Analysis of the Effects and Current Treatments of Laminin Deficiency

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Abstract

Laminin (LM) is a network of proteins that functions as a connective framework of most cells in the body. It is composed of multiple different subunits and therefore has many different variations. It is a trimeric protein, meaning that it is composed primarily of α, β, and γ chains. The differentiation of these subunits is what gives the different variants their functions. In addition, although LM is the primary molecule in scope, the network of other connective proteins involved in LM-associated diseases will also be covered in lesser detail because molecules like dystrophin, dystroglycan, collagen, and integrin are vital to the understanding of how LM can influence the body. Current treatments are typically symptomatic, and if cures are to be found, the focus needs to be on the root cause.

Analysis of the Effects and Current Treatments of LM Deficiency

Background

Structure of LM

The most crucial aspect of learning the function and role of LM is first knowing and understanding the structure. LM is a diverse molecule with a unique structure. It consists of three chains that are wrapped around one another forming a cross or t-shape (Figure 1). At the point where they meet, the chains form a coiled coil domain which is perhaps the most distinguishable feature of LM. The chains are labeled α, β, and γ because each one has its own role. Each molecule of LM has one of each kind of chain. There have been different types of each chain that have been identified: five α, three β, and three γ chains (Durbeej, 2010). This variation is what gives LM its diversity. Each LM isoform can consist of a combination of the chains, and therefore is named accordingly. For example, LM-511 contains an α5, a β1, and a γ1 chain.

The chains all have unique domains within their structure that have specific interactions. The shorter arms contain a sequence of domains: the N-terminus is occupied with the globular LM N-terminal (LN) domain, globular LM 4 (L4) domains, and LM four (LF), which are all spaced out with rod-like sections made of LM epidermal growth factor-like (LE) domains (Durbeej, 2010). Next, the long arm is considered one domain, the LM coiled-coil domain (LCC) with a large globular (LG) domain on the end. In one place along the LCC, specifically the β chain, there is an interruption named LM β-knob (Lβ) domain (Aumailley et al., 2005).

It should be noted that although theoretically there could be multiple combinations of LM chains, only certain ones are seen. According to research, only

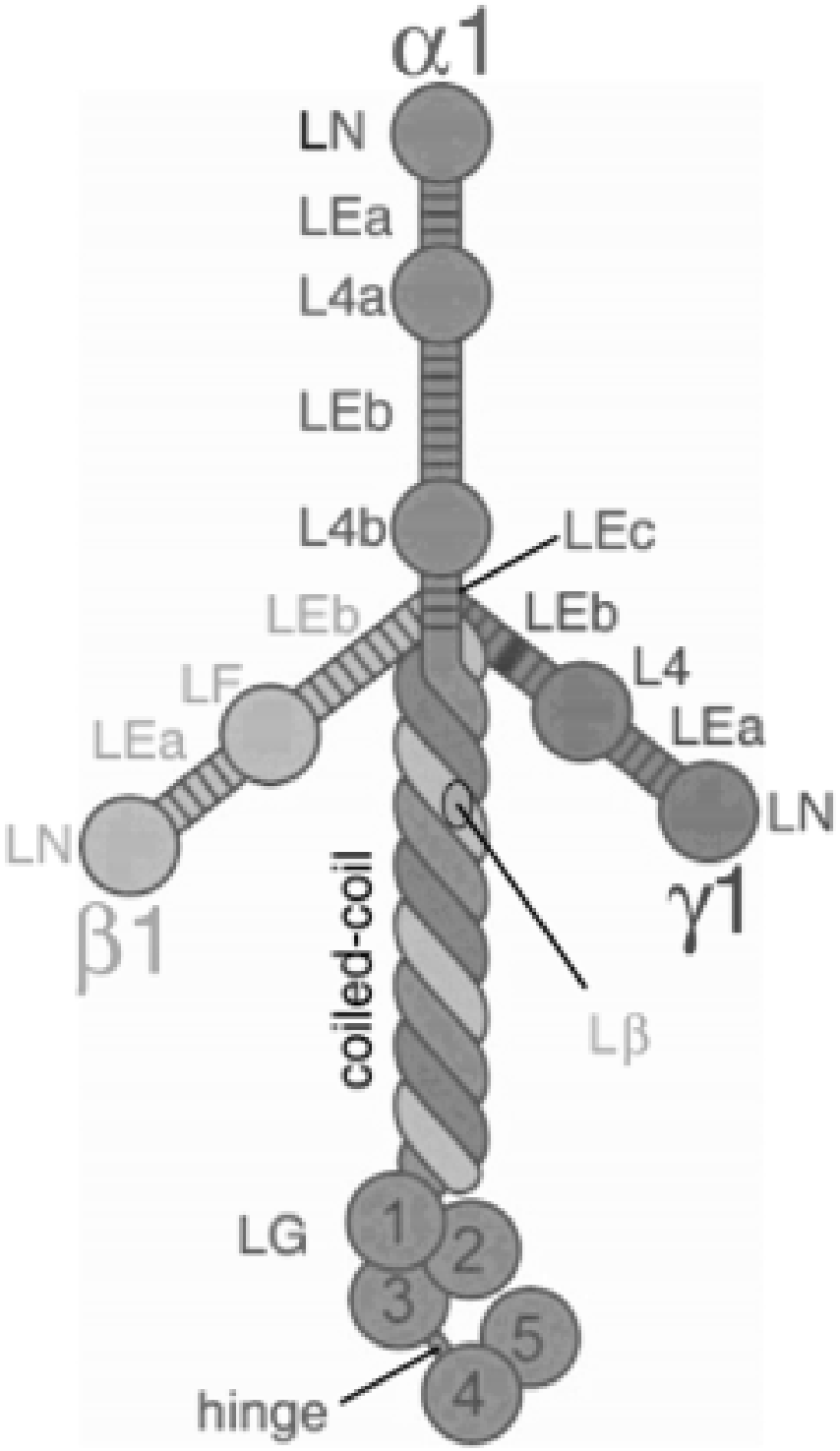

Figure 1. Structure of LM (Durjeeb, 2010).

twelve trimeric LM isoforms have been clearly observed in the body. In addition, the γ2 chain is seen to only assemble with the α3 and β3 forming LM-332 (Tunggal, 2000).

Functions of LM

LM has a primary role of structural support. It forms a lattice-work of repeats acts as an anchor for other structural proteins in the basement membrane (BM). LM forms (along with other components of the BM) the largest polymer in the body; the sheet of membrane under the skin runs continuously throughout the body (Scheele et al., 2007). This self-polymerization is perhaps the most crucial characteristic of LM, as it allows the embryonic membranes to initiate assembly immediately. Providing this structure in the BM is essential in the epithelium, endothelium, muscle, Schwann, and fat cells (Durbeej, 2010). While the primary role of LM is structure, it is also crucial in a number of different aspects. It is an important cell-signaling molecule responsible for neurite outgrowth, embryonic development and organogenesis (Timpl, 2000). Each domain (LN, L4, LF, LE, LG and LCC) in their respective chains has a particular role in the molecule. Each binds to molecules unique from each other, thus changing the role it has in the body.

LN domain. The LN domain is a calcium dependent binding region. Being positioned at the end of the short arms, the LN domain is responsible for binding many different kinds of molecules. This distal domain is primarily responsible for cell surface anchorage. It is seen to bind to sulfated carbohydrates (S-CHO), heparin, and receptor interactions mediated by integrins (Miner & Yurencho, 2004). This domain greatly influences the incorporation of the whole molecule into the BM. It supports neurite outgrowth and has been seen to exhibit self-assembly (Tunggal et al., 2000). This self

assembly can occur because of the LN domain being on the ends of the short arms. The β and γ chains of many LM molecules will bind to one another in a horizontal fashion while the α chain connects to this junction vertically. This self-assembly forms a lattice-like framework.

LG domain. The LG domain is made of five independent units, or subdomains, that can be designated LG1 to LG5 (Tunggel et al., 2000). It is also responsible for cell-anchorage of principal ligand interactions with the cell surface. The most common interactions with the LG domain are heparins, sulfides, and integrins (Miner & Yurencho, 2004). There is also evidence showing a tendency for α-dystroglycan to bind. It does so with negatively charged oligosaccharides binding to the positive calcium ion. This binding can happen at more than one site on the LG domain, forming a vital component of the trans-membrane complex that provides the link from the extracellular matrix to the cytoskeleton.

LCC domain. The LCC domain is the longest arm in LM, providing the structural link to the molecule. The α-helix is made of seven repeats (*abcdefg*) which have distinct characteristics; *a* and *d* are hydrophobic, *e* and *g* are charges, and *b, c* and *f* are hydrophilic (Tunggel et al., 2000). The strength in this domain comes from the interactions of the repeats with their preferred environment. Since the LCC is made of three chains, each chain has repeats interacting with one another. On the inside of the chain, the hydrophobic repeats *a* and *d* interact with one another in a non-polar fashion. The strength of the non-polar interactions increases with number. Next, the charged particles *e* and *g* align, further stabilizing the length of the chains to each other. Finally, the hydrophilic regions *b, c* and *f* are oriented facing outward because of any polar

interactions they may encounter. For further stability, all three chains are supported to one another by disulfide crossbridges on the N-terminal end. On the C-terminal end, the β and γ chains are supported by one additional disulfide crossbridge (Tunggel et al., 2000). This arrangement exclusively forms heterotrimers. Having the primary role of structure, the LCC is limited in other interactions.

LF and L4 domains. The LF and L4 domains are globular segments located on the short arms and are contained in short sequences. On either side of the L4 globular domain there lies an LE domain (Tunggel et al., 2000). Although precise functions of these domains have not been properly characterized, it is hypothesized that these domains still play a major role in muscular integrity. This is because a mutation lacking regions of the LF and L4 domains results in forms of congenital muscular dystrophy (Allamand et al., 1997).

LE domain. The LE domain provides rod-like spacers between the other domains on the short arms of LM. Although the role of the LE domain is relatively uncertain, it has been experimentally tested and suggested that it binds to nidogen, forming an essential bond in the BM (Willem et al. 2002). The nidogen acts to connect preexisting, independently assembled LM networks with collagen networks (Tunggel et al., 2000).

Functions of Proteins Associated with LM

In order to provide the strength and support necessary, LM must be highly cross-linked to other matrix proteins in the BM. LM connects to receptor proteins, other structural proteins, and glycolipids in order to provide the necessary support in the BM.

Integrin. The most common LM receptor is integrin; all others are called *non-integrin* receptors. Integrin is also the primary receptor for collagen where it mediates cell

binding (Tuckwell & Humphries, 1996). Similar to LM, these molecules are primarily involved in cell adhesion. They are responsible for attaching certain cells to the extracellular matrix (ECM). Composed of 24 αβ heterodimers, they can also be important factors in cell-cell interactions (Barczyk, Carracedo, & Donald Gullberg, 2010). Integrins have a cytoplasmic domain responsible for this interaction through activation of signal transduction. Also, these domains interact with the cytoskeleton influencing cell shape and migration (Belkin & Stepp, 2000).

Integrin is a diverse molecule that is able to bind to many different ligands such as nidogen, collagen, fibronectin, and LM. There are at least eight integrin isoforms that bind to LMs. These include α1β1, α2β2, α3β1, α6β1, α6β4, α7β1, α9β1, and αvβ3 which usually bind to the αLG domains. It is also noted that β1 integrins lend to the organization of BM organization (Durbeej, 2010, Köhling et al., 2006). Of these, some are involved with LM to the extent of being seen exclusively as LM receptors (Belkin & Stepp, 2000). LM-integrin interactions are seen greatly in epithelial cells, muscle cells, and nervous cells.

Dystroglycan. Dystroglycan (DG) is a cell surface protein that is expressed by cells in contact with the BM. It is necessary for embryonic tissue development and for the deposition of other BM proteins (Henry & Campbell, 2000). DG has been characterized in a complex of dystrophin and glycoprotein along with other minor proteins such as syntrophins, sarcoglycans, dystrobrevins, and sarcospan. Within this complex, the two components of dystroglycan (α and β) form a link between LM, muscle membrane, and dystrophin within the actin cytoskeleton (Bozzi et al, 2009, Henry & Campbell, 2000). Although it is not a main contributor to congenital disorders, the absence of DG is largely

noticed in mouse models. Phenotypes are seen in neuromuscular regions, skeletal muscle, the central nervous system and the brain (Bozzi et al., 2009). In addition immunocytochemistry reveals a nearly complete absence of αDG in dystrophic muscles (Mercuri et al., 2002). As well as integrin, DG (a non-integrin) is a major LM receptor in the nervous and muscular tissue, primarily at the neuromuscular junction. The LM binding aspect of DG is known as αDG and has been observed to bind primarily to the LG domain of LM. αDG is also similar to integrin, being a major contributor to the organization of the BM (Belkin & Stepp, 2000, Köhling et al., 2006).

Nidogen. Nidogen is a structural protein that is involved in the assembly and structure of the BM. Unlike LM, it is not essential for architecture of the membrane because although nidogens are important structural components, they are not a prerequisite for BM formation. This is seen in the early embryonic death of mice that have the γ chain of LM absent; they are not present for the binding of nidogen (Ho et al, 2008, Köhling et al., 2006). The two isoforms of nidogen, nidogen-1 and niogen-2, have the same principal structure consisting of three globular domains. These are named G1, G2, and G3, of which the latter is seen to bind to the LE domain in short arm of the γ chain of LM (Köhling et al., 2006). This interaction is mechanistically and structurally unique. The G3 domain of nidogen forms 6 propeller-like structures that form into a concave depression. All six blades of this mold fit onto the repeats of the γ chain, forming a joint that is strengthened by hydrogen bonds (Ho et al, 2008). It has been noticed that LM and nidogen aid one another in certain signaling processes. Although this makes it difficult to distinguish the origin of the signal, their collaboration increases the overall

intensity. In addition, cell adhesion seen in nidogen is markedly reduced when apart from LM (Ho et al, 2008).

Collagen. A large component of the BM is collagen. Collagens are abundant molecules that are found throughout the body, providing strength and structure to connective tissues such as cartilage, tendons, ligaments, and skin. Having many places of function, there are many slightly different variations of collagen. For example, type XVII collagen helps with the attachment of the outer and inner layers of skin providing both strength and flexibility (Junctional, 2009). Together with LM, collagen's absence from the skin results in blistering upon contact. Mutations in the gene COL17A1 that codes for type XVII collagen results in a less severe form of junctional epidermolysis bullosa, as discussed later.

Being such a large part of the structural framework, like LM, its absence is largely noticed. This molecule works together with LM to assemble in the BM. Collagen relies upon LM for assembly, while LM can independently self assemble into a polymer. This dependence on LM means that the BM cannot form in the absence of LM (Miner & Yurencho, 2004). Studies have shown that the main type of collagen present in the BM is type IV which binds to perlecan, nidogen, and LM (Köhling et al., 2006). Cardiac dysfunction resulting in mid-gestational lethality is a product of having deficient collagen IV (specifically the α1 and β2 chains) (Köhling et al., 2006).

Dystrophin. Dystrophin can be normally found in the cytoskeleton of the muscle and works with a protein complex in connecting the sarcolemma to the actin (Figure 1). The other proteins in the complex include utrophin and α-dystrobrevin, which aid in the maintenance of muscle strength (Blake et al., 2002). The dystrophin acts as an anchor in

the framework of the muscle cells, and without it muscles become weak, degenerating quickly.

Dystrophin is most commonly known for its association with Duchenne muscular dystrophy (DMD), a severe muscle wasting disease. LM is also greatly involved with muscular dystrophy, as both these molecules are vital for structural maintenance. In this pathology, wedge-shaped areas of abnormal cytoplasm are underlying disrupted or even absent pieces of sarcolemma. This abnormality is the primary pathology of DMD muscle because it is the source of the fragility and leakiness of the cell membrane (Mokri & Engel, 1975). The dystrophin deficient muscle has increased permeability to macromolecules, allowing them to flow relatively freely in and out of the cell. In addition, this permeability is made worse with physical activity because the movement puts stress on deficient muscle (Rowland, 1976). Dystrophin is connected to LM via dystroglycan, and the disruption of this link can cause symptoms of DMD (Ehmsen, Poon & Davies, 2002).

Perlecan. Perlecan is a diverse molecule that is key in the structure of the BM, in the genesis and stability of cartilage, and in the function of the neuromuscular junction (NMJ). In the BM it binds primarily to integrins, dystroglycan, collagen, and LM aiding in cell adhesion and integrity (Sasse et al., 2008 and Iozzo, 1994). The role that perlecan has in the cartilage is extensive. This proteoglycan is involved in the maintenance of collagen where it has protective role of binding and regulating (Gustafsson et al. 2003). In the NMJ, perlecan mediates muscle contraction by localizing acetylcholine, enabling muscle relaxation (Smith & Hassell, 2006). Studies have shown that when mice lack perlecan 70 percent die within ten to twelve days of becoming an embryo while the other

30 percent suffer from severe skeletal defects (Costell et al., 1999). Human perlecan deficiency results in disorders such as Dyssegmental Dysplasia and Schwartz-Jampel syndrome, where symptoms similar to those of the null mice are lethal (Gustafsson et al. 2003). Perlecan has a structure consisting of five domains, two of which bind to LM. Domain three binds to regions on the short arms, and domain five binds to the globular domain of the α chain (Smith & Hassell, 2006).

Pathological Analysis

A disease or disorder from an LM deficiency (lamininopathy) can have a range of different effects. The diversity that LM exhibits in the body creates a number of different pathological disorders. LM is composed of multiple components that are each genetically coded with a different sequence. With each component having different roles it is understandable that many different outcomes could be seen from an LM disorder. For example, a mutation in a gene coding for one LM chain produces muscular dystrophy while another mutation leads to skin blistering and another to kidney defects (Scheele et al., 2007). There are also many problems associated with lamininopathies that do not have official names, or are secondary effects of existing diseases. Symptoms such as egression of bone marrow and mature blood cells in the hematopoietic system as age increases. In addition, some disorders that are associated with other things could also have LM deficiencies. For example, vascular and pulmonary diseases such as atherosclerosis are a major problem in the West. Under examination, atherosclerotic plaques have been found to have reduced amounts of LM β2 and LM α5 chains. The deficiency in LM could be a major contributor to the malfunctioning hearts in these disorders (Scheele et al., 2007). Furthermore, the role that LM plays in microbial and

viral diseases is largely overlooked. In order for a pathogen to have any detrimental effect in the body, it must first anchor itself to something to provide surface for colonization. LM is an example of a molecule that a bacterial adhesin could interact with (Ljungh, Moran, & Wadstrom, 1996). This is not an LM deficiency; however, it is a health issue associated with LM.

Congenial Muscular Dystrophy

Many types of muscular dystrophy are seen in the body, each with varying genetic malfunctions. This type of disease is known for muscle weakness and early childhood fatality. Congenital muscular dystrophy (CMD) is best classified as symptoms of "severe muscle weakness, hypotonia, joint contractures, white matter abnormalities, peripheral neuropathy, respiratory compromise, and failure to thrive" (classified as weak and uncoordinated swallowing) due to feeding difficulties (Scheele et al., 2007). In addition to this, features of CMD include severe mental retardation, eye impairment, rigidity of the spine, and limp distal joints (Mercuri et al., 2002). There are several types of CMD diseases, with the most common one having LM deficiency. The others are a result of insufficient ukutin, integrin, fukutin-related protein, and collagen-VI. Two main sub categories of these different types of CMD are present: with mental retardation and without mental retardation (Mercuri et al., 2002). The type of with LM deficiency, called MCD1A (without mental retardation), is linked to chromosome 6q2 (Hillaire et al., 1994). Severity of MCD1A is a result of the type of genetic mutation present. Some milder cases are seen to have either a homozygous frame mutation of the LAMA2 (coding for LM-α2 chain) gene or a compound heterozygosity in certain splice mutations paired with a null allele (Allamand et al., 1997, Naom et al., 2000). These milder cases of

CMD delay the onset of symptoms until the individual is past the twentieth year (Mercuri et al., 2002). In more severe cases symptoms occur neonatally, resulting from missense mutation in the LAMA2 and other highly functional genes. Other less severe cases of CMD are known as MDC1B and MDC1C where normal intelligence and peripheral nerve electrophysiology is apparent (Mercuri et al., 2002)

LM deficiency. Perhaps the most abundant molecule in the muscular BM is LM α2. This part of LM is seen in the NMJ surrounding muscle fibers and nervous fibers (Scheele et al., 2007). Mutations in the gene that codes for LM-α2 cause neuromuscular effects like congenital muscular dystrophy (Guo et al, 2003). The mutated LAMA2 gene greatly affects the LG domain, thus affecting ligand binding (Mercuri et al., 2002). Although CMD is a muscular disorder, the phenotypes are attributed to both muscular and nervous LM. CMD is as much a nervous disorder as it is a muscular one. An example of this comes from a study done where LM specific to the Schwann cells was removed resulting in severe muscular dystrophy (Chen & Strickland, 2003). The phenotypes of this disease, therefore, seem to stem from nervous abnormality which then results in muscular impairment (Yu, W., Yu, H., & Chen, Z., 2007).

LM deficient nervous tissue. Since LM is significantly involved in Schwann cell (SC), CMD can be further understood by analyzing the process of radial axon sorting. This is the process in which SCs proliferate and engage in direct one-on-one contact with axons (Yu, W., Yu, H., & Chen, Z., 2007). One particular experiment (Yu et al. 2009) demonstrates the importance of LM in SC morphogenesis. SCs must undergo proliferation and morphogenic changes to accomplish radial axon sorting. SCs lacking LMs do not myelinate, nor do they form a bipolar shape (which is the first step of SC

differentiation). Since SCs that do not have LM are defected in both proliferation and extension, it is hypothesized that the signaling pathway is the cause. The enzyme Rho GTPase has two members Cdc42 and Rac1 that are involved in the signaling pathways of axonal sorting process. In normal nervous tissue, Cdc42 and Rac1 levels are high during the initiation of myelination, but this is not the case in LM deficiencies. Since LM regulates Cdc42 and Rac1 pathways, SCs that lack LM had a decreased amount of activity of Rho GTPase, and thus, decreased proliferation and extension. Furthermore, when treated with Cdc42 and Rac1, these nerves showed phenotypic improvement. The Cdc42 and Rac1 inhibition or activation directly controlled the myelination activity of the LM deficient SCs.

Junctional Epidermolysis Bullosa

LM runs in a lattice framework all throughout the body--especially the skin. Junctional epidermal bullosa (JEB) is one of many different kinds of epidermal bullosa. It is an LM associated skin disorder that results in severe blistering. Within JEB there are two similar main sub-groups, Herlitz JEB and non-Herlitz JEB. Although these have nearly the same mutated genes and symptoms, they differ greatly in severity (Junctional, 2009). Herlitz JEB often results in fatality one year after birth, whereas patients with non-Herlitz JEB are more likely to have a normal life span. The blistering that occurs is prevalent on the visible skin, but is also present in the mucous membranes of the throat, oral and nasal cavities, and digestive lining (Pfendner & Lucky, 2008) making it more difficult to eat. Granulation tissue also beings to develop as red, bumpy patches after repeated blister formation. This tissue is susceptible to infection and loss of nutrients because it bleeds easily and profusely. Also, when a build-up of the granulation tissue

occurs in the airway, it can cause difficulty breathing (Junctional, 2009). Secondary issues regarding JEB (especially Herlitz) include growth retardation via malnutrition, anemia, infection, sepsis, electrolyte imbalance, osteoporosis, squamous cell carcinoma, and enamel dysplasia (Pfendner & Lucky, 2008, Fewtrell et al, 2006, Mallipeddi et al., 2004, Kirkham et al., 2000).

In order to affirm a case of JEB, transition electron microcopy (TEM) or immunofluorescent antibody mapping is used to view a recently acquired blister sample (Charlesworth et al., 2003). TEM is beneficial in examining the structural aspects of the basement membrane such as anchors and hemidesmosomes. Although they both have aspects that will look the same, this technique will differentiate between Herlitz JEB and non-Herlitz JEB. For example, in both cases splitting in the lamina lucida (the "layer of the basement membrane lying next to the basal surface of the adjoining cell layer" [Lamina, 2012]) of the basement membrane is seen. In Herlitz JEB, hemidesmosomes are significantly low in number, and anchoring filaments are either very low or gone completely. In non-Herlitz JEB, hemidesmosomes and anchoring filaments are low, but still helpful. Using immunofluorescent antibody mapping leads to the finding of absent LM (Pfendner & Lucky, 2008).

LM deficiency. The most crucial LM isoform present in the junction between the dermal and the epidermal layers is LM-332 (Scheele et al., 2007). All chains α3, β3, and γ2 are vital for survival; any loss of these chains results is Herlitz JEB, which is fatal (Mitsuhashi & Hashimoto, 2003). The immunofluorescent antibody mapping technique allows mutations in the LAMA3, LAMB3 (coding for LM-β3 chain), and LAMC2 (coding for LM-γ2) genes to be seen via stain. To further test for JEB, a sequence

analysis test can be done on LAMA3, LAMB3, and LAMC2 detecting ninety-eight percent of the total mutations. LAMA3 mutations are also found in Shabbir syndrome, which is another disorder involving skin fragility (McLean et al., 2003). Emphasis is placed on the LAMB3 gene as it accounts for a seventy-percent attribution to JEB (Pfendner & Lucky, 2008). Most of the mutations that take place in LAMB3 are located at R635X and R42X, both resulting in a premature stop codon (Uitto & Richard, 2004). These premature stop codons result in truncated LM polypeptides that are unable to assemble into the trimeric shape, resulting in a compromised assembly of anchoring proteins (Uitto & Richard, 2004). A second proposed mechanism is that the mutations accelerate mRNA decay, resulting in no polypeptide synthesis (Frischmeyer & Dietz, 1999). The mechanisms are not testable because in both cases there is no LM molecule to examine; both fail to produce LM-332.

Congenital Nephrotic Syndrome

Congenital nephrotic syndrome (CNS) is a genetic (some non-genetic cases are less severe) disorder that primarily affects the kidneys, however, there are four distinct identifies types: Finnish, Pierson syndrome, Denys-Drash syndrome, and mesangial sclerosis. This disorder places the patient at high risk of infection, diseases associated with malnutrition, blood problems, acute and chronic kidney failure, and impaired growth and development (Jackson, 2007). Components such as nephrin and podocin, which are key in glomerular filtration, are the most affected (Jalanko, 2009). Although the complete classification of CNS is not fully understood, some constants appear between cases. Genetic mutations coding for nephrin and podocin appear in CNS resulting in dysfunctional filtration slits. These slits are formed with extracellular proteins that create

a meshwork anchor (Zenker et al. 2004). Symptoms of CNS include proteinuria, hypoproteinemia, and edema that begin soon after birth.

LM Deficiency. Normal function of the glomerulus hinges on LM. Composed of tufts of capillaries, the glomerulus constantly filters out waste (forming urine) from the blood. Already mentioned, one symptom of CNS is proteinuria where excess protein, such as albumin, leaks through the glomerulus into the urine (Scheele et al., 2007). Although this can be attributed to dysfunctional LM in the epithelial filtration slits, it can also be attributed to the LM in the glomerular BM (Jarad et al., 2006). The BM of a mature, healthy glomerulus is composed of α5, β2, and γ1 chains. It has been observed that all three LM chains are distinctly important in this pathogenesis. The LM α5 chain is necessary for the embryonic development of the glomerulus, while the β2 chain is essential for the function of glomerular filtration (Miner & Le, 2000 and Jarad et al., 2006). CNS is a result of a variety of genetically mutated proteins; however the major one observed is LM β2 (LAMB2) (Zenker et al. 2004). The defected LAMB2 gene has dramatic effects on the BM of the glomerulus. It is hypothesized that it is necessary so that the renal filtration apparatus functions and matures properly (Jackson, 2007). Furthermore, symptoms of CNS can be attributed to deficient kidney genesis when the disruption of LM and nidogen-1 is seen (Willem et al., 2002). Analyzing the disrupted LM γ1 chain shows significant defects in the BM directly affecting the branching morphogenesis of the kidney (Köhling et al., 2006).

Current Treatments

Treating cases of lamininopathy is a complicated process. Current treatments are symptomatic and primarily deal only with maintenance and prevention. In most cases,

LM deficiency has serious detrimental effects that result in fatality. Less severe cases provide opportunity for treatment and therapy.

CMD

Treatment and therapy. CMD is a difficult disease to deal with. The progression of the deficiency rapidly develops, and immediate treatments need to begin. Without a "cure" it is often difficult to extend the life of a patient past what is expected, and in most cases symptomatic treatment is the only option. One particular complication that can be treated in MDC1A is respiratory dysfunction (Mercuri et al., 2002). In most individuals hypoventilation occurs during sleep due to impaired restrictive respiratory syndrome. This can be amended with positive pressure ventilation via facemask resulting in long-term success (Simonds et al., 2000). CMD with mental retardation is often more difficult to treat because of early onset and severity.

Potential cures. Current research is making progress on potential cures using laboratory techniques. Success is seen in mouse models that are used in therapeutic strategies. In these models, transgenes of LM α1 and β1 demonstrate the ability to successfully compensate for lack of LM α2 (Gawlik et al., 2006). Typically LM α2 deficient mice die in five weeks; however, when treated with LM α1 the mice are as normal as the wild-type mice. Other mouse models used include overexpression of mini-agrin to help compensate for the LM deficiency (Bentzinger, Barzaghi, & Ruegg, 2005).

Currently, cures for secondary effects of MDC1A are also being researched. These secondary tactics increase overall health and life-span. Since increased apoptosis has been suggested as a cause for dystrophic phenotype, reduction of apoptosis in LM α2 deficient mice may result improved dystrophic phenotype (Girgenrath, 2004).

Inactivation of the apoptotic Bax protein and overexpression of the antiapoptotic protein Bcl-2 both improve the health of the mouse (Gawlik & Durbeej, 2011). This procedure did not have the same effect in dystrophin-related muscular dystrophy, which would imply that apoptosis is a contributor more to MDC1A than it is to DMD (Dominov, et. al., 2005).

JEB

Treatment and therapy. Treatment of JEB depends on the severity. Due to the short life expectancy of Herlitz-JEB, management of wounds is the extent of care. In non-Herlitz cases, symptoms seem to subside after the initial year of life. In both cases, caesarian section is recommended so that minimal abrasion occurs in the birth process (Pfendner & Lucky, 2008). Blistering is treated delicately (after lancing) with three layers of protection: a soft, non-adhesive layer, a second stabilizing layer, and a third elastic layer. Also, preventative measures must be taken to minimize abrasions: the use of everyday bandages, loosely fitting shoes, coarsely-textured clothing, and abrasive activities. These primary blisters are treated similarly as any wound, but secondary precautions must be taken: treatment of infectious wounds with antiseptic, replenishment of fluid electrolytes, nutritional support, and screening for anemia (Pfendner & Lucky, 2008).

Potential Cures. Finding a cure for JEB is seemingly more likely than other lamininopathies. One group of researchers (Igoucheva et al., 2008) was able to restore the trimeric LM-332 assembly in human keratinocytes. These cells lacking LM-β3 chain were restored using recombinant LM-β3 subunit from an LM-332 molecule. This process "suggest[s] that recombinant individual chain of laminin 332 can be utilized for the

restoration of trimeric laminin and, potentially, for treatment of JEB and alleviation and prevention of the blister formation associated with this devastating skin disorder (Igoucheva et al., 2008).

CNS

In most cases, CNS is identifiable through prenatal screening. In doing so, proper measured are taken so the infant will be placed on treatment immediately. However, even with immediate aggressive treatment, most patients with CNS will not live past a year. Immunosuppressive drugs have been tried, but are not effective in treating CNS. Since the disorder is mostly localized to the kidney, having a kidney transplant is a common therapy. In some cases, to prevent life-threatening edema, daily albumin shots are given. Part of this therapeutic procedure is a hypercaloric diet, and mineral consumption to prevent thrombosis (Jalanko, 2009). Considering that kidney transplants are not always readily available, maintenance measures need to be taken to sustain kidney function. Blood pressure is normalized and dialysis is practiced. In addition, abnormal growths are removed to prevent future cancer. Some medical treatments include angiotensin-converting enzyme (ACE) and prostaglandin inhibitors to avoid the use of dialysis before a transplant becomes available (Jackson, 2007).

Summary

LM

The diversity of LM is exquisite. Made up of three different chains, LM has the ability to influence many different parts of the body. Variations of the chains provide even further complexity as these chains are made up of different domains. These domains bind to certain molecules and provide structure and integrity to the BM. Polymerizing

into lattice sheets, the LM molecule holds cells together in a flowing continuous membrane. This integral part of the structure in our tissue is coded for by many different genes. Each of these genes codes for a different component of LM. This means that a mutation of any one of these genes produces a different phenotype. There are many disorders linked to LM deficiency, each with a unique affected gene.

CMD

CMD is perhaps the most prevalent of the disorders linked to LM deficiency. This congenital disease can be classified as an LM α2 chain neuromuscular disorder. The lack of LM α2 is the cause of many different symptoms, most of which lead to childhood fatality. The mutation in this specific LM chain is devastating to the LG domain on the distal end of the long arm. This globular region is primarily responsible for ligand binding; thus, a mutation in that region greatly affects interactions between LM and other structural proteins. Being a neuromuscular disorder, much of the phenotype can be linked to LM in the nervous tissue. LM is significantly used in the morphogenesis of SCs, and without it certain nerves cells are impaired in proliferation and myelination. These nervous defects are a direct result of needed LM regulation. Instability in the nervous tissue then directly affects the muscular tissue, initiating dystrophic phenotypes.

JEB

JEB is a rare and devastating skin-blistering disorder. Fortunately, this disorder is not very prevalent, affecting only one in a million Americans (Junctional, 2009). This disorder can be classified as an LM-332 disorder, as the trimeric LM does not assemble in patients with JEB. The major mutation in LAMB3 (along with other smaller mutations in LAMA3 and LAMC2) results in truncated or non-existent chains that cannot form a

trimeric molecule. The dermal layer that is deficient in the LM molecule is very weak and sensitive blistering upon any slight abrasion. In addition, the more severe type, Herlitz JEB, involves blistering in the mucosal membranes, making feeding very difficult. Due to these combined problems, a child with Herlitz JEB normally does not survive part one year of age. Hope for this disease lies partially in recombinant DNA treatment that replaces truncated and absent β chain molecules with functional ones so that polymerization can take place producing healthy skin cells.

CNS

CNS is a genetic disorder that primarily affects the glomerulus of the kidneys. This malfunctioning filter can produce infection, malnutrition, blood issues, kidney failure, and impaired growth and development. This disease can be classified as a defected LAMB2 gene having dramatic effects on the BM of the glomerulus. This defect can impact the filtration slits, allowing nutrition to be leaked from the blood. In addition, a deficient LM molecule will not properly bind to other connective proteins such as nidogen. Disruption of LM and nidogen-1 is seen to affect the branching and morphogenesis of the kidney cells. Kidney transplants are an effective treatment, as the deficiency is (for the most part) localized to the kidney. While waiting for a transplant, however, proper measures need to be taken so that replenishment of proteins and proper blood filtration is practiced.

Conclusion

LM is a complicated and intricate molecule that has a multitude of different roles. One glance might give the appearance of a simple cell-adhesion molecule, but a deeper look provides insight to the diversity it holds. Shaped like a cross, this molecule has the

capability to bind many different things at once, including other LMs. The cross shape that contains the three unique chains has a wide array of necessary tasks. These responsibilities are not entirely appreciated until the potential of their absence is presented. The disorders that result from not having parts of LM are extremely unfortunate, if not lethal. Modern research is improving with time as technological advances provide more efficient and accurate techniques.

One study found that vitamin C deficiency could be influencing the major connective proteins collagen, LM and elastin. Although this study found that vitamin deficiency only affects collagen and elastin genes (not LM), there is hope that something as simple as vitamin C could help with disease (Mahmoodian & Peterkofsky, 1999). Granted, the severity of the disorders would probably overwhelm a small increase of expression due to vitamin C, but there is hope that current knowledge of LM and other structural proteins will enhance the path towards finding a cure for LM deficiencies.

Most of the disorders could be cured through stem-cell or gene therapy procedures, and the hope is that current knowledge will aid the process of finding clinical cures. As seen in the current mouse models for CMD, transgene implantation results in direct improvement. Although these transgene procedures are not yet clinically feasible, the benefit of understanding molecular treatment is already noticeable. With new research constantly being done it is not far off to believe that a cure will be found for LM deficiencies.

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