The laminin family
Founding members of the basement membrane

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Basement membranes (BMs) are extracellular sheet-like structures present in all multi-organisms. The major components are laminins, collagen type IV, nidogens and heparan sulfate proteoglycan perlecan. The pioneer in the field of laminins (LMs) was Ruppert Timpl who characterized in 1979 the first member of a new protein family.[1] At that time the authors concluded in the publication: "At present, it is not clear what role laminin plays in basement membranes." They had not realized to what extent the LM molecules would later be recognized for their dynamic role in diverse cellular processes. The constant interest in LMs is proven by the high number of published papers (20,384 publications in 2012) with the term "laminins" and which also reflects their importance in cellular signaling. Aumailley (schooled in the R. Timpl thought) provides a historical view of the LM family pointing out the new LM members that were successively discovered over the years.[2] The community proposed and agreed on a new terminology that simplifies LM nomenclature. For example, LM-111 replaced LM-1 to better reflect its $\alpha1\beta1\gamma1$ subunit composition. There are more than 50 theoretically possible heterotrimeric associations between all the α, β and γ chains but only a smaller number of LMs has been suggested. The exact number of proven isoforms is still under debate ranging from 15 to 18 isoforms. Two new potential LM isoforms 321 and 422 have been recently identified by biochemical and biophysical techniques.[3] Whether these two latter isoforms exist in vivo remains to be confirmed. LM chains differ at the level of the amino acid sequences with predicted molecular masses ranging from ~130 (LMβ3 chain) to ~400 kDa (LMα5 chain) with actual sizes being larger due to post-translational modifications. LMs possess structural domains that are conserved among all the isoforms despite this apparent heterogeneity in structure as emphasized by Aumailley and by Yurchenco.[2,4] The mapping of the major functions of LMs in relation to their structure is provided and discussed by Aumailley, among which cell-adhesion promoting activity and basement membrane assembly.[2]

Formation, Stability and Stiffness of the LM-Containing Basement Membranes

LMs form a network that is the foundation of all basement membranes. In this issue, Hohenester and Yurchenco recapitulate observations showing that polymerization of LM-111 act in concert with its cell-anchoring activities (via receptors) to assemble a functional BM on a cell surface.[5] Although the β

About Dr Patricia Simon-Assmann

Dr Patricia Simon-Assmann received her PhD in Biochemistry from the Université Louis Pasteur (Strasbourg, France) in 1979. Under the supervision of Dr Katy Haffen (a disciple of the famous embryologist E. Wolff) she studied the control of maturation of digestive enzymes by in vitro organ cultures and in vivo. After that, she joined James C. Thompson, professor and chairman of the Department of Surgery, University of Texas Medical Branch (Galveston, TX) as a postdoctoral fellow and elucidated the physiological and pathological roles of gastrin and other gut peptides. Upon her return to France she obtained a permanent position at CNRS in 1982 and has worked since then in an INSERM laboratory in Strasbourg dedicated to development and cancer research. Together with M. Kedinger she has contributed to the important discovery that epithelial-mesenchymal cell interactions are required for cell differentiation and intestinal morphogenesis. She is known for her work in the field of extracellular matrix, having discovered the importance of fibroblasts in the deposition and assembly of basement membrane components by creating chimeric intestines, a strategy developed by her. A major focus of her work are laminins. She demonstrated that these secreted molecules play an essential role in tissue architecture, epithelial cell differentiation, signaling and transcription factor activity. Her results are highly relevant for intestinal pathologies such as inflammatory bowel diseases and colon cancer where laminin expression is deregulated.

and γ chains of LM-111 primarily play a structural role mediating polymerization, the α chain also contributes to its self-assembly. Such a model, which is now extended to LM-211 and LM-511, shows that the process of polymerization involves the LN (for laminin N-terminal) domains at each end of the three short arms and is defined as "the three-arm interaction model"

as described by Hohenester and Yurchenco, and discussed by Aumailley.[2,5] Therefore, improving our understanding of the amino acid residues involved in each type of interaction will help in defining the biological roles of different LM ligands. While studying LM polymerization in solution has largely improved our knowledge of the domains involved, genetic studies, supported by in vitro observations clearly established that LMs are uniquely required for ECM assembly as stated by Hohenester and Yurchenco.[5] In parallel Halfter et al. discuss the new techniques that have been developed to analyze the protein composition and biochemical properties of in vivo-derived BMs.[6] For example, improvement in isolation procedures of laminin containing-BMs made them accessible to atomic force microscopy (AFM)-based measurements of thickness and elasticity. These recent developments lead to new concepts. Indeed, AFM measurements unravel the underestimated thickness and mechanical properties of native BMs.[6,7] Interestingly, Halfter et al. discuss the relation of BM mutations with the reduced stiffness of BMs, which might explain some of the associated pathological phenotypes.[6] The dramatic changes in thickness/stiffening of the BM observed during aging are also mentioned. Such a comprehensive evaluation of BM composition has been performed by mass spectrometry-based proteome in the chick retinal BM.[8] Future research should aim to quantify ratio of the individual molecules and all the possible functional interactions within the BM that will affect its stiffness, an important determinant of cell behavior.

Diversities in LM Expression in Tissues

It is important to consider that each BM contains a variety of LM components whose composition and organization change throughout development. Deciphering these dynamic remodelling and compositional changes are critical for the understanding of LM functions during development. Besides that, each LM has a preferential role in tissue structure and architecture. As emphasized in this special issue, two key LM isoforms, namely LM-111 and LM-511, are strongly expressed and functional during embryonic development as confirmed by invalidated knockout mouse models.[9-11]

The review proposed by Borycki illustrates the critical role of LM-111 in BM assembly and function in embryonic muscle cells.[9] Several persuasive arguments lead to conclude for the first time that the Sonic hedgehog pathway controls LM-111 synthesis as well as the subsequent upregulation of the α6β1 laminin receptor (driven by the Myf5 transcription factor) during myotomal BM assembly. As stated by the author, it remains now to establish whether Sonic hedgehog controls *Lama1* transcription directly or indirectly. Such a study of myotomal BM provides a novel insight into the possible relationship between transcription factors, LM and growth factors from a developmental point of view that should be extended to other organs. The review from Edwards and Lefebvre focuses on retina development and brings further arguments for the essential role of LM-111 in tissue integrity and function.[10] An extensive overview on the available mutant models for LMα1 is included which clearly demonstrates its key

role in early development and retinal development. Moreover, the authors reviewed literature reporting the expression of the other LM chains within the retina and described the retinal phenotypes associated with their loss owing to LM mutant models. Potential links with human pathologies are discussed, which could open some new avenues of investigation and carry hope for possible therapeutics strategies.

In contrast to the LMα1 chain, which has a very limited expression at least in adult organs, the LMα5 chain is widely expressed in developing and mature tissues. Spenlé et al. provide a very detailed review on the sites of expression of the LMα5-containing isoforms and, more importantly, on their functional roles.[11] They compiled data from studies of *Lama5* transgenic mice and cultures on LMα5-containing substrates. We also learn that signaling pathways can be modulated by LM-511, in particular the PI3-kinase/Akt, Wnt and Sonic hedgehog pathways. In the field of regenerative medicine, stem cell research becomes an emerging field. Linked to necessity to set-up methods to support the self-renewal of embryonic stem cells, interest in the LM-511 field has recently increased as discussed in the review. Interestingly, this LM-511 substrate allows to culture mouse and human embryonic stem cells in a defined cell culture environment. This approach may be useful for the development of cell lineages for therapeutic purposes. Lastly, it is emphasized that BM assembly often requires close contacts between heterotypic cell types and interesting examples of such cooperation for LM deposition are here mentioned by Spenlé et al. and by Borycki.[9,11]

Blood vessels are other sites of LM expression where these molecules provide structural support and promote endothelial cell adhesion, migration and survival.[12] As reported by Yousif et al., α4- and α5-containing LMs are the major isoforms found in the vessel walls, with the added contribution of LMα2 in larger vessels.[13] We also learn that the vessel structure (endothelial cells with pericytes or smooth muscle cells) varies according to tissue type with associated changes in LM isoform expression. In vitro data confirmed by *Lama4* knockout mice show that LMα4 could promote endothelial Dll4/Notch signaling and are of potent interest.[10,13] A schematic representation of LM functions in the endothelium and smooth layers of the vessel wall emphasizes the diversity of the physiological responses to LMs, i.e., leukocyte extravasation, mechanotransduction and contractility relayed by defined specific intracellular information.[13]

The active regulatory functions of LMs arise from their interactions with membrane receptors that will subsequently activate different signaling pathways. Four major transmembrane receptors are responsible for cell binding to LMs: integrins, dystroglycan, syndecans and Lutheran blood group glycoprotein.[14] These bindings are mediated via different domains of the LM molecule and are more or less specific to each isoform as notified in several reviews of this special focus.[2,5,11,13,15,16]

In summary, the balance between the different LM isoforms in tissues is likely to be important for the homeostatic regulation of normal organs. Yet, the molecular mechanisms that drive the developmental transitions in LM expression and deposition until adulthood (when LMα1 is largely supplanted by the LMα5

chain for example) have not yet been clearly found and deserve attention.

A Single Molecule but Multiple Functions

LM and tissue integrity. The importance of LMs in maintaining tissue integrity is demonstrated in diseases known as congenital muscular dystrophy and junctional epidemolysis bullosa. As stated by Holmberg and Durbeej, LM-211 is the main LM isoform in the BM of adult skeletal muscle.[15] Mutations in the gene encoding the LMα2 chain (*LAMA2*) cause the severe form of congenital muscular dystrophy type 1A. Analyses of the various LM-deficient mouse models have led to a significant improvement in our understanding of the function of the individual LM molecules. This is exemplified by the phenotypes associated with the spontaneous, targeted or induced mutations in mouse in the *Lama2* gene. These models are described in detail and compared with other genetic mouse models of muscular dystrophies by Holmberg and Durbeej.[15] LM-211 binds to two major classes of receptors in the skeletal muscle, the integrins and the α-dystroglycan. Holmberg and Durbeej discuss the signaling pathways triggered by LM-211 binding to α7β1 integrin which include the PI3-kinase/Akt cell survival pathway and genes known to be part of the ubiquitin-proteasome system.[15] Nevertheless much less is known regarding LMα2 signaling through dystroglycan. The mentioned authors further discuss on the importance of LM-211 for transmission of muscle force and the necessity to create bioengineered skeletal muscle for future treatment strategies.

In the skin, LM-332 is an essential dermal-epidermal component that maintains tissue cohesion providing skin integrity and resistance against external mechanical forces. As described in the review of Kiritsi et al. mutations in the *LAMA3*, *LAMB3* and *LAMC2* genes that encode respectively the three constituent chains of LM-332 will lead to human disorders.[17] They correspond to the junctional epidemolysis bullosa (JEB) with two subtypes, JEB-Herlitz (complete absence of LM-332 with severe skin fragility and blistering) and JEB-other (moderate phenotype), or to laryngo-onycho-cutaneous syndrome caused by specific mutations in the *LAMA3* gene. Immunofluorescence mapping with antibodies to all three LM-332 chains appears as the first diagnostic tool, as negative signals (meaning complete lack of the molecule) indicate the diagnosis of JEB-Herlitz and a severe prognosis. The review summarizes the mutational spectrum reported in LM-332-deficient JEB patients where *LAMB3* is affected in 80% of the cases and unusual phenotype-genotype correlations are included. Understanding the genetic basis is a major goal as it will facilitate the development of molecular therapy approaches. As a proof-of-concept, in a human pilot trial, gene therapy for JEB partially restored LM-332 in the skin and allowed to regenerate a functional epidermis in vivo.[18]

Structure of LMs may modify biological activity. The review of Rousselle and Beck points to an interesting concept which is that the processing of a LM molecule can impact the cellular responses.[16] This is exemplified by the LM-332 molecule: this molecule is synthesized as a precursor molecule that undergoes cleavage by proteolytic processing at the N- and C-terminus of the

α3A chain as well as at the γ2 chain N-terminal extremity. Much is known about the related enzymes involved in this proteolytic process. They include various classes of proteases that specifically cleave defined domains of the LM-332 molecule (plasmin, BMP-1, thrombin, mTLD and possibly metalloproteases). The authors describe the structure of the LM-332 molecule as deduced from homology modeling and provide structural models of the human LMβ3 LN-LE1–4 domains and of the LMα3 LG domains. They further discuss the physiological relevance of complex post-translational processing of the LM-332 and conclude that the balance between unprocessed and processed forms regulate several physiological functions. For example, a form of LM-332 that lacks the LG45 domain is found in mature BM, while LM-332 with intact LG45 is linked to migratory/remodeling situations. Furthermore, a defective α3 and γ2 processing was observed in pathological situations in skin disease or can be exacerbated in cancer in conjunction with increased proteolytic activity. As mentioned in the literature and reviewed here, the generated cleaved fragments will either retain their original biological activity or display cryptic biological property.[16] The most obvious example is the domain III of the γ2 chain that directly interacts with the EGF receptor and activates migration.[19] Furthermore, processing of the LM-332 will affect its binding ability to integrins and to syndecans as mentioned by Rousselle and Beck.[16]

LMs in Tumorigenesis

Pouliot and Kusuma highlight the novel importance of LM-511 in cancer and its pro-metastatic function.[20] Their data on breast (and other) tissue highly support the key role played by LM-511 in cancer progression and metastasis. They proposed a sequential integrated model that includes deregulation of other LM isoforms (LM-111, LM-332), activation of metalloproteases (MMP2/9), involvement of myofibroblasts and upregulation of a defined subset of integrins. Their data are also consistent with the contention that LM-511 can contribute to breast cancer metastasis through autocrine mechanisms. They propose that targeting tumor-LM-511 interactions may have therapeutic potential in advanced cancer patients providing that LM-511 expression could clearly define the tumors with a tendency to metastasize to bone.

Proeolytic disruption of the BM architecture via degradation of its individual components (such as by metalloproteases) occurs in tissue remodeling and tumorigenesis. Therefore, the proteolytic processing of LMs could lead to small fragments that could have biological properties by themselves and thus differ from the entire native molecule. Thus the goal was to identify synthetic peptides derived from the LM-111 molecule able to inhibit or activate tumorigenesis. Kikkawa et al. described a strategy to screen for active sites allowing identification of LM-111 derived peptides that would affect malignancy.[21] The rationale to focus on LM-111 in tumorigenesis is discussed and linked to data from previous literature. Moreover, the experimental design to produce the synthetic peptides and the methods to test their cell-adhesion properties are described. Four peptides are of potent interest: two of them (IKVAV and AG73) are found on the α1

chain, one (YIGSR) on the β1 chain and one (C16) on the γ1 chain. Kikkawa et al. integrate in vivo experiments that allowed them to discover three tumor-promoting peptides.[21] These negative or positive activities are mediated through different sets of cellular receptors, 67 kDa, integrins, syndecans, with defined mechanisms raising a possible tissue specificity of action. Ways to deliver such peptides to target tumors are under study for cancer therapy application.

Conclusion and Prospects

The BM with its diversity in LM composition is a multifunctional and dynamic entity that can influence diverse biochemical, physiological and mechanical processes simultaneously. This focus highlights the difficulty of analyzing the precise contribution of LM to morphogenesis and cell behavior due to the variety of LM isoforms, to the transient combination of LMs with other BM molecules and to the various sets of cell receptors. This difficulty is further accentuated by the multiplicity of possible molecular interactions that may exist in vivo in a given BM, which eventually affect tissue patterning as well as cell behavior. Besides that, we can also assume that the release of bioactive molecules, such as growth factors known to be sequestered in the BM, will depend on the structural organization of LMs. This remains an understudied question. In order to facilitate and extend our knowledge on LMs, development of other animal models such as zebrafish and *C. elegans* is certainly an important task. The zebrafish is an outstanding model system for imaging cell behavior in vivo and in particular the optically transparent retina. As an example it was nicely shown that Lama1 contact is sufficient to transform a neurite into an axon in vivo.[22] The *C. elegans* model has allowed to realize a real-time analysis of BM dynamics in vivo providing evidence that the BM itself can have plastic properties.[23]

Many questions still remain unanswered among which the regulation of the LMs. Some advances have been made regarding the control at the transcriptional levels by studies on gene promoters such as those encoding the LMα1 or LMγ2 chain.[24] In particular, it will be of potent interest to pay more attention to the epigenetic regulation of the LM genes. Indeed, promoter hypermethylation of some genes has been associated with their silencing and methylation frequencies allowed to distinguish invasive from noninvasive tumors as exemplified for *LAMA3*.[25] The potential use of methylation of LM-encoding genes as molecular markers in clinics might be useful.

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