled 4 (Marneros and Olsen 2005). Altered vascular endothelial growth factor (VEGF) signalling (Fukai et al. 2002; Hurskainen et al. 2005) may then cause the retinal vasculature defects, as VEGF mutant animals exhibit persistence of hyaloid vasculature (Stalmans et al. 2002), and VEGF can bind to heparan sulphate suggesting a role for collagen type XVIII in VEGF signalling (Hurskainen et al. 2005). Normal retinal vascularization is driven by hypoxia-induced VEGF expression in astrocytes. The persistent hyaloid vasculature could then cause an absence of hypoxia (Fukai et al. 2002) and/or alterations in endothelial cell or astrocyte function or behaviour (Hurskainen et al. 2005), thereby affecting VEGF signalling and retinal vasculature development.

These vascular defects probably do not underlie the progressive loss of vision in Knobloch syndrome patients and *Col18a1*$^{-/-}$ animals (Marneros and Olsen 2005), as they do not affect retinal perfusion or function (Marneros et al. 2004). A more likely explanation may be the accumulation of deposits between the retinal pigment epithelium and Bruch's membrane, which are also observed in age-related macular degeneration, possibly attributable to BM defects in Bruch's membrane (Marneros et al. 2004).

Eye diseases and collagen type IV mutations

Given the wide expression pattern of α1.α1.α2(IV), it is not surprising that *COL4A1* mutations affect multiple tissues, including the eye. The ocular aspects of HANAC syndrome include retinal tortuosities (a tortuous appearance of the retinal vasculature) and haemorrhaging (Plaisier et al. 2007), which have also been observed in adult small vessel disease and porencephaly caused by *COL4A1* mutations (Gould et al. 2005, 2006), indicating that retinal tortuosities are a common and characteristic phenotype of *COL4A1* mutations. Retinal tortuosities represent one of a number of vascular phenotypes that affect the eye and that include a silvery appearance of the arterioles, retinal bleeding and neovascularization (Favor et al. 2007; Van Agtmael et al. 2005). In addition to the vascular BM, *COL4A1* and *Col4a2* mutations also affect BMs of the anterior structures of the eye resulting in cataract formation and anterior segment dysgenesis including Axenfeld-Rieger anomaly (Favor et al. 2007; Gould et al. 2007; Sibon et al. 2007; Van Agtmael et al. 2005).

Although Alport's syndrome is primarily a renal disease, patients can display ocular phenotypes including dot fleck retinopathy, lenticonus and cataract (Hudson et al. 2003). The formation of cataracts in patients with Alport's syndrome or *COL4A1* mutations indicates that the different collagen type IV networks present in the lens capsule are not redundant. However, the mechanisms underlying

cataract formation remain elusive as both BM defects (Van Agtmael et al. 2005) and ER stress (Gould et al. 2007; Van Agtmael et al. 2005) might contribute to the phenotype. Currently, the manner in which collagen type IV mutations result in the ocular phenotypes is unclear.

Vascular disease and collagen type IV mutations

The vascular BM surrounds smooth muscle cells and separates them from endothelial cells. The importance of BM proteins in vascular disease was identified by the identification of *COL4A1* mutations in porencephaly (cerebral spinal fluid filled cavities caused by haemorrhagic stroke), recurrent cerebral and retinal microbleeds, leukoencephalopathy, retinal tortuosities and ischaemic lacunar infarcts (Breedveld et al. 2006; Favor et al. 2007; Gould et al. 2005; Plaisier et al. 2007; van der Knaap et al. 2006). *Col4a1* mutations also weaken the vessel wall of the major vasculature as shown by intracranial aneurysms in the carotid artery (Plaisier et al. 2007; van der Knaap et al. 2006). These vascular defects may occur in isolation or be part of a wider syndrome affecting the eye and kidneys (Plaisier et al. 2007; Sibon et al. 2007; Vahedi et al. 2007a).

Thus far, no human *COL4A2* mutations have been identified but, in mice, *Col4a2* mutations result in similar but less severe phenotypes than *Col4a1* mutations. The milder phenotype may be attributable to $\alpha1.\alpha1.\alpha2$(IV) network composition, with the mutant alpha chain being present in 50% of protomers, as compared with 75% in the case of a *Col4a1* mutation.

The variable clinical severity of patients with *COL4A1* mutations depends on the type and position of the mutation and on genetic and environmental modifiers (Gould et al. 2006, 2007; Van Agtmael et al. 2005). The reduced penetrance of perinatal cerebral haemorrhaging (Gould et al. 2005) and the increased survival in mouse pups born by Caesarean delivery (Gould et al. 2006) indicate that birth trauma is an environmental risk factor, at least in some cases, as embryonic porencephaly does occur (Favor et al. 2007). In addition, the occurrence of cerebral haemorrhages during sport activities (Vahedi et al. 2007b) also suggests exercise as a risk factor.

These data, combined with the identification of a *COL4A1* mutation in patients without a family history of haemorrhagic stroke (Vahedi et al. 2007b), and the association of extracellular matrix proteins with intracranial aneurysms (Ruigrok et al. 2006) raise the important question of whether collagen type IV plays a role in stroke in general.

Muscle and the neuromuscular junction

Each muscle fibre is ensheathed by a BM and, at the neuromuscular junction (NMJ), a highly specialized BM is present in the synaptic cleft. The BM is not only important for NMJ development, but also for the functions of the NMJ and the muscle.

Merosin-deficient congenital muscular dystrophy

Merosin-deficient congenital muscular dystrophy 1A (MDC1A) is the most frequent form of congenital muscular dystrophy in which patients present with hypotonia, muscle weakness and joint contractures at birth or within the first months of life and is caused by *LAMA2* mutations (Helbling-Leclerc et al. 1995; Xu et al. 1994b). MDC1A patients also display central and peripheral neuropathy including white matter changes and neuromigration and nerve conduction abnormalities probably attributable to myelination defects, which have also been observed in mouse models. The majority of *LAMA2* mutations (Helbling-Leclerc et al. 1995; Xu et al. 1994b) result in the absence or severe reduction of LM-211 staining in the BM, although recently a mouse model has been described carrying a *Lama2* missense mutation with normal Lama2 expression (Patton et al. 2008). In muscle, the dystrophin-glycoprotein complex links the cytoskeleton to the BM through the binding of dystroglycan with the LG domains of laminin $\alpha2$. Similar to mutations that affect the structure of the complex and the glycosylation of dystroglycan (which binds laminin $\alpha2$; Jimenez-Mallebrera et al. 2005), *LAMA2* mutations may result in muscular dystrophy by affecting the interaction between the dystrophin-glycoprotein complex and the BM (Hall et al. 2007; Moll et al. 2001) by the failure to form a laminin network (Colognato and Yurchenco 1999) and by forming an abnormal BM because of defects in LN domain function (Patton et al. 2008). The description of mutations in integrin $\alpha7$, which binds laminin, in muscular dystrophy patients (Jimenez-Mallebrera et al. 2005) underlines the central role of the interactions between laminin and its cell surface receptors. Importantly, improvement of the interaction between laminin and dystroglycan through the administration of linker molecules or agrin ameliorates the dystrophy in mice (Meinen et al. 2007; Moll et al. 2001); this has also been observed through the inhibition of apoptosis by increased BCL2 expression (Dominov et al. 2005; Girgenrath et al. 2004), thereby providing potential therapeutic avenues.

Schwartz-Jampel syndrome and dyssegmental dysplasia Silverman-Handmaker type

Two allelic disorders, Schwartz-Jampel syndrome (SJS) and dyssegmental dysplasia Silverman-Handmaker type (DDSH), are caused by *HSPG2* mutations. SJS is a non-lethal recessive disorder characterized by myotonia (muscle stiffness) with repetitive muscle discharges and

chondrodysplasia (Nicole et al. 2000). DDSH is a neonatal lethal autosomal recessive syndrome characterized by short-limbed dwarfism and encephalocele. The clinical outcome is determined by the amount of perlecan in the tissue, as the absence of perlecan secretion results in DDSH (Arikawa-Hirasawa et al. 2001), whereas reduced secretion and a partially functional perlecan underlies SJS (Nicole et al. 2000).

DDSH and SJS patients and *Hspg2*$^{-/-}$ and *Hspg2*^{C1532Y} mice (carrying a C1532Y *HSPG2* mutation causing SJS) (Arikawa-Hirasawa et al. 1999; Rodgers et al. 2007; Stum et al. 2008) display chondrodysplasia characterized by severe disorganization of chondrocytes and reduced extracellular matrix deposition. The requirement of perlecan for collagen fibrillogenesis in chondroplasts (Kvist et al. 2006) suggests that defective deposition of fibrillar collagen underlies the reduced matrix deposition and that perlecan anchors the BM to connective tissue (Bengtsson et al. 2002).

In the NMJ, acetylcholine excites the muscle and is degraded by acetylcholinesterase (AchE). The clustering of AchE in the NMJ is dependent on perlecan (Arikawa-Hirasawa et al. 2002). The permanent myotonia in SJS patients is caused by reduced perlecan secretion leading to partial AChE deficiency. This may result in re-excitation of muscle leading to myotonia in SJS, and muscle potentiation and a prolonged decay time of the action potential in *Hspg2*^{C1532Y} mice (Stum et al. 2008). In mice, both myotonia and chondrodysplasia phenotype severity is determined by the level of perlecan secretion and may also be influenced by age and genetic modifiers (Rodgers et al. 2007; Stum et al. 2008). However, the spontaneous muscle activity at rest in SJS patients might not be caused by AChE deficiency, as it does not result in spontaneous activity in diaphragm muscle in mice (Stum et al. 2008), but might be attributable to axonal defects (Echaniz-Laguna et al. 2009).

Although NMJ disorganization does not underlie the myotonia in SJS patients, the BM plays a crucial role in NMJ organization, maturation and function (Patton 2003; Sanes 2003). A complete review of this is outside the scope of this article but a few examples will be mentioned. For example, laminin α4 is important for the alignment of pre- and post-synaptic nerve endings (Patton et al. 2001), whereas laminin α5 is required for post-synaptic maturation (Nishimune et al. 2008). *Lamb2* mutations result in pre-synaptic defects (Noakes et al. 1995a) leading to failure to thrive in *Lamb2*$^{-/-}$ mice, a model for Pierson syndrome. In addition, *Col18a1* mutations result in neuromigration and axon guidance defects (Ackley et al. 2001), which may underlie the exencephaly in Knobloch syndrome, and collagen type IV is important for the clustering of synaptic vesicles and the maintenance of mature synapses (Fox et al. 2007).

Skin

The DEJ in skin is an example of a highly complex form of BM and of specific divergence in its structure. This type of BM underlies stratifying squamous epithelia in the skin and mucous membranes of the orifices. It binds the basal cells of the epidermis with the dermis, provides structural adhesion and regulates epithelial-mesenchymal interactions (Aumailley et al. 2006). The DEJ contains ultrastructurally recognizable suprastructures, such as the hemidesmosome complex in the basal cells, the anchoring filaments extending throughout the lamina lucida and the anchoring fibrils emanating into the dermis. The molecular components of hemidesmosomes, anchoring filaments and anchoring fibrils in the DEJ are well characterized and mutations in their genes lead to the diminished adhesion of the epidermis and the dermis and to skin blistering.

Epidermolysis bullosa

The term epidermolysis bullosa (EB) covers genetic skin disorders characterized by muco-cutaneous blistering and chronic epithelial fragility induced by minor shearing forces or trauma (Aumailley et al. 2006). The clinical symptoms vary from mild to severe and include continuous skin blistering, persistent erosions and mucosal involvement, nail dystrophy, alopecia, progressive soft tissue scarring, mutilating deformities and increased risk of epithelial cancer. Complications occur, since many organs are secondarily affected, e.g. the eye, the gastrointestinal, respiratory and urogenital tracts, scarring contractures of the joints and even muscles to some extent.

Depending on the affected molecular suprastructure, four main categories of EB are recognized: EB simplex, junctional EB, dystrophic EB and Kindler syndrome (Fine et al. 2008). Whereas EB simplex and Kindler syndrome result from intracellular anomalies in keratinocytes, the junctional and dystrophic forms can be considered true BM diseases.

Junctional EB

Junctional EB is caused by mutations in the genes encoding LM-332 (*LAMA3*, *LAMB3*, and *LAMC2*), and collagen XVII (*COL17A1*; Kern and Has 2008). Dermal-epidermal separation occurs at the level of the lamina lucida within the BM, and the hemidesmosome-anchoring filament complex appears rudimentary. The most severe EB subtype, the Herlitz variant, results from the absence of LM-332 from the skin because of null mutations in the laminin genes *LAMA3*, *LAMB3*, or *LAMC2*. The majority of mutations are found in *LAMB3* (Varki et al. 2006) and the disease is characterized by congenital muco-cutaneous fragility lead-

ing to widespread blisters and erosions. At some stage, the healing of the skin ceases because of the depletion of epidermal stem cells (Mavilio et al. 2006) and large chronically erosive areas lead to extensive fluid and protein loss, infection and lethal complications within the first year of life.

In less-severe junctional EB cases, the symptoms range from localized to generalized skin blistering, with onset at birth or later in life. These milder cases are caused by missense mutations in *LAMA3*, *LAMB3*, *LAMC2* or *COL17A1* resulting in truncated or misfolded LM-332 or collagen type XII proteins. These may become degraded intracellularly (Bateman et al. 2009) or, if secreted, interfere with ligand interactions between LM-332 and collagen XVII or other extracellular matrix proteins (McGrath et al. 1995; Nakano et al. 2002). Alternatively, misfolded molecules might be subjected to secondary degradation by tissue proteinases (Tasanen et al. 2000).

Rare variants include junctional EB with pyloric atresia and the laryngo-onycho-cutaneous (LOC) syndrome. The former results from mutations of $\alpha_6\beta_4$ integrin, a LM-332 ligand, and the latter from *LAMA3* frame-shift mutations, which abrogate the laminin α3A isoform. This isoform is secreted by the basal keratinocytes of stratified epithelia suggesting that LOC is caused by the dysfunction of keratinocyte-mesenchymal communication (McLean et al. 2003).

Dystrophic EB

Mutations in the gene for collagen type VII, *COL7A1*, cause recessive and dominant forms of dystrophic EB and the spectrum of biological and clinical phenotypes is broad (Fig. 6; Kern and Has 2008). Tissue separation occurs at the level of the anchoring fibrils in the uppermost dermis, and the fibrils, if present, exhibit an altered ultrastructure. In all forms of dystrophic EB, the blisters heal with scarring, i.e. dermal fibrosis. Patients with dominant dystrophic EB have a mild phenotype, mostly limited to their hands and feet, which is usually caused by dominant negative interference by glycine substitutions within the triple-helical domain. Recessive dystrophic EB is caused by homozygosity or compound heterozygosity for two different *COL7A1* mutations of the nonsense, missense or splice-site type. In the majority of severe generalized recessive dystrophic EB cases, both mutations generate premature termination codons leading to nonsense-mediated decay and complete absence of collagen type VII. In this severe EB subtype, progressive blistering leads to chronic wounds, extensive scarring and mitten deformities of hands and feet. Oral and gastrointestinal involvement leads to malnutrition, which, in combination with protein loss through ulcerations, results in growth retardation and anaemia.

Mouse models for EB

Ablation or disruption of genes coding for the α3, β3 or γ2 chains of LM-332, or for the integrin α6 or β4 subunits in mice lead to junctional EB with the absence of hemidesmosomes, severe detachment of the epidermis from the dermis and perinatal death (Aumailley et al. 2006). Deficiency of collagen type XVII leads to a milder phenotype of junctional EB displaying moderate skin blistering, nail dystrophy, alopecia and dental anomalies (Nishie et al. 2007), similar to the human pathologies caused by *COL17A1* mutations. The clinical and morphological characteristics of recessive dystrophic EB in humans are recapitulated in two different mouse models with complete (Heinonen et al. 1999) or partial (Fritsch et al. 2008) deficiency of collagen type VII. The mice with abrogation of LM-332, α6β4 integrin or collagen type VII expression and having phenotypes of severe blistering and perinatal lethality have been helpful for understanding the functional role of these proteins in skin morphogenesis and homeostasis. Mice with milder phenotypes, e.g. the collagen type VII hypomorph or the collagen type XVII knockout mouse, are particularly interesting as they are useful for testing novel, biologically valid, therapeutic strategies, including protein-, cell- and gene-based therapies, which are showing some promising results (Fritsch et al. 2008; Kern et al. 2009; Mavilio et al. 2006).

Acquired autoimmune diseases targeting BMs

Goodpasture's syndrome

Goodpasture's syndrome is an autoimmune disease with anti-GBM antibodies and results in glomerulonephritis and frequently in lung haemorrhaging. The renal injury is the consequence of complement and protease activation, which disrupts the filtration barrier leading to proteinuria, and an immune response ultimately causing fibrosis (Hudson et al. 2003). The antibodies react with two major epitopes in the NC1 domain of the α3(IV) chain. These are located near the junction of the NC1 and triple-helical domains where they are masked by the hexamer structure (Borza et al. 2002). Consequently, antibodies only gain access to the epitopes following hexamer dissociation (Borza et al. 2002). Unmasking of the epitopes may be influenced by additional external factors such as reactive oxygen species (Kalluri et al. 2000) but in vitro evidence also shows that hexamer structure and stability determines, at least in part, the ability of the antibodies to unmask the epitopes (Borza et al. 2005). Moreover, the class of the major histocompatabilty complex is a modifier of the disease (Hudson et al. 2003), as the severity of

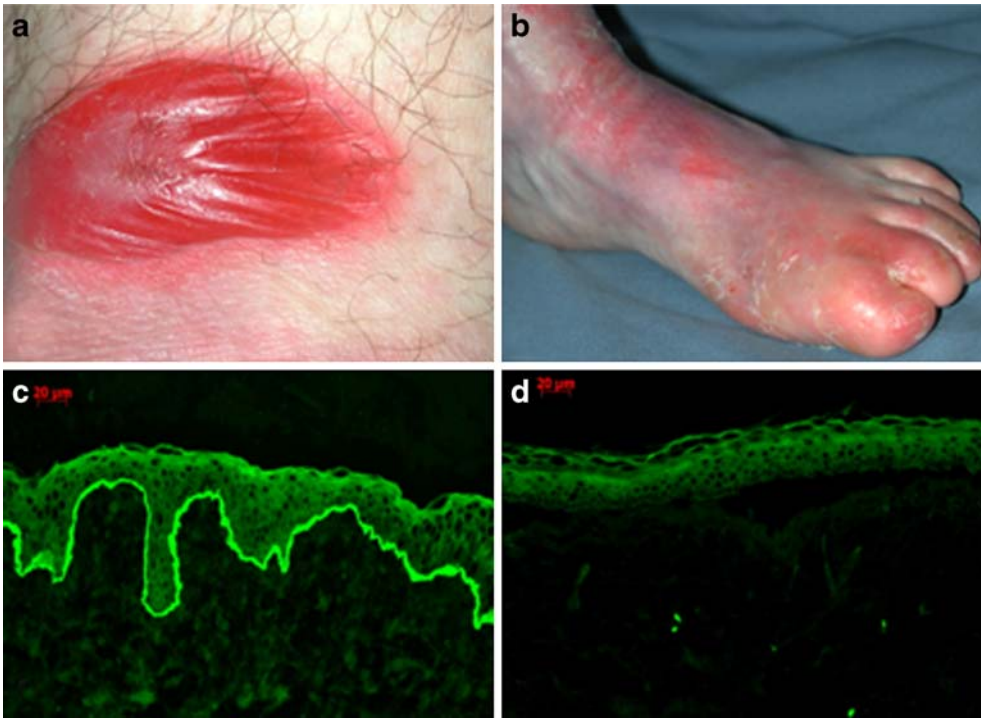

Fig. 6 Dystrophic EB. Consequences of mutations of the epidermal BM in the skin. **a** A trauma-induced skin blister. **b** Scarring, inflammation and loss of nails are consequences of repeated blistering. **c** Immunofluorescence staining of normal human skin with antibodies to collagen type VII. Note the linear signal along the epidermal BM. **d** In the skin of an individual with severe dystrophic EB, collagen type VII is missing as a consequence of nonsense mutations in the *COL7A1* gene

Goodpasture's syndrome is determined by the human leukocyte antigen class.

The production of antibodies against the GBM can also occur in Alport's syndrome patients. Following kidney transplantation, the presence of "foreign" collagen IV chains can cause Alport's post-transplantation anti-GBM nephritis. In autosomal recessive Alport's syndrome, the epitopes are located in the NC1 domains of Col4a3 or Col4a4 (Hudson et al. 2003; Wang et al. 2005), whereas X-linked patients develop alloantibodies targeting epitopes on COL4A5 (Brainwood et al. 1998; Kang et al. 2007). However, in contrast to the cryptic Goodpasture epitopes, the epitopes are accessible in the NC1 hexamer indicating that separate mechanisms may exist (Kang et al. 2007).

Bullous pemphigoid

Bullous pemphigoid (BP) is the most common autoimmune blistering skin disease that typically affects the elderly and is associated with circulating and tissue-bound autoantibodies to collagen XVII. Clinically, tense serous blisters on inflamed skin give rise to erosions and wounds. During the course of the disease, localized blister formation progresses into generalized erosive dermatitis. The pathogenicity of autoantibodies against collagen type XVII in BP is supported by extensive clinical and experimental evidence (Sitaru 2009). The major pathogenic epitopes are located in the juxtamembranous NC16A domain of collagen type XVII (Fig. 2d). The NC16 domain is also the site of cleavage for the ectodomain shedding (Franzke et al. 2009; Franzke et al. 2002) and this shedding and epitope recognition by pemphigoid autoantibodies is regulated by the phosphorylation status of collagen type XVII (Zimina et al. 2008).

EB acquisita

EB acquisita is a rare acquired autoimmune subepidermal blistering disease of the skin and derives its name from the similar phenotype to dystrophic EB (Mihai and Sitaru 2007; Woodley et al. 2007). It is characterized by IgG autoantibodies against epitopes located in the NC1 domain of collagen type VII, the major component of anchoring fibrils. Murine models have demonstrated the pathogenicity of the autoantibodies (Sitaru et al. 2005), which are likely to perturb the functions of ligand-binding sites within the NC1 domain, e.g. for LM-332.

Therapeutic perspectives

The rapid increase of our knowledge concerning the causes and mechanisms of certain BM diseases has allowed the scientific community to embark on the development of molecular therapy strategies. Indeed, many genetic BM disorders are extremely severe and the development of molecular therapy approaches is urgently needed. Although the clinical application of such remedies still lies in the future, new technologies hold promise for individually designed and biologically valid, evidence-based treatments.

Based on our present knowledge, protein-, cell- and gene-based therapies seem possible. However, a large gap clearly remains between laboratory experiments and the successful treatment of a patient. For most diseases, a higher level of understanding of the molecular and cellular consequences of the mutations needs urgently to be acquired. However, despite this, some marked progress has been made.

Skin disorders have been the prime focus of therapy developments, because the target organ is easily accessible for both therapeutic measures and for analysis of their macroscopic, microscopic and molecular effects. Recent studies in animal models have revealed that relatively small biological changes, e.g. moderately increased levels of a missing protein in the skin, can have substantial clinical effects. Therefore, a large number of patients with genetic skin disorders will, at this point, not expect a complete cure but welcome any biologically valid advances that reduce symptoms, improve functionality or increase their quality of life.

A relatively broad spectrum of approaches ranging from protein therapy (Ortiz-Urda et al. 2003; Remington et al. 2009) and viral- and non-viral-mediated gene transfer (Baldeschi et al. 2003; Ortiz-Urda et al. 2002; Seitz et al. 1999) to cell therapy (Fritsch et al. 2008; Kern et al. 2009; Wong et al. 2008) and bone marrow transplantation (Tolar et al. 2009) has been employed in mouse models and single patients. The correction of junctional EB by the transplantation of genetically modified epidermal stem cells in one patient represents the first long-term clinical therapeutic success in genetic BM disease (Mavilio et al. 2006). A cell-therapy approach with intradermal injections of allogeneic wild-type or gene-corrected fibroblasts in mice and humans with recessive dystrophic EB has shown that these cells can increase collagen VII content at the DEJ (Fritsch et al. 2008; Kern et al. 2009; Wong et al. 2008; Woodley et al. 2003). A long-term study with fibroblast treatment of the collagen VII hypomorphic mouse, a model for recessive dystrophic EB, has demonstrated that the high stability of collagen VII contributes to positive long-term effects. The injected fibroblasts synthesize collagen VII for 3–4 weeks and increase its levels to about 35% of normal. The newly synthesized collagen persists in the skin for up to 4 months, stabilizes it substantially against shearing forces and ameliorates the phenotype significantly (Kern et al. 2009) without notable adverse effects. As similar observations have been made in a pilot study in human skin (Wong et al. 2008), intradermal injections of normal fibroblasts may represent a first causal therapy that can be expected to increase the resistance of the skin against external shearing forces and alleviate skin blistering and scarring in trauma-exposed skin areas in patients with dystrophic EB.

Yet another approach has been the direct intradermal injection of recombinant human collagen type VII into the *Col7a1*$^{-/-}$ mouse (Remington et al. 2009). The injected collagen is present as the DEJ but also diffuses into distant sites and persists in the skin for about 2 months. This improves the phenotype, as shown by decreased skin blistering and prolonged survival of the animals. The findings are intriguing as they suggest that injections of monomeric collagen type VII, a component of highly specific supramolecular polymers, can result in phenotypic improvement. However, the way that collagen type VII homes to the DEJ and the length of time that it and its specific aggregates, the anchoring fibrils, would remain stable and functional in the skin remain unclear (Bruckner-Tuderman 2009).

Recent reports have suggested bone marrow/stem cell transplantation as a promising therapeutic approach in mouse models with various forms of EB or in Alport's syndrome. The *Col7a1*$^{-/-}$ mouse, which dies within days after birth if untreated (Heinonen et al. 1999), has been transplanted with SLAM family receptor-positive (CD150+/CD48-) adult bone marrow or mesenchymal stem cells. This leads to the survival of some mice, to skin engraftment of donor cells and to improved skin stability (Tolar et al. 2009). However, collagen type VII has not been detected at the DEJ. Embryonic bone marrow transplantation in *Col7a1*$^{-/-}$ mice results in detectable bone-marrow-derived fibroblasts in the dermis, ameliorates the clinical phenotype of the mice and leads to collagen type VII deposition primarily in the follicular BM (Chino et al. 2008).

Similarly, wild-type bone-marrow-derived cells can ameliorate the phenotype in Alport mice by secreting the missing collagen a3.α4.α5(IV) network (Gross et al. 2009). Circulating bone-marrow-derived cells are thought to be recruited to glomeruli where they can cross the GBM, become podocytes, secrete the missing collagen chains, repair the GBM defects and slow (if not reverse) the disease. Intriguingly, Katayama et al. (2008) have reported that bone marrow transplantation following lethal irradiation with either wild-type or Col4a3$^{-/-}$ bone marrow prolongs the lifespan of Alport mice with similar efficiencies. Sublethal irradiation alone also provides significant benefits (Gross et al. 2009).

Taken together, the exciting advancements in the design of effective therapies, such as those described above, should provide new avenues for research into these incurable diseases. This work will ultimately facilitate the translation of possible treatments to clinics and thus establish suitable therapy protocols for the management of genetic BM diseases.

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