Kevin J. Hamill, PhD, BSc

Submitted for publication March 14, 2014.
Accepted in revised form April 27, 2014.

*Correspondence: Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, University of Liverpool, UCD Building, 3rd Floor, Daulby St., Liverpool L69 3GA, United Kingdom (e-mail: kevin.hamill@liverpool.ac.uk).

Laminins: Roles and Utility in Wound Repair

Valentina Iorio, Lee D. Troughton, and Kevin J. Hamill*

Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom.

Significance: Laminins are complex extracellular macromolecules that are major players in the control of a variety of core cell processes, including regulating rates of cell proliferation, differentiation, adhesion, and migration. Laminins, and related extracellular matrix components, have essential roles in tissue homeostasis; however, during wound healing, the same proteins are critical players in re-epithelialization and angiogenesis. Understanding how these proteins influence cell behavior in these different conditions holds great potential in identifying new strategies to enhance normal wound closure or to treat chronic/nonhealing wounds.

Recent Advances: Laminin-derived bioactive peptides and, more recently, laminin-peptide conjugated scaffolds, have been designed to improve tissue regeneration after injuries. These peptides have been shown to be effective in decreasing inflammation and granulation tissue, and in promoting re-epithelialization, angiogenesis, and cell migration.

Critical Issues: Although there is now a wealth of knowledge concerning laminin form and function, there are still areas of some controversy. These include the relative contribution of two laminin-based adhesive devices (focal contacts and hemidesmosomes) to the re-epithelialization process, the impact and implications of laminin proteolytic processing, and the importance of laminin polymer formation on cell behavior. In addition, the roles in wound healing of the laminin-related proteins, netrins, and LaNts are still to be fully defined.

Future Directions: The future of laminin-based therapeutics potentially lies in the bioengineering of specific substrates to support laminin deposition for *ex vivo* expansion of autologous cells for graft formation and transplantation. Significant recent advances suggest that this goal is within sight.

SCOPE AND SIGNIFICANCE

DEFECTIVE WOUND REPAIR IS a major clinical burden. Developing a greater understanding of the fundamental processes involved in normal and pathogenic wound repair is a prerequisite to the development of new or improved therapeutic approaches. The laminins and their related proteins, the netrins, and the LaNts, have been demonstrated to be involved in regulating core cell behaviors required for wound repair. In

this review, we will describe some of the important findings indicating the specific roles of different laminins in wound re-epithelialization and angiogenesis, and describe some of the ways in which this knowledge is beginning to be exploited for the generation of novel therapeutics.

TRANSLATIONAL RELEVANCE

Laminins, netrins, and LaNts are extracellular matrix (ECM) proteins and that makes them excellent

candidate targets for therapy development, as any treatment modality can be directly applied to wound environments. In practical terms, laminin biology could be translated into therapy through directly replacing the entire protein or mimicking aspects of their signaling activities to promote epithelial migration over a wound bed (re-epithelialization) or to enhance blood vessel growth into the wound microenvironment (angiogenesis). A further potential utility of the research into these proteins would be exploiting the garnered information regarding their normal biological role to functionalize biomaterials for *ex vivo* expansion of epidermal keratinocytes for transplantation purposes.

CLINICAL RELEVANCE

Defective wound repair specifically related to laminins is a feature of the genetic skin fragility disorder junctional epidermolysis bullosa (JEB) and rare, related variant laryngo-onycho-cutaneous syndrome (LOCS).¹⁻⁴ Defective laminin deposition or processing has also been shown to be a feature of the chronic cutaneous ulcers of diabetic patients. In addition, since laminins are critical players in processes associated with normal wound repair, therapeutics based on either enhancing or mimicking laminin functionality could have widespread clinical utility.

BACKGROUND

Laminins are large cross-shaped molecules that are deposited by cells and are assembled along with collagen IV, nidogens, agrin, and perlecan into a

structured region of the ECM, termed the basement membrane (BM).⁵⁻⁷ During homeostasis, BMs provide the attachment substrate for epithelial and endothelial cell sheets and effectively separate those cells from the interstitial matrix.^{5,6} In addition to this, laminins have been shown to be important players in wound repair. First, changes in laminin expression, processing, distribution, or deposition have been identified during normal wound repair and defects in this distribution have been correlated with delayed or impaired wound closure.⁸⁻¹² Second, inherited disorders have been identified where the pathogenic mutations reside in laminin encoding genes and the patients' phenotypes include defects in wound repair.¹⁻⁴ Third, fundamental molecular and cell biology studies have directly demonstrated laminins' abilities to influence cell behavior, including their involvement in determining cell motility and angiogenesis.¹³⁻¹⁷

Each laminin is a heterotrimer comprising an α , a β , and a γ chain, each of which is derived from individual genes.⁷ In higher organisms, 5 α (*LAMA1-5*), 3 β (*LAMB1-3*), and 3 γ (*LAMC1-3*) chains have been identified.⁷ In addition, use of an alternate promoter gives rise to two major isoforms from the *LAMA3* gene, a short $\alpha3A$ form and a longer $\alpha3B$ form, which effectively increase the number of α chains to 6.^{8,18} Although theoretically the 6 α chains, 3 β and 3 γ chains could enable generation of 51 different $\alpha\beta\gamma$ trimers, restrictions in interaction potential, as well as differential tissue distribution patterns, means that only 16 laminin trimers have been demonstrated to exist *in vivo*.⁷ These are named based on their chain composition; for example, laminin $\alpha5\beta1\gamma1$ is termed LM511 (Fig. 1).^{7,19}

Figure 1. Diagrammatic representation of laminins and laminin-related proteins involved in wound repair. Schematic depicting structural features of laminins expressed by epithelial and endothelial cells. Open triangles denote processing points; LN (coloured circles), laminin N-terminal domain; LE, laminin-type epidermal growth factor-like repeats; L4/LF, laminin globular domains; LCC, laminin coiled coil; LG, laminin globular domains. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

The 11 laminin encoding genes have arisen through gene duplication and rearrangement from single ancestral precursors and, therefore, share common structural motifs.^{20,21} Working from the amino terminus, these are a large globular laminin N-terminal domain (LN domain), a rod-like stretch of laminin-type epidermal growth factor-like repeats (LE domain) that is interspersed with globular domains, one in β or γ chains (termed L4 or LF, respectively), or two in α chains (L4a and L4b). This is followed by a laminin coiled coil (LCC) domain through which trimerization occurs, and finally, in α chains only, a C-terminal stretch of 5 laminin globular domains (LG1–5) (Fig. 1).²² It should be noted that not all laminin chains contain all of these conserved domains. Indeed four, the $\alpha 3A$, $\alpha 4$, $\beta 3$, and $\gamma 2$ chains, contain much shorter amino termini and are, therefore, sometimes described as “truncated.”²³ Of these, $\alpha 3A$, $\alpha 4$, and $\gamma 2$ specifically lack a LN domain and are sometimes therefore described as “headless” (Fig. 1).²²

Of the conserved domains described earlier, the LG domains of the α chain harbor the majority of cell-surface binding sites, notably the highest affinity integrin, dystroglycan, and syndecan binding sites.¹³ However, differential affinities for these receptors have been demonstrated based not only on the specific α chain but also on the precise $\alpha\beta\gamma$ combination.¹³ In other words, the interaction potential of the α chain is fine-tuned by the β and γ chains. The major role for the LN domains, in contrast, is in mediating laminin–laminin interactions and assembly of laminin heterotrimers into higher-order networks or polymers.^{24,25} In this regard, it has been demonstrated that laminins can only self- or co-polymerize when all three of the constituent chains contain an LN domain; that is, any laminin heterotrimer containing one or more “headless” chain is unable to directly polymerize.²⁶ It is hypothesized that incorporation of these nonpolymerizing laminins into functional BMs requires the activity of additional nonlaminin proteins.²²

Laminins contribute to two major aspects of wound repair, re-epithelialization and angiogenesis, with specific laminins playing different roles during each process. For re-epithelialization, laminins are involved in providing the substrate for epithelial keratinocytes to move to cover the wound and re-establish an intact epithelial barrier. Within epithelial tissues, including the skin and cornea, the major laminin component is $\alpha 3A\beta 3\gamma 2$ (LM3A32 or LM332, formerly laminin-5) with lesser amounts of LM511, LM3A11, and LM3B32 also being expressed. Each of these laminins has been implicated in regulating keratinocyte motile behavior.^{15,27} La-

minins are also critical players in blood vessel growth and maturation, and, therefore, they are important players in angiogenesis. In endothelial BMs, the predominant laminin is LM411 with smaller vessels and, specifically, dermal vessels also containing LM511 and LM3B11.^{15,28–31}

Laminins in wound healing LM3A32 (LM332, previously laminin 5)

Increased LM332 expression is one of the earliest events in wound re-epithelialization, with expression in epidermal keratinocytes occurring within hours of injury. Indeed, LM332 appears to be the first BM component laid down onto the wound bed, as its expression has been shown to precede that of other BM components, including LM511/LM521, type IV, or type VII collagen.^{9–11,18,32} Moreover, as demonstrated by Kainulainen *et al.*, increased LM332 expression occurs, irrespective of the injury mechanism as suction blisters, full-thickness mucosal wounds, and an animal model in which human epidermal keratinocytes were injected into the back of athymic mice; all showed LM332 expression in the leading edge of the migrating epithelial tongue and that LM332 was the only BM component deposited between the clot and the migrating keratinocytes (Fig. 2).¹²

Keratinocytes are able to interact with LM332 through a number of different receptors with the two most studied being integrin-based complexes. Specifically, $\alpha_6\beta_4$ integrin in the intermediate filament associated hemidesmosome (HD) and $\alpha_3\beta_1$

Figure 2. LM332 is upregulated at the leading front of migrating epithelial sheets and promotes rapid migration over the wounded surface. Hemidesmosome (HD) and focal adhesion (FA) proteins localize toward the leading front of migrating keratinocytes. LM332 then terminates vascularized granulation tissue formation via signaling from the N-terminal part of laminin $\alpha 3A$. In laryngo-onycho-cutaneous (LOC) syndrome, this signal is absent and leads to excessive vascularized granulation tissue formation. In junctional epidermolysis bullosa (JEB), loss of function mutations in any of the LM332 encoding genes leads to skin fragility. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

integrin in the actin filament-based focal adhesion (FA). Both of these complexes have been demonstrated to regulate different aspects of keratinocyte migration.

HDs are electron dense plaques found along the basal aspect of basal keratinocytes where they interact with the BM. They are often described as spot welds, both for their appearance by transmission electron microscopy and also for their function in buttoning down the epidermal keratinocyte layers onto the underlying BM.³³ In this sense, it was long believed that $\alpha_6\beta_4$ integrin interaction with LM332 was only required for stable attachment, and, therefore, HDs were negative mediators of migration and needed to be dissolved for re-epithelialization to proceed.^{34–37} However, recent work by the Jones and Isseroff groups have shown that genetic absence or knockdown of core hemidesmosomal components, including $\alpha_6\beta_4$ integrin, type XVII collagen, or bullous pemphigoid antigen 1e, resulted in a loss of directional persistence in migration and slower *in vitro* wound closure rates.^{38–41}

Compared with HDs, FAs are transient, highly dynamic structures found predominantly toward the periphery of motile keratinocytes particularly within and beneath cell extensions.³⁶ These actin-rich extensions (lamellipodia/filopodia) enable the cell, via integration with the ECM and activation of downstream signaling pathways, to generate traction forces that drive migration.⁴² Integrin $\alpha_3\beta_1$ is at the core of FAs associated with LM332 and is, therefore, considered absolutely required for migration to proceed, a hypothesis that was supported by a number of knockdown and functional inhibition studies.^{43–47} However, there is still some controversy, as apparently opposing results were presented by the Sonnenberg group, who reported that keratinocytes from an α_3 integrin knockout mice display increased migration velocities relative to controls.^{44,48} It should also be noted that there is increasing evidence indicating cross-talk between HDs and FAs and, as such, any therapeutic designed to specifically influence the action of one complex will almost certainly induce off-target effects on the other.³⁶

In addition to integrin-based interactions, laminin chains also harbor binding sites for members of the syndecan family. Epithelial tissues express two syndecans family members, syndecans-1 and -4, both of which have been implicated as being important for wound repair as null mice of either syndecan display delayed wound closure rates.^{49,50} Through binding their extracellular ligands, which include LM332, the syndecans influence signaling cascades and matrix proteases that induce prolif-

eration, activate cell motility machinery, or result in remodeling of the ECM.^{51,52} As such, some of the laminin-based therapeutics described next are based on peptides that directly activate these syndecans.

There is also some controversy regarding the contribution of proteolytic processing on LM332 function in supporting either stable attachment or migration. LM332 is produced with full-length α_3A , β_3 , and γ_2 chains, and this form is found at the leading edge of acute wounds. However, in mature, unwounded skin, a processed form predominates that lacks the C-terminal LG4 and LG5 domains from α_3A chain and the amino terminus of the γ_2 chain. *In vitro*, processing of the α_3A chain has been demonstrated to be necessary for formation of HD-like complexes;⁴⁷ in addition, the presence of the LG4/LG5 region also apparently aids deposition of LM332.⁵³ Moreover, chronic diabetic cutaneous ulcers display reduced expression of the uncleaved form of LM332.⁵⁴ Therefore, it was largely held that processing is a negative regulator of re-epithelialization. Recently, however, it has been demonstrated in a work by Senyurek *et al.* that the released LG4/LG5 module undergoes further processing, which results in a release of bioactive peptides.⁵⁵ Notably, processing of this region was shown to correlate with the speed of wound closure and functionally that LG4-derived peptides from α_3 , α_4 , and α_5 laminin possess broad antimicrobial activities.⁵⁵ As described next, some of the therapeutics in development involve peptides derived from this region of the α_3 chain and, although they are capable of interacting directly with syndecans, it is possible that some of the observed effects are due to supporting or stimulating increased LM332 deposition.⁵³

Processing of the γ_2 chain may also regulate LM332 function. Specifically, the second stretch of LE repeats in the γ_2 chain has been shown to be capable of interacting with the epidermal growth factor receptor, and it has been suggested that the proteolytic removal of the γ_2 amino terminus exposes this region and enables an interaction to occur, thereby stimulating signaling from this receptor.¹⁶ Processing of the γ_2 chain also removes a syndecan-1 binding site, which has been shown to regulate cell adhesion and migration.⁵⁶ In addition to these signaling roles, the amino terminus of the γ_2 chain, specifically the L4 domain, has also been demonstrated to be required for LM332 deposition into the ECM.¹⁷

Junctional epidermolysis bullosa. JEB is an inherited skin fragility disorder, where patients

present with blistering in response to mild mechanical trauma as a result of separation at the epidermal/dermal junction. Inheritance is usually recessive, and mutations have been identified in all three subunits of LM332.¹⁻⁴ Disease severity generally reflects the genotype, with nonsense mutations leading to diminished LM332 production, widespread blistering, and often early lethality; while missense mutations are generally associated with a milder presentation.¹ For an in-depth discussion of the genotype/phenotype correlation in JEB, we refer readers to two excellent recent reviews that focus specifically on this disorder.^{1,57}

Although skin fragility is the predominant phenotype of JEB patients, an additional aspect is chronic, slow-healing cutaneous erosions with excessive granulation tissue.¹⁻⁴ In this regard, it has been demonstrated by Jiang *et al.* that treating large erosions of JEB patients in the early stage of the disease with an artificial skin bioequivalent which releases LM332, along with other factors, supported the healing of acute or chronic wounds and resolution of protein, iron, and hemoglobin levels.⁵⁸

Laryngo-onycho-cutaneous syndrome. LOCS (also known as Shabbir syndrome) is a variant of JEB where patients do not display the blistering aspect of the JEB phenotype but rather present only with increased granulation tissue production in exposed epithelia, which manifests as slow-healing erosions in the skin.⁸ Following a genome-wide linkage analysis study by McLean *et al.*, the first genetic mutation underlying LOCS was identified.⁸ This mutation, a single base pair insertion in exon 39 of the *LAMA3* gene, led to frameshift and introduction of a premature termination codon. However, surprisingly, this mutation did not lead to nonsense-mediated mRNA decay but rather to transcription from a downstream initiation codon and production of an amino terminally truncated version of laminin $\alpha 3A$.⁸ Subsequently, a missense mutation in the same exon and a second $\alpha 3A$ specific nonsense mutation have been identified.^{59,60} Interestingly, in all of these patient cohorts, the expression and deposition of LM332 into the cutaneous BM does not seem to be affected. Together, these data specifically implicate the N-terminal domain of $\alpha 3A$ in regulating termination of the granulation tissue response (Fig. 2).⁸ The precise mechanism through which this regulation occurs has yet to be elucidated.

More recently, the Marinkovich group has demonstrated that $\beta 3$ laminin is also involved. Specifically, through expressing amino terminally

truncated forms of laminin $\beta 3$ in a $\beta 3$ integrin null JEB background, forming skin equivalents from the mutant cells and grafting those constructs onto immunodeficient mice, they identified that the mutant skin not only displayed significant subepidermal blistering but also increased granulation tissue production.⁶¹ These data suggest that in addition to the $\alpha 3A$ N-terminus, the N terminus and specifically the LN domain of laminin $\beta 3$ is involved in the regulation of the granulation response. Dissecting this process could potentially have therapeutic implications, not only for the treatment of chronic wounds but also for other disorders of overproduction of granulation tissue.

LM3B32 (laminin 5B), LM3B11 (laminin 6B)

Laminin $\alpha 3B$, as described earlier, is generated through use of an alternate promoter from the *LAMA3* gene.¹⁸ As a result, the $\alpha 3A$ and $\alpha 3B$ chains share LCC and LG domains and, therefore, are capable of interacting with the same repertoire of cell surface receptors.¹⁸ The large shared region between these two chains means that many of the antibodies and other reagents are not specific to a single form, and, therefore, some of the data described earlier may be relevant to either or both LM3A32 and LM3B32. However, there is a potentially large difference between the two chains in that $\alpha 3B$ contains a much longer amino terminal arm which, in contrast to $\alpha 3A$, contains all the structural motifs found in full-length laminins. This has implications regarding the cell behavior supported by substrates coated with the different forms. For example, a work by Kariya *et al.* demonstrated that for epidermal keratinocytes in culture, LM3B32 supports significantly higher cell adhesion and migration activity than LM3A32, and that the 3B form can stimulate proliferation when added directly in culture medium.¹⁵ Moreover, they demonstrated that proteolytic processing results in release of the amino terminal $\alpha 3B$ arm, which, in itself, could support adhesion, migration, and proliferation via $\alpha 3\beta 1$ integrin.¹⁵

The "extra" N-terminal sequence of $\alpha 3B$ chain includes an LN domain through which laminin-laminin interactions occur. LN-LN interactions have been shown to only be stable as ternary nodes between LN domains from α , β , and γ chains (Fig. 3).^{62,63} Therefore, LM3B11, which is present in epithelial and likely in endothelial BMs, may be capable of assembling into a network with LM511. Again, this is in contrast to LM3A32, which, according to the current model, cannot polymerize

Figure 3. LaNts and netrins putative influence on laminin network formation. Laminin polymerization requires ternary node formation between LN domains from an α , a β , and a γ LN domain. Netrin-4 and possibly the LaNts may compete for these interactions, destabilizing the network. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

directly.⁶² Functionally, this may modify the response of cells to a matrix, as even though they may interact with the substrate using the same cell surface receptors, the mechanical properties of the formed matrix may be different depending on the level of polymer formed. In turn, this would influence processes such as the cells' ability to generate traction forces or the strength of those forces and hence their motile behavior.

LM511/521 (laminin 10/11)

The $\alpha 5$ laminin chain displays the widest expression profile of any of the laminins in adult tissues, including being expressed in the dermal vasculature and abundantly in the BM underlying the interfollicular epidermis.^{64,65} Laminin $\alpha 5$ is developmentally regulated in vessels, with expression in mouse only detected at 3–4 weeks after birth.⁶⁶ Laminin $\alpha 5$ knockout mice display widespread defects, including reduced blood vessel stability.⁶⁷ Changes in laminin deposition, particularly LM511, have been associated with delayed corneal epithelial wound closure associated with diabetes.^{68,69} For example, in a streptozocin-induced mouse model of diabetes, Sato *et al.* demonstrated that laminin expression was delayed, fragmented, and irregular in corneal debridement wounds⁶⁸ while previously, Ljubimov *et al.* reported reduced LM111 and LM511 expression in corneal BMs of patients with diabetes.⁶⁹ Interestingly, they also reported a correlation with diabetic retinopathy (DR), where changes to LM111 or LM511 expression were observed in 10 out of 12 corneas in patients with DR compared with only three from 10 in patients without DR.⁶⁹

Immunogold analyses demonstrated that pericytes in human skin synthesize laminin $\alpha 5$, which contributes to the BM of both the epidermis and the dermal vasculature.⁷⁰ Consistent with this, coculture with pericytes in an organotypic culture enhanced LM511/521 deposition at the dermal–epidermal junction. Moreover and excitingly, coculture with pericytes was shown to enhance the tissue regeneration capacity of differentiated human epidermal cells (independent of angiogenesis).⁷⁰ In a preceding study, Pritinder Kaur's group also demonstrated that the tissue regenerative capacity of keratinocytes could be directly enhanced through exogenous addition of LM511/521.⁷¹

The $\alpha 5$ laminin chain has been shown to display the widest repertoire of interacting cell surface proteins, and this is reflected by LM511 being a very good substrate for a variety of cell behaviors.^{13,14} For example, recombinant LM511 has been shown to support adhesion and migration of saphenous vein endothelial cells to a greater extent than LM411.⁷² Similarly, both neonatal and adult keratinocytes have been shown to be able to attach rapidly via $\alpha_3\beta_1$ integrin to LM511/LM521.³¹ In two-dimensional scratch assays, keratinocytes seeded on LM511/521-coated plates closed wounds more rapidly than those grown on BSA-coated plates.³¹ The interpretation of these latter experimental findings are potentially very interesting. As the scratching process of this assay is likely to have damaged the original coating, these data may indicate that keratinocytes on a predominantly LM511/521 substrate are primed and ready to begin moving. Since the cells will have contributed to the composition of their substrates before scratching, these data may also say that it is the relatively higher ratio of LM511/521 to LM332 which is supporting the enhanced wound closure rates

Figure 4. LaNt $\alpha 31$ decorates regions of LM332 deposition in migrating epidermal keratinocytes. Human epidermal keratinocytes were plated overnight on glass coverslips, then fixed, and processed for indirect immunofluorescence microscopy with antibodies against LaNt $\alpha 31$ (magenta, left), or LM332 (green, middle). Phase-contrast image of cells to the right. Scale bar 20 μm . To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

rather than the absolute LM511/521 level alone. A recent study by the Matlin group has developed on this theme; they demonstrated that knockdown of LM511 in MDCK cells inhibited directional migration and destabilized cell–cell contacts, however simultaneous knockdown of both the $\alpha 5$ and $\alpha 3$ chains in these cells restored their directional migration.⁷³ These data further support that it is relative LM511/521 to LM332 ratio that determines cell migration characteristics rather than the absolute level of either.⁷³

In practical terms, the combination of the corneal wound data, the *in vitro* functional data, and the coculture findings suggest that inducing expression of LM511/521, exogenously supplying it at wound sites, or mimicking its function may simultaneously promote re-epithelialization and angiogenesis and, thus, provide a double-hit strategy to restart stalled wounds.

LM411 (laminin 8)

LM411 is the major laminin component of capillary and larger vessel BMs with LM411 and LM511 being produced and deposited from the stalk cells and from the leading tip cell during sprouting angiogenesis.^{74,75} Studies of laminin $\alpha 4$ -deficient mice revealed that, although this chain is not absolutely required for blood vessel formation, knockout mice exhibit vascular abnormalities, notably weakened and unstable capillary BMs.⁷⁶ Similarly, the $\gamma 1$ chain is not absolutely required for vasculogenesis, with vessels still being formed by null mice, albeit with reduced vessel diameter.⁷⁷

Similar to the $\alpha 3A$ chain, $\alpha 4$ is one of the “headless” laminins and, therefore, cannot polymerize directly into laminin networks; rather, its integration in vascular BMs requires the action of proteins such as nidogen, which binds $\gamma 1$ chain and to the collagen IV network.^{78–80} Moreover, similar to laminin $\alpha 3$ chain, the laminin $\alpha 4$ chain appears to be processed in tissue through proteolytic cleavage at the junction of the LG3-L4 domains.⁸¹ As described earlier, these released fragments have been shown to have antimicrobial functionality.^{55,82}

The $\alpha 4$ laminin chain has been shown to have the lowest affinity for various cell surface receptors; however, despite this, a number of studies have demonstrated its importance for angiogenesis.¹³ Functionally, LM411 has been implicated in regulating endothelial cell survival,⁸³ migration, and adhesion^{84,85} and overexpression of LM411 in human dermal microvascular cells was shown to promote their migration and accelerate angiogenic

tubule formation in collagen gel overlay assays.⁸⁶ A study by Gonzales *et al.* demonstrated that LM411, similar to LM332 in HDs, integrated the intermediate filament cytoskeleton at $\alpha_v\beta_3$ integrin-containing adhesions.⁸⁴ These adhesions also display focal contact-like features, as they decorate the periphery of the cells in culture, are enriched in vinculin, and are associated with the actin microfilament network.⁸⁴ Using deletion mutants of the $\alpha 4$ LG2 domain, an adhesion site for human vascular endothelial cells was mapped to amino acids 1121–1139 and a peptide derived from that sequence was demonstrated to promote angiogenesis in an $\alpha_v\beta_3$ integrin-dependent manner.⁸⁷ Interestingly however, and in close similarity to the LM332-based cell-matrix interactions described earlier, there appears to be differential integrin usage dependent on the specific behavior of the cell with a suggestion that $\alpha 6\beta 1$ -dependent adhesions are utilized for scratch wound closure.⁸⁶

Laminin-related proteins netrins

The netrins are a family of extracellular signaling molecules that share structural features and genetic ancestry with the laminins.²⁰ Each netrin consists of a LN domain followed by three LE repeats and a basic C-terminal domain or a glycosylphosphatidylinositol (GPI) anchor.⁸⁸ There are five netrin genes in higher organisms: netrins-1 and –3 are by sequence homology closest to laminin γ chains, while netrins-4 and the GPI anchored netrins –G1 and –G2 are closer to β chains.⁸⁸

The netrins are best known for their roles as neuronal guidance cues; however, they have been demonstrated to play significant roles outside the central nervous system, particularly in organogenesis and tissue morphogenesis.^{88,89} These functions include directing cell migration, cell–cell interactions, and cell–ECM adhesion, processes that are important in wound repair.⁸⁹ The majority of netrin functions have been attributed to signaling via deletion in colorectal cancer (DCC), its paralogue neogenin, and the UNC5 homolog receptors (UNC5A-D).⁸⁹ In addition, in the absence of their netrin ligand, DCC and neogenin mediate signaling, resulting in apoptosis and, hence, have tumor suppressor roles.^{90–92}

Netrin's roles in angiogenesis appear to be isoform and also context specific. For example, netrin-4 is found in vascular BMs and corneal epithelial BM, and it was recently shown that netrin-4 null mice display increased proliferation in their corneal epithelium and increased branching of the deep capillary plexus, suggesting that netrin-4 may be a negative regulator of proliferation and angiogene-

sis.⁹³ A separate study indicated that netrin-1 and its receptor UNC5B may be important mediators of neovascularization in corneal wounds.⁹⁴ Specifically, both netrin-1 and UNC5B have been shown to be downregulated after an alkali burn, and that exogenous addition of netrin-1 resolved the burn-induced inflammation and reversed neovascularization *via* upregulating epidermal growth factor (EGF) expression, accelerating epithelial wound healing, inhibiting neutrophil and macrophage infiltration, and reducing apoptosis.⁹⁴ Together, these data raise the possibility of developing therapeutics based around either targeting the function of netrin-4 or mimicking that of netrin-1 in corneal wounds.

The cellular responses to netrin exposure are determined both by the local netrin concentration and by the precise combinations of receptors expressed by the cell. Intriguingly, signaling via DCC and UNC5a in response to netrin has also been demonstrated to induce dramatic rearrangement of the actin cytoskeleton by modulating the activity of the small Rho GTPases Rac1 and Cdc42.^{95,96} Netrin-4 has also been demonstrated to regulate migration and proliferation of glioblastoma multiforme cells via an interaction with β_4 integrin and the induction of downstream Akt and mTOR signaling.⁹⁷ In addition, netrin-4 has been shown to regulate the mitogen-activated protein kinase pathway via ligating $\alpha_6\beta_1$ integrin, a process that requires an interaction with $\gamma 1$ laminin.⁹⁸

In addition to these signaling roles, netrin-4 is capable of inhibiting laminin polymer assembly and inducing disassembly of formed networks likely via competing for LN-LN domain interactions (Fig. 3).⁹⁹ At this time, the implications of such an impact on cell behavior are unclear. However, changing the level of laminin polymerization within a BM is likely to impact the mechanical properties of that matrix, which, in turn, could influence the ways in which cells interact with it. The netrins, therefore, may act as a switch in certain conditions, converting a BM from a supporting stable attachment to one that supports migration or vessel growth. An elegant, recent study supports this hypothesis; using *Caenorhabditis elegans*, Hagedorn *et al.* demonstrated a role for netrins in invasion where the UNC-40 (DCC homologue) netrin receptor focally enriches at the site of BM breach and directs focused F-actin formation.¹⁰⁰ Interestingly, live imaging of laminin in the BM during this process revealed invasion to involve transient physical displacement of the laminin network, which subsequently reassembles after cellular passage.¹⁰⁰ Whether these findings could be exploited and a netrin-based treatment used to

initiate a stalled wound remains to be seen; however, such an approach could have widespread potential therapeutic utility.

LaNts

The LaNts (Laminin N-terminus) are a family of short secreted proteins that are generated from the 5' end of laminin encoding genes but which lack the LCC domain and, therefore, are not able to trimerise into bona fide laminins.¹⁰¹ Four different LaNt encoding transcripts have been described to date, two each from the amino terminus of LAMA3 and LAMA5. These transcripts display widespread tissue distribution, including high expression in epidermal keratinocytes.¹⁰¹ Each LaNt consists of an LN domain followed by a short stretch of LE repeats and, therefore, resembles netrins in both size and structural architecture. Based on this architecture, it is predicted that the LaNts may share some netrin functions, potentially including the netrin signaling behavior and also in regulating laminin–laminin interactions (Fig. 3). Consistent with this, initial functional analyses of one LaNt family member, LaNt $\alpha 31$, demonstrated that this protein colocalizes with regions of major LM332 deposition in epidermal keratinocytes (Fig. 4).¹⁰¹ Moreover, *in vitro* scratch wound data indicate that LaNt $\alpha 31$ expression increases during times of keratinocyte motility and siRNA-mediated targeting of LaNt $\alpha 31$ expression results in impaired scratch closure rates.¹⁰¹ Together, these data suggest that members of the LaNt family may play important roles in the re-epithelialization process and, similar to the netrins, could potentially become therapeutic targets. As with the netrins, these may be direct targets by mimicking or inhibiting their signaling function, or through an indirect mechanism by modifying laminin polymer organization and therefore the mechanical properties of the cellular microenvironment.

Laminin-based therapies

Due to their extracellular localisation, which enables exogenous targeting or a microinjection, and due to their established biological importance in the wound repair process, the laminins are appealing molecules around which to develop strategies to enhance normal wound closure or to treat chronic/nonhealing wounds. Indeed, one early study demonstrated the validity of this approach through direct application of a preparation of laminins derived from human placenta to a superficial rat skin wound that displayed an enhanced rate of re-epithelialization.¹⁰² Similarly it was shown that a coating of cell-derived LM332 on

polytetrafluoroethylene (PTFE) scaffolds (a porous polymer that permits vessel ingrowth through the pores of the material) increased tissue vascularization and accelerated neovascularization on subcutaneous implantation.^{103,104}

Although there has also been some success using amniotic membrane as a source of a mixture of BM components, including laminins to treat wounds, the majority of the laminin-specific therapeutics designed to date have focused on the use of laminin-derived peptides. This reflects the challenge of producing functional, large, multi-gene proteins with appropriate post-translational modifications under good manufacturing practice (GMP) conditions. The goal of using peptide-based therapy, therefore, is to directly stimulate specific activities that are usually mediated by these laminins, rather than mimicking the complex three-dimensional (3D) architecture of a 600 kDa+ structural protein. There has been some progress in this regard, and we will describe here a few of the peptides that have shown promise as an illustration of how this area is progressing (See Fig. 5 for a synopsis of peptide location and identified functional roles).

Peptide-based approaches

One of the first peptides widely used was IKVAV from the LCC domain of laminin $\alpha 1$, which was shown to induce angiogenesis *in vivo* when subcutaneously injected into mice.¹⁰⁵ Further studies analyzing the angiogenic potential of peptides derived from $\alpha 1$ and $\gamma 1$ laminin chains identified two

with particularly strong properties RQVFQVAYI IKA and KAFDITYVRLKF.¹⁰⁶ Each of these peptides is located within the LN domains of their respective chains, are highly conserved across different family members, and are demonstrated to be capable of directly interacting with $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin.¹⁰⁶ *In vivo* functionality was demonstrated through topical application to rat punch biopsies, where the $\alpha 1$ peptide was shown to stimulate wound re-epithelialization at low concentrations but inhibit at higher; while the $\gamma 1$ -derived peptide induced a constant 8–11% increase in re-epithelialization compared with controls.¹⁰⁶ Interestingly, both peptides were shown to promote production of granulation tissue in the early stages (approximately 4 days) after wounding.¹⁰⁷

Several other laminin-derived peptides have also been shown to be capable of improving wound healing. Rousselle *et al.* demonstrated that a peptide mimicking the KKLRIKSKEK sequence of the $\alpha 3$ LG4/LG5 module was effective in enhancing the wound repair response.¹⁰⁸ In *in vitro* studies, they showed that this peptide could support attachment and migration of epidermal keratinocytes, while topical application to partial-thickness cutaneous wounds in pigs led to a significant increase in skin-re-epithelialization with reduced inflammation and decreased granulation tissue production.¹⁰⁸ Similarly, treating keratinocytes using the synthetic peptide KIPKPSSVPTELSAISML from the laminin $\alpha 3$ LG4 domain was shown to lead to increased migration rates via a syndecan-4 medi-

Figure 5. Location of laminin-derived peptides described in the text. Schematic representation of archetypal laminin with laminin chain, structural location, and function of peptide sequences indicated. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

ated increase in integrin β_1 -dependent cell-ECM adhesion.¹⁰⁹

Peptide-conjugated scaffolds

Although effective in soluble form, sustained exposure of bioactive peptides to treatment sites will require conjugation to scaffolding materials to aid delivery and increase their longevity at wound sites. However, this may not be as simple as attaching the previously studied peptides to a suitable scaffold, as the peptides may produce different effects when added in solution versus when attached to a carrier molecule. For example, in the former case, the peptide may compete for the binding sites of intact laminins; while when tethered, they might instead mimic matrix-bound laminin by serving as points for cell attachment. The choice of substrate will, therefore, also have dramatic effects on the functional outcomes.

In recent years, there have been considerable advances utilizing a variety of different carrier molecules. For example, the PPFLMLLKSTR peptide derived from LG3 domain of α_3 laminin, which has been shown to bind to $\alpha_3\beta_1$ integrin, has been conjugated to chitin, a long-chain polymer formed by the glucose derivative N-acetylglucosamine.^{110,111} Application of the Chitin-LG3 peptide to rabbit and rat full-thickness cutaneous wounds resulted in increased rates of skin re-epithelialization.¹¹¹ Similar positive results in a rat wound model were recently observed when the same peptide sequence was tethered to a type I collagen scaffold using microbial transglutaminase.¹¹² In this case, the biomimetic conjugate was demonstrated to stimulate neovascularization, decrease inflammatory cell infiltration, and enhance fibroblast proliferation.¹¹² Using a similar mechanism, the laminin α_1 -derived AG73 peptide, RKRLQVQLSIRT from the LG4 domain, has been demonstrated as biologically active in stimulating cell migration and adhesion when conjugated to a chitosan membrane formed by a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine.¹¹³ In a separate study, human keratinocytes seeded onto such a membrane showed 80% attachment within 2h, increasing the possibility of using this as a delivery system of *ex vivo* expanded epidermal sheets to wounded tissue.¹¹⁴

The IKVAV peptide described earlier has also been used with a range of scaffolds. A particularly innovative approach has utilized a fusion protein consisting of a combination of different functionally relevant ECM proteins: the IKVAV sequence from laminin, an elastin-derived structural unit, an

RGD sequence, and the collagen-binding domain from fibronectin.¹¹⁵ This fusion was demonstrated to enhance the angiogenic activity of a collagen gel and promote endothelial cell migration.¹¹⁵

The design of the current sets of peptide-based therapeutics are predicated on direct stimulation of specific cell behavior via cell surface receptor interactions and although peptide-based approaches can never truly mimic the entire 3D molecule, they hold enormous potential. Ultimately, the goal for such therapeutics would be for the implanted material to be cleared from the body. An innovative approach to address this issue has been recently reported, which involved incorporation of a matrix metalloproteinase-sensitive peptide into a poly(ethylene glycol) polymer backbone that was then functionalized through addition of laminin-derived peptides.¹¹⁶ Using this combination, the hydrogel is degraded, as matrix metalloproteinases (MMPs) are released by the growing endothelial cells, enabling transient stimulation by the conjugated peptide which, ultimately, is replaced by nascent deposition of BM components.¹¹⁶

Substrates supporting laminin deposition

Peptide-based approaches may not be effective when wound repair problems occur as a result of defects in the deposition or processing of laminins, such as in chronic diabetic cutaneous ulcers. The future of laminin-based therapeutics may, therefore, lie in the bioengineering of substrates that will either support or ideally promote "normal" laminin deposition either for direct application to wound sites or for *ex vivo* expansion of patient-derived cells for transplantation. A recent work by Fu *et al.* has demonstrated that polycaprolactone/collagen nanofibrous matrices coated with a thin layer of collagen gel stimulated LM332 deposition and matrix metalloproteinase activity, as well as the activation of β_1 -integrin-mediated actin remodeling.¹¹⁷ Another exciting new development is the use of micropatterned substrates to promote a specific cell behavior. For example, a work by Clement *et al.* recently showed that when keratinocytes were grown in 400 μ m channels, their LM332 deposition rates increased compared with cells grown on flat substrates.¹¹⁸ Future developments based around these approaches are an exciting new direction for the next generation of biomaterials and one that holds great promise for the future. This is also one area where a deeper understanding of netrin/LaNT biology could prove beneficial. Specifically, if, as hypothesized, these small proteins are capable of providing a nucleation point for laminin network formation, then

functionalizing a scaffold with these proteins, or peptides derived from them, may enhance laminin deposition rates onto those substrates.

SUMMARY

The laminins are key players in re-epithelialization and angiogenesis, processes that are critical to wound repair. Through molecular genetics, biochemistry, molecular, and cellular biology approaches, many of the fundamental aspects of laminin biology have been identified and these are beginning to be exploited in therapeutic development.

ACKNOWLEDGMENTS AND FUNDING SOURCES

Work in the Hamill is supported by funding from the Biotechnology and Biological Sciences Research Council, Fight For Sight, and the British Skin Foundation.

AUTHOR DISCLOSURE AND GHOSTWRITING

The authors disclose no commercial associations or known conflicts of interest in connection with

TAKE-HOME MESSAGES

- The laminins are critical structural components of underlying epithelial and endothelial BMs.
- After wounding, expression of specific laminins increases and they are deposited at wound margins where they provide the substrate for re-epithelialization and blood vessel growth.
- Receptors on epithelial and endothelial cells interact directly with laminins, and these interactions determine behavioral responses to laminin exposure.
- Some laminin functions may be mimicked by the laminin-related proteins, the netrins, and the LaNts, and these proteins may also influence laminin network assembly.
- Peptides derived from laminins that can mimic aspects of laminin function have been identified.
- Current work is developing scaffolding mechanisms that enable stable delivery of laminin-derived functional peptides to wound environments.

this article. No ghostwriters were used to write this article.

ABOUT THE AUTHORS

The authors are members of the Department of Eye and Vision Science within the Institute of Aging and Chronic Disease at the University of Liverpool, Liverpool, UK. Their research focuses on the impact of BM composition, assembly states, and mechanical properties on cell behavior.

REFERENCES

1. Kiritsi D, Has C, Bruckner-Tuderman L. Laminin 332 in junctional epidermolysis bullosa. *Cell Adh Migr* 2013;7:135–141.
2. Kivirikko S, McGrath JA, Pulkkinen L, Uitto J, Christiano AM. Mutational hotspots in the LAMB3 gene in the lethal (Herlitz) type of junctional epidermolysis bullosa. *Hum Mol Genet* 1996;5:231–237.
3. Muhle C, Jiang QJ, Charlesworth A, Bruckner-Tuderman L, Meneguzzi G, Schneider H. Novel and recurrent mutations in the laminin-5 genes causing lethal junctional epidermolysis bullosa: molecular basis and clinical course of Herlitz disease. *Hum Genet* 2005;116:33–42.
4. Pulkkinen L, Christiano AM, Airenne T, Haakana H, Tryggvason K, Uitto J. Mutations in the gamma 2 chain gene (LAMC2) of kalinin/laminin 5 in the junctional forms of epidermolysis bullosa. *Nat Genet* 1994;6:293–297.
5. Ko MS, Marinkovich MP. Role of dermal-epidermal basement membrane zone in skin, cancer, and developmental disorders. *Dermatol Clin* 2010;28:1–16.
6. Timpl R, Brown JC. Supramolecular assembly of basement membranes. *Bioessays* 1996;18:123–132.
7. Aumailley M, Bruckner-Tuderman L, Carter WG, et al. A simplified laminin nomenclature. *Matrix Biol* 2005;24:326–332.
8. McLean WH, Irvine AD, Hamill KJ, et al. An unusual N-terminal deletion of the laminin alpha3a isoform leads to the chronic granulation tissue disorder laryngo-onycho-cutaneous syndrome. *Hum Mol Genet* 2003;12:2395–2409.
9. Ryan MC, Lee K, Miyashita Y, Carter WG. Targeted disruption of the LAMA3 gene in mice reveals abnormalities in survival and late stage differentiation of epithelial cells. *J Cell Biol* 1999;145:1309–1323.
10. Laplante AF, Germain L, Auger FA, Moulin V. Mechanisms of wound reepithelialization: hints from a tissue-engineered reconstructed skin to long-standing questions. *FASEB J* 2001;15:2377–2389.
11. Kurpakus MA, Stock EL, Jones JC. Analysis of wound healing in an *in vitro* model: early appearance of laminin and a 125×10(3) Mr polypeptide during adhesion complex formation. *J Cell Sci* 1990;96(Pt 4):651–660.
12. Kainulainen T, Hakkinen L, Hamidi S, et al. Laminin-5 expression is independent of the injury and the microenvironment during reepithelialization of wounds. *J Histochem Cytochem* 1998;46:353–360.
13. Taniguchi Y, Ido H, Sanzen N, et al. The C-terminal region of laminin beta chains modulates the integrin binding affinities of laminins. *J Biol Chem* 2009;284:7820–7831.
14. Kikkawa Y, Sanzen N, Fujiwara H, Sonnenberg A, Sekiguchi K. Integrin binding specificity of laminin-10/11: laminin-10/11 are recognized by alpha 3 beta 1, alpha 6 beta 1 and alpha 6 beta 4 integrins. *J Cell Sci* 2000;113(Pt 5):869–876.

15. Kariya Y, Yasuda C, Nakashima Y, Ishida K, Tsubota Y, Miyazaki K. Characterization of laminin 5B and NH2-terminal proteolytic fragment of its alpha3B chain: promotion of cellular adhesion, migration, and proliferation. *J Biol Chem* 2004;279:24774–24784.
16. Schenk S, Hintermann E, Bilban M, et al. Binding to EGF receptor of a laminin-5 EGF-like fragment liberated during MMP-dependent mammary gland involution. *J Cell Biol* 2003;161:197–209.
17. Gagnoux-Palacios L, Allegra M, Spirito F, et al. The short arm of the laminin gamma2 chain plays a pivotal role in the incorporation of laminin 5 into the extracellular matrix and in cell adhesion. *J Cell Biol* 2001;153:835–850.
18. Ryan MC, Tizard R, VanDevanter DR, Carter WG. Cloning of the LamA3 gene encoding the alpha 3 chain of the adhesive ligand epiligrin. Expression in wound repair. *J Biol Chem* 1994;269:22779–22787.
19. Macdonald PR, Lustig A, Steinmetz MO, Kammerer RA. Laminin chain assembly is regulated by specific coiled-coil interactions. *J Struct Biol* 2010;170:398–405.
20. Fahey B, Degnan BM. Origin and evolution of laminin gene family diversity. *Mol Biol Evol* 2012;29:1823–1836.
21. Domogatskaya A, Rodin S, Tryggvason K. Functional diversity of laminins. *Annu Rev Cell Dev Biol* 2012;28:523–553.
22. Hamill KJ, Kligys K, Hopkinson SB, Jones JC. Laminin deposition in the extracellular matrix: a complex picture emerges. *J Cell Sci* 2009;122:4409–4417.
23. Kallunki P, Sainio K, Eddy R, et al. A truncated laminin chain homologous to the B2 chain: structure, spatial expression, and chromosomal assignment. *J Cell Biol* 1992;119:679–693.
24. Yurchenco PD, Tsilibary EC, Charonis AS, Furthmayr H. Laminin polymerization *in vitro*. Evidence for a two-step assembly with domain specificity. *J Biol Chem* 1985;260:7636–7644.
25. Schittny JC, Yurchenco PD. Terminal short arm domains of basement membrane laminin are critical for its self-assembly. *J Cell Biol* 1990;110:825–832.
26. Yurchenco PD, Cheng YS. Self-assembly and calcium-binding sites in laminin. A three-arm interaction model. *J Biol Chem* 1993;268:17286–17299.
27. Miner JH, Li C, Mudd JL, Go G, Sutherland AE. Compositional and structural requirements for laminin and basement membranes during mouse embryo implantation and gastrulation. *Development* 2004;131:2247–2256.
28. Miner JH, Patton BL, Lentz SI, et al. The laminin alpha chains: expression, developmental transitions, and chromosomal locations of alpha1-5, identification of heterotrimeric laminins 8–11, and cloning of a novel alpha3 isoform. *J Cell Biol* 1997;137:685–701.
29. Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM. Expression and function of laminins in the embryonic and mature vasculature. *Physiol Rev* 2005;85:979–1000.
30. Kariya Y, Mori T, Yasuda C, et al. Localization of laminin alpha3B chain in vascular and epithelial basement membranes of normal human tissues and its down-regulation in skin cancers. *J Mol Histol* 2008;39:435–446.
31. Pouliot N, Saunders NA, Kaur P. Laminin 10/11: an alternative adhesive ligand for epidermal keratinocytes with a functional role in promoting proliferation and migration. *Exp Dermatol* 2002;11:387–397.
32. Nguyen BP, Gil SG, Carter WG. Deposition of laminin 5 by keratinocytes regulates integrin adhesion and signaling. *J Biol Chem* 2000;275:31896–31907.
33. Jones JC, Yokoo KM, Goldman RD. Is the hemidesmosome a half desmosome? An immunological comparison of mammalian desmosomes and hemidesmosomes. *Cell Motil Cytoskeleton* 1986;6:560–569.
34. Wilhelmsen K, Litjens SH, Kuikman I, Margadant C, van Rheenen J, Sonnenberg A. Serine phosphorylation of the integrin beta4 subunit is necessary for epidermal growth factor receptor induced hemidesmosome disruption. *Mol Biol Cell* 2007;18:3512–3522.
35. Margadant C, Frijns E, Wilhelmsen K, Sonnenberg A. Regulation of hemidesmosome disassembly by growth factor receptors. *Curr Opin Cell Biol* 2008;20:589–596.
36. Tsuruta D, Hashimoto T, Hamill KJ, Jones JC. Hemidesmosomes and focal contact proteins: functions and cross-talk in keratinocytes, bullous diseases and wound healing. *J Dermatol Sci* 2011;62:1–7.
37. Riddelle KS, Hopkinson SB, Jones JC. Hemidesmosomes in the epithelial cell line 804G: their fate during wound closure, mitosis and drug induced reorganization of the cytoskeleton. *J Cell Sci* 1992;103(Pt 2):475–490.
38. Hamill KJ, Hopkinson SB, DeBiase P, Jones JC. BPAG1e maintains keratinocyte polarity through beta4 integrin-mediated modulation of Rac1 and cofilin activities. *Mol Biol Cell* 2009;20:2954–2962.
39. Hamill KJ, Hopkinson SB, Jonkman MF, Jones JC. Type XVII collagen regulates lamellipod stability, cell motility, and signaling to Rac1 by targeting bullous pemphigoid antigen 1e to alpha6beta4 integrin. *J Biol Chem* 2011;286:26768–26780.
40. Sehgal BU, DeBiase PJ, Matzno S, et al. Integrin beta4 regulates migratory behavior of keratinocytes by determining laminin-332 organization. *J Biol Chem* 2006;281:35487–35498.
41. Pullar CE, Baier BS, Kariya Y, et al. beta4 integrin and epidermal growth factor coordinately regulate electric field-mediated directional migration via Rac1. *Mol Biol Cell* 2006;17:4925–4935.
42. Beningo KA, Dembo M, Kaverina I, Small JV, Wang YL. Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. *J Cell Biol* 2001;153:881–888.
43. DiPersio CM, Shah S, Hynes RO. alpha 3A beta 1 integrin localizes to focal contacts in response to diverse extracellular matrix proteins. *J Cell Sci* 1995;108(Pt 6):2321–2336.
44. Choma DP, Pumiglia K, DiPersio CM. Integrin alpha3beta1 directs the stabilization of a polarized lamellipodium in epithelial cells through activation of Rac1. *J Cell Sci* 2004;117:3947–3959.
45. Kligys KR, Wu Y, Hopkinson SB, Kaur S, Platanias LC, Jones JC. alpha6beta4 integrin, a master regulator of expression of integrins in human keratinocytes. *J Biol Chem* 2012;287:17975–17984.
46. Frank DE, Carter WG. Laminin 5 deposition regulates keratinocyte polarization and persistent migration. *J Cell Sci* 2004;117:1351–1363.
47. Goldfinger LE, Hopkinson SB, deHart GW, Collawn S, Couchman JR, Jones JC. The alpha3 laminin subunit, alpha6beta4 and alpha3beta1 integrin coordinately regulate wound healing in cultured epithelial cells and in the skin. *J Cell Sci* 1999;112(Pt 16):2615–2629.
48. Margadant C, Raymond K, Kreft M, Sachs N, Janssen H, Sonnenberg A. Integrin alpha3beta1 inhibits directional migration and wound re-epithelialization in the skin. *J Cell Sci* 2009;122:278–288.
49. Stepp MA, Gibson HE, Gala PH, et al. Defects in keratinocyte activation during wound healing in the syndecan-1-deficient mouse. *J Cell Sci* 2002;115:4517–4531.
50. Woods A, Couchman JR. Syndecan-4 and focal adhesion function. *Curr Opin Cell Biol* 2001;13:578–583.
51. Bass MD, Roach KA, Morgan MR, et al. Syndecan-4-dependent Rac1 regulation determines directional migration in response to the extracellular matrix. *J Cell Biol* 2007;177:527–538.
52. Zimmermann P, David G. The syndecans, tuners of transmembrane signaling. *FASEB J* 1999;13 Suppl:S91–S100.
53. Sigle RO, Gil SG, Bhattacharya M, et al. Globular domains 4/5 of the laminin alpha3 chain mediate deposition of precursor laminin 5. *J Cell Sci* 2004;117:4481–4494.
54. Usui ML, Mansbridge JN, Carter WG, Fujita M, Olerud JE. Keratinocyte migration, proliferation, and differentiation in chronic ulcers from patients with diabetes and normal wounds. *J Histochem Cytochem* 2008;56:687–696.
55. Senyurek I, Kempf WE, Klein G, et al. Processing of laminin alpha chains generates peptides involved in wound healing and host defense. *J Innate Immun* 2014 [Epub ahead of print]. (DOI:10.1159/000357032)
56. Ogawa T, Tsubota Y, Hashimoto J, Kariya Y, Miyazaki K. The short arm of laminin gamma2

- chain of laminin-5 (laminin-332) binds syndecan-1 and regulates cellular adhesion and migration by suppressing phosphorylation of integrin β 4 chain. *Mol Biol Cell* 2007;18:1621–1633.
57. Laimer M, Lanschuetzer CM, Diem A, Bauer JW. Herlitz junctional epidermolysis bullosa. *Dermatol Clin* 2010;28:55–60.
 58. Jiang QJ, Izakovic J, Zenker M, et al. Treatment of two patients with Herlitz junctional epidermolysis bullosa with artificial skin bioequivalents. *J Pediatr* 2002;141:553–559.
 59. Figueira EC, Crotty A, Challinor CJ, Coroneo MT, Murrell DF. Granulation tissue in the eyelid margin and conjunctiva in junctional epidermolysis bullosa with features of laryngo-onycho-cutaneous syndrome. *Clin Exp Ophthalmol* 2007;35:163–166.
 60. Barzegar M, Mozafari N, Kariminejad A, Asadikani Z, Ozoemena L, McGrath JA. A new homozygous nonsense mutation in LAMA3A underlying laryngo-onycho-cutaneous syndrome. *Br J Dermatol* 2013;169:1353–1356.
 61. Sakai N, Waterman EA, Nguyen NT, Keene DR, Marinkovich MP. Observations of skin grafts derived from keratinocytes expressing selectively engineered mutant laminin-332 molecules. *J Invest Dermatol* 2010;130:2147–2150.
 62. Hohenester E, Yurchenco PD. Laminins in basement membrane assembly. *Cell Adh Migr* 2013;7:56–63.
 63. Hussain SA, Carafoli F, Hohenester E. Determinants of laminin polymerization revealed by the structure of the α 5 chain amino-terminal region. *EMBO Rep* 2011;12:276–282.
 64. Pouliot N, Kusuma N. Laminin-511: a multifunctional adhesion protein regulating cell migration, tumor invasion and metastasis. *Cell Adh Migr* 2013;7:142–149.
 65. Pouliot N, Nice EC, Burgess AW. Laminin-10 mediates basal and EGF-stimulated motility of human colon carcinoma cells via α 3(3) β 1 and α 6(6) β 4 integrins. *Exp Cell Res* 2001;266:1–10.
 66. Sorokin LM, Pausch F, Frieser M, Kroger S, Ohage E, Deutzmann R. Developmental regulation of the laminin α 5 chain suggests a role in epithelial and endothelial cell maturation. *Dev Biol* 1997;189:285–300.
 67. Miner JH, Cunningham J, Sanes JR. Roles for laminin in embryogenesis: exencephaly, syndactyly, and placental pathology in mice lacking the laminin α 5 chain. *J Cell Biol* 1998;143:1713–1723.
 68. Sato N, Nakamura M, Chikama T, Nishida T. Abnormal deposition of laminin and type IV collagen at corneal epithelial basement membrane during wound healing in diabetic rats. *Jpn J Ophthalmol* 1999;43:343–347.
 69. Ljubimov AV, Huang ZS, Huang GH, et al. Human corneal epithelial basement membrane and integrin alterations in diabetes and diabetic retinopathy. *J Histochem Cytochem* 1998;46:1033–1041.
 70. Paquet-Fifield S, Schluter H, Li A, et al. A role for pericytes as microenvironmental regulators of human skin tissue regeneration. *J Clin Invest* 2009;119:2795–2806.
 71. Li A, Pouliot N, Redvers R, Kaur P. Extensive tissue-regenerative capacity of neonatal human keratinocyte stem cells and their progeny. *J Clin Invest* 2004;113:390–400.
 72. Doi M, Thyboll J, Kortessmaa J, et al. Recombinant human laminin-10 (α 5 β 1 γ 1). Production, purification, and migration-promoting activity on vascular endothelial cells. *J Biol Chem* 2002;277:12741–12748.
 73. Greciano PG, Moyano JV, Buschmann MM, et al. Laminin 511 partners with laminin 332 to mediate directional migration of Madin-Darby canine kidney epithelial cells. *Mol Biol Cell* 2012;23:121–136.
 74. Kortessmaa J, Yurchenco P, Tryggvason K. Recombinant laminin-8 (α 4(4) β 1 γ 1). Production, purification, and interactions with integrins. *J Biol Chem* 2000;275:14853–14859.
 75. Wondimu Z, Geberhiwot T, Ingerpuu S, et al. An endothelial laminin isoform, laminin 8 (α 4 β 1 γ 1), is secreted by blood neutrophils, promotes neutrophil migration and extravasation, and protects neutrophils from apoptosis. *Blood* 2004;104:1859–1866.
 76. Thyboll J, Kortessmaa J, Cao R, et al. Deletion of the laminin α 4 chain leads to impaired microvessel maturation. *Mol Cell Biol* 2002;22:1194–1202.
 77. Jakobsson L, Domogatskaya A, Tryggvason K, Edgar D, Claesson-Welsh L. Laminin deposition is dispensable for vasculogenesis but regulates blood vessel diameter independent of flow. *FASEB J* 2008;22:1530–1539.
 78. Aumailley M, Nurcombe V, Edgar D, Paulsson M, Timpl R. The cellular interactions of laminin fragments. Cell adhesion correlates with two fragment-specific high affinity binding sites. *J Biol Chem* 1987;262:11532–11538.
 79. Paulsson M, Aumailley M, Deutzmann R, Timpl R, Beck K, Engel J. Laminin-nidogen complex. Extraction with chelating agents and structural characterization. *Eur J Biochem* 1987;166:11–19.
 80. Kittelberger R, Davis PF, Stehbins WE. Distribution of type IV collagen, laminin, nidogen and fibronectin in the haemodynamically stressed vascular wall. *Histol Histopathol* 1990;5:161–167.
 81. Talts JF, Sasaki T, Miosge N, et al. Structural and functional analysis of the recombinant G domain of the laminin α 4 chain and its proteolytic processing in tissues. *J Biol Chem* 2000;275:35192–35199.
 82. Senyurek I, Klein G, Kalbacher H, Deeg M, Schitteck B. Peptides derived from the human laminin α 4 and α 5 chains exhibit antimicrobial activity. *Peptides* 2010;31:1468–1472.
 83. DeHahn KC, Gonzales M, Gonzalez AM, et al. The α 4 laminin subunit regulates endothelial cell survival. *Exp Cell Res* 2004;294:281–289.
 84. Gonzales M, Weksler B, Tsuruta D, et al. Structure and function of a vimentin-associated matrix adhesion in endothelial cells. *Mol Biol Cell* 2001;12:85–100.
 85. Gonzalez AM, Gonzales M, Herron GS, et al. Complex interactions between the laminin α 4 subunit and integrins regulate endothelial cell behavior *in vitro* and angiogenesis *in vivo*. *Proc Natl Acad Sci U S A* 2002;99:16075–16080.
 86. Li J, Zhou L, Tran HT, et al. Overexpression of laminin-8 in human dermal microvascular endothelial cells promotes angiogenesis-related functions. *J Invest Dermatol* 2006;126:432–440.
 87. Lian J, Dai X, Li X, He F. Identification of an active site on the laminin α 4 chain globular domain that binds to α 5 β 3 integrin and promotes angiogenesis. *Biochem Biophys Res Commun* 2006;347:248–253.
 88. Castets M, Mehlen P. Netrin-1 role in angiogenesis: to be or not to be a pro-angiogenic factor? *Cell Cycle* 2010;9:1466–1471.
 89. Rajasekharan S, Kennedy TE. The netrin protein family. *Genome Biol* 2009;10:239.
 90. Castets M, Broutier L, Molin Y, et al. DCC constrains tumour progression via its dependence receptor activity. *Nature* 2012;482:534–537.
 91. Kelker W, Van Dyke DL, Worsham MJ, et al. Loss of 18q and homozygosity for the DCC locus: possible markers for clinically aggressive squamous cell carcinoma. *Anticancer Res* 1996;16:2365–2372.
 92. Park HL, Kim MS, Yamashita K, et al. DCC promoter hypermethylation in esophageal squamous cell carcinoma. *Int J Cancer* 2008;122:2498–2502.
 93. Li YN, Pinzon-Duarte G, Dattilo M, Claudepierre T, Koch M, Brunken WJ. The expression and function of netrin-4 in murine ocular tissues. *Exp Eye Res* 2012;96:24–35.
 94. Han Y, Shao Y, Lin Z, et al. Netrin-1 simultaneously suppresses corneal inflammation and neovascularization. *Invest Ophthalmol Vis Sci* 2012;53:1285–1295.
 95. Shekarabi M, Kennedy TE. The netrin-1 receptor DCC promotes filopodia formation and cell spreading by activating Cdc42 and Rac1. *Mol Cell Neurosci* 2002;19:1–17.
 96. Picard M, Petrie RJ, Antoine-Bertrand J, Saint-Cyr-Proulx E, Villemure JF, Lamarche-Vane N. Spatial and temporal activation of the small GTPases RhoA and Rac1 by the netrin-1 receptor UNC5a during neurite outgrowth. *Cell Signal* 2009;21:1961–1973.
 97. Hu Y, Ylivinkka I, Chen P, et al. Netrin-4 promotes glioblastoma cell proliferation through

- integrin beta4 signaling. *Neoplasia* 2012;14:219–227.
98. Staquicini FI, Dias-Neto E, Li J, et al. Discovery of a functional protein complex of netrin-4, laminin gamma1 chain, and integrin alpha6beta1 in mouse neural stem cells. *Proc Natl Acad Sci U S A* 2009;106:2903–2908.
 99. Schneiders FI, Maertens B, Bose K, et al. Binding of netrin-4 to laminin short arms regulates basement membrane assembly. *J Biol Chem* 2007;282:23750–23758.
 100. Hagedorn EJ, Ziel JW, Morrissey MA, et al. The netrin receptor DCC focuses invadopodia-driven basement membrane transmigration *in vivo*. *J Cell Biol* 2013;201(6):903–913.
 101. Hamill KJ, Langbein L, Jones JC, McLean WH. Identification of a novel family of laminin N-terminal alternate splice isoforms: structural and functional characterization. *J Biol Chem* 2009;284:35588–35596.
 102. Blinova MI, Paramonov BA, Kukhareva LV, Gor-elik lu V, Nikitina lu M, Voronkina IV. [Effect of fibroblasts, collagen, and laminin on the healing of wounds formed after dissection of split skin flaps in rats]. *Biull Eksp Biol Med* 1997;124:229–232.
 103. Kidd KR, Dal Ponte D, Stone AL, Hoying JB, Nagle RB, Williams SK. Stimulated endothelial cell adhesion and angiogenesis with laminin-5 modification of expanded polytetrafluoroethylene. *Tissue Eng* 2005;11:1379–1391.
 104. Kidd KR, Williams SK. Laminin-5-enriched extracellular matrix accelerates angiogenesis and neovascularization in association with ePTFE. *J Biomed Mater Res Part A* 2004;69:294–304.
 105. Kibbey MC, Grant DS, Kleinman HK. Role of the SIKVAV site of laminin in promotion of angiogenesis and tumor growth: an *in vivo* Matrigel model. *J Natl Cancer Inst* 1992;84:1633–1638.
 106. Ponce ML, Kleinman HK. Identification of redundant angiogenic sites in laminin alpha1 and gamma1 chains. *Exp Cell Res* 2003;285:189–195.
 107. Malinda KM, Wysocki AB, Koblinski JE, Kleinman HK, Ponce ML. Angiogenic laminin-derived peptides stimulate wound healing. *Int J Biochem Cell Biol* 2008;40:2771–2780.
 108. Rousselle P, Carulli S, Chajra H, Dayan G, Pin D, Herbage B. The syndecan binding sequence KKLRIKSKEK in laminin alpha3 LG4 domain promotes epidermal repair. *Eur J Dermatol* 2013 [Epub ahead of print].
 109. Araki E, Momota Y, Togo T, et al. Clustering of syndecan-4 and integrin beta1 by laminin alpha 3 chain-derived peptide promotes keratinocyte migration. *Mol Biol Cell* 2009;20:3012–3024.
 110. Kim JM, Park WH, Min BM. The PPFLMLLKSTR motif in globular domain 3 of the human laminin-5 alpha3 chain is crucial for integrin alpha3beta1 binding and cell adhesion. *Exp Cell Res* 2005;304:317–327.
 111. Min SK, Lee SC, Hong SD, Chung CP, Park WH, Min BM. The effect of a laminin-5-derived peptide coated onto chitin microfibers on re-epithelialization in early-stage wound healing. *Biomaterials* 2010;31:4725–4730.
 112. Damodaran G, Tiong WH, Collighan R, Griffin M, Navsaria H, Pandit A. *In vivo* effects of tailored laminin-332 alpha3 conjugated scaffolds enhances wound healing: a histomorphometric analysis. *J Biomed Mater Res Part A* 2013;101:2788–2795.
 113. Mochizuki M, Kadoya Y, Wakabayashi Y, et al. Laminin-1 peptide-conjugated chitosan membranes as a novel approach for cell engineering. *FASEB J* 2003;17:875–877.
 114. Ikemoto S, Mochizuki M, Yamada M, et al. Laminin peptide-conjugated chitosan membrane: Application for keratinocyte delivery in wounded skin. *J Biomed Mater Res Part A* 2006;79:716–722.
 115. Nakamura M, Mie M, Mihara H, Nakamura M, Kobatake E. Construction of multi-functional extracellular matrix proteins that promote tube formation of endothelial cells. *Biomaterials* 2008;29:2977–2986.
 116. Ali S, Saik JE, Gould DJ, Dickinson ME, West JL. Immobilization of cell-adhesive laminin peptides in degradable PEGDA hydrogels influences endothelial cell tubulogenesis. *Biores Open Access* 2013;2:241–249.
 117. Fu X, Xu M, Liu J, Qi Y, Li S, Wang H. Regulation of migratory activity of human keratinocytes by topography of multiscale collagen-containing nanofibrous matrices. *Biomaterials* 2014;35:1496–1506.
 118. Clement AL, Moutinho TJ, Jr., Pins GD. Micro-patterned dermal-epidermal regeneration matrices create functional niches that enhance epidermal morphogenesis. *Acta Biomater* 2013;9:9474–9484.

Abbreviations and Acronyms

BM	= basement membrane
DCC	= deleted in colorectal cancer
DR	= diabetic retinopathy
ECM	= extracellular matrix
FA	= focal adhesion
HD	= hemidesmosome
JEB	= junctional epidermolysis bullosa
LCC	= laminin coiled coil
LE domain	= laminin-type epidermal growth factor-like repeats
LG	= laminin globular
LM	= laminin
LN	= laminin N-terminal
LOCS	= laryngo-onycho-cutaneous syndrome