



HAL
open science

Laminins and matrix metalloproteinases connection: a subtle relationship that can go wrong in a tumor context, particularly if CD44 gets involved

Patricia Rousselle, Konrad Beck

► To cite this version:

Patricia Rousselle, Konrad Beck. Laminins and matrix metalloproteinases connection: a subtle relationship that can go wrong in a tumor context, particularly if CD44 gets involved. The Extracellular Matrix and the Tumor Microenvironment. *Biology of Extracellular Matrix*, vol 11. Springer, Cham., 2022, 978-3-030-99708-3. hal-03834467

HAL Id: hal-03834467

<https://hal.science/hal-03834467v1>

Submitted on 29 Oct 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Laminins and matrix metalloproteinases connection: a subtle relationship that can go wrong in a tumor context, particularly if CD44 gets involved

Patricia Rousselle¹ and Konrad Beck²

¹ Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique, Institut de Biologie et Chimie des Protéines; UMR 5305; CNRS; Univ. Lyon 1; SFR BioSciences Gerland-Lyon Sud; 7 passage du Vercors, 69367, Lyon, France

² Cardiff University School of Dentistry, Heath Park, Cardiff CF14 4XY, U.K.

Corresponding author: Patricia Rousselle, LBTI, 7 passage du Vercors – 69367 Lyon Cedex 07, France. Tel: (33) 4 72 72 26 39; Fax: (33) 4 72 72 26 02; E-mail: patricia.rousselle@ibcp.fr

Keywords: laminin, basement membrane, CD44, matrix metalloproteinase, cancer, protein domain modeling

Abbreviations

ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; FAK, focal adhesion kinase; GAG, glycosaminoglycan; HA, hyaluronic acid (hyaluronan); HBS, heparin binding site; HS, heparan sulfate; LG, laminin globular domain; LM, laminin; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; PI3K, phosphatidylinositol-3-kinase.

ABSTRACT

Laminins (LM) are large extracellular glycoproteins involved in several biological processes, including cellular interactions, self-polymerization, and binding with other extracellular matrix proteins. LMs influence cell function by inducing various signaling pathways *via* cell membrane receptors and have multiple, often cell type-specific, functions in, for example, adhesion, differentiation, migration, and phenotype maintenance, and they also provide resistance to apoptosis. They are also important components of basement membranes. The basement membrane is partly degraded in the course of tumor growth, facilitating the invasion of budding cells and their migration to lymphatic or blood vessels. In this context, LMs undergo proteolytic cleavage, which disrupts their involvement in maintaining the structural and biological properties of the basement membrane. LMs are also involved *via* their participation in cancer cell adhesion and migration processes. These events are either supported by their major cell binding domains or triggered by cryptic interaction sites revealed by matrix metalloproteinase (MMP) induced proteolytic cleavage. While being ideal targets for MMPs, LM can enhance their expression and activity. They appear to be key matrix elements in the regulation of MMP activity *via* the recruitment of the CD44 receptor, a multiple MMP-interacting and activating platform playing an important role in cancer progression.

1. The laminin protein family

Laminins (LMs) are a family of glycoproteins found in basement membranes with biological and structural properties that attract a growing interest. As essential structural constituents of the extracellular matrix (ECM), LMs also maintain close relationships with cells and transmit important morphogenetic information (Miner and Yurchenco 2004) (Domogatskaya et al. 2012). All LMs are composed of three different gene products, termed α , β , and γ chains, which are assembled into a cross-shaped heterotrimer $\alpha\beta\gamma$. The three chains assemble within the endoplasmic reticulum *via* their C-terminal regions to form a triple stranded α -helical coiled coil rod and are covalently linked by disulfide bonds at both extremities of the coiled coil (Beck et al. 1993) (Matsui et al. 1995). The primary structures of five α chains ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$), four β chains ($\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$), and three γ chains ($\gamma 1$, $\gamma 2$, $\gamma 3$) were identified by cDNA sequencing, leading to at least 18 heterotrimeric isoforms (**Table 1, Figure 1**). Some genes encoding LMs may produce more than one subunit isoform due to alternative splicing; short-chain and long-chain splice variants are annotated with A and B, respectively (Aumailley et al. 2005). All basement membranes contain at least one LM isoform, and some may have two or three different isoforms. The expression and assembly of the various subunits vary in a spatio-temporal manner, which suggests biological and structural roles specific to each isoform (Yap et al. 2019). Some isoforms appear very early in embryogenesis and are markers for a given ECM, whereas others appear later and in a tissue-specific manner. In general, expression of the $\alpha 5$ subunit is the most widespread, whereas that of the $\alpha 1$ subunit is the most restricted (Miner et al. 1997). The $\alpha 2$ subunit is particularly abundant in tissues of mesodermal origin (cardiac and skeletal muscle), and the $\alpha 4$ subunit is abundant in endothelial and mesenchymal cells. The

$\alpha 3$ subunit, similar to $\beta 3$ and $\gamma 2$, is concentrated in epithelial basement membranes. The $\beta 1$ and $\beta 2$ subunits often have mutually exclusive expression, whereas the $\gamma 1$ chain is present in all basement membranes (Durbeej 2009). LMs are essential at various stages of development. Thus, most LM mutants are fatal to the embryo and have made it difficult to study their functions at later stages (Yao 2017).

Although LMs are differentially recognized by cellular receptors, they all share a molecular organization based on the repetition of structural modules, giving them a common molecular organizational pattern. LM-111, which was initially extracted from the Engelbreth-Holm-Swarm tumor, was the first LM described as an asymmetric cross comprising one long arm and three short arms carrying globular domains linked by linear segments (**Figure 2A**). Other LMs do not exhibit all of the short arms, as they include "truncated" subunits at the N-terminal ends ($\alpha 3$, $\alpha 4$, $\beta 3$, and $\gamma 2$) (**Figures 1 and 2B**). The N-terminus of each full-length LM chain consists of cysteine-poor sequences and are named LN domain. The short-arm rod-like portions consist of tandem arrays of LE modules, which sequences are related to the epidermal growth factor but contain one additional C-terminal disulfide bond. Inner globular structures within the short arms are the L4 domains resulting from a long insert between cysteines 3 and 4 of the canonical 8-cysteine pattern of an LE domain. The LF domains have a distant structural similarity to L4 but are unique with respect to their cysteine pattern (Pulido et al. 2017). The LFx domains share similar sequences at the N- and C-termini with LF, but have further insertions of two internally similar sequence regions. C-terminal regions of the α , β , and γ chains assemble into a three-strand α -helical coiled coil forming the long arm (Macdonald et al. 2010). The amino acids involved in this structure are organized in a heptad repeat in which the first and fourth residue are of hydrophobic, and the fifth and seventh

frequently of charged character (Paulsson et al. 1985). Within the β chains, the coiled coil region is interrupted by domain $L\beta$ consisting of 30 to 40 residues including six cysteines which biological function is not yet known. All LM α chains possess a large globule at the C-terminal end that consists of five similar domains (LG1 to LG5), each containing approximately 200 residues (Timpl et al. 2000) (Hohenester 2019). LG3 and LG4 are separated by a flexible hinge providing access for proteolytical attack. All LMs are glycosylated, which regulates their biological functions in addition to stabilizing them (Morita et al. 1985) (Inamori et al. 2016).

2. Laminins as multifunctional elements within basement membranes

Early molecular and *in vivo* studies in *Drosophila*, *C. elegans*, and mice support the idea that LMs are the basic building block for initiating basement membrane formation (McKee et al. 2007) (Jayadev and Sherwood 2017). Most LMs can self-associate into polymeric sheet-like networks that are tightly associated with the cell surface through their LG domains (Yurchenco et al. 1992) (Li et al. 2003). This polymerization process is reversible and depends on both the concentration and presence of divalent cations. This assembly model involves and absolutely requires the N-terminal domain LN of the α , β , and γ subunits (Cheng et al. 1997) (Hohenester and Yurchenco 2013) (**Figure 2A**). LM networks are non-covalent in nature and appear more dynamic than the cross-linked collagen IV networks described as being responsible for the mechanical strength of basement membranes. Thus, collagen IV recruitment and further basement membrane assembly appear to depend on LM self-assembly and its LG-mediated cell surface anchorage. LMs containing at least one truncated subunit, and therefore lacking one LN domain (*i.e.*, the $\alpha3A$, $\alpha4$, and $\gamma2$

chains), are non-polymerizing LMs and form other types of supramolecular arrays to integrate the basement membrane (**Figure 2B**). The epithelial LM heterotrimers $\alpha 3A\beta 3\gamma 2$, $\alpha 3A\beta 1\gamma 1$, and $\alpha 3A\beta 2\gamma 1$ establish specific molecular interactions leading to the formation of anchoring structures characteristic of epithelial basement membranes (Champlaud et al. 1996; Rousselle and Beck 2013). The matrix integration of LMs comprising a truncated α chain assembled with the $\beta 1$ and $\gamma 1$ subunits, such as LM-3A11 and LM-411, may involve known interactions between the $\beta 1$ and $\gamma 1$ chains with agrin, nidogen (entactin), and perlecan.

LMs provide interaction sites for many other constituents including cell surface receptors. Some LM isoforms are modified extracellularly by proteolytic processing at the N- or C-terminal ends prior to binding cellular receptors or other matrix molecules (Tzu and Marinkovich 2008). Cellular receptors, such as integrins, syndecans, α -dystroglycan, Lutheran glycoprotein, or sulfated glycolipids, predominantly bind to the five LG domains at the C-terminal end of the α chain. The LG1-LG3 region contains the integrin binding domains, which often require the context of the LM heterotrimer and, most importantly, the C-terminal end of the γ chain to be fully effective (Deutzmann et al. 1990; Rousselle et al. 1995; Ido et al. 2004; Ido et al. 2007; Ido et al. 2008; Navdaev et al. 2008). The major LM-binding integrins are $\alpha 3\beta 1$, $\alpha 6\beta 1$, $\alpha 7\beta 1$, and $\alpha 6\beta 4$ (Nishiuchi et al. 2006). The LG1-LG3 region of the LM $\alpha 5$ chain also binds to the Lutheran blood group antigen/basal cell adhesion molecule, a cell surface protein consisting of five immunoglobulin-like domains (Udani et al. 1998; Vainionpää et al. 2006; Kikkawa et al. 2007). The LG45 region contains binding sites for α -dystroglycan, syndecans, and sulfated glycolipids (Gee et al. 1993; Talts et al. 1999; Smirnov et al. 2002; Wizemann et al. 2003; Yamashita et al. 2004; Utani et al. 2003; Hozumi et al. 2009; Carulli et al. 2012; Suzuki et al. 2005). The N-terminal

globular LN domains of the $\alpha 1$ (Cognato-Pyke et al. 1995) and $\alpha 2$ chains (Cognato et al. 1997) as well as LN (Nielsen and Yamada 2001) and L4 (Sasaki and Timpl 2001) of the $\alpha 5$ chain can bind to $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 3\beta 1$, and $\alpha V\beta 3$ integrins.

Deregulated expression and involvement of LMs and their integrin receptors in tumor development and aggressiveness have been demonstrated in recent years. They will not be the subjects of this review as they have been exhaustively covered recently (Maltseva and Rodin 2018; Qin et al. 2017; Ramovs et al. 2017; Rousselle and Scoazec 2020). Instead, here we will focus on the link between LMs and matrix metalloproteinases (MMPs) and demonstrate how it can become toxic in cancer by amplifying the tumor process. We will also examine the current state of understanding the status of LMs as ligands for the CD44 receptor and seek for a potential link with MMP activity.

3. Laminins are involved in the regulation of MMP expression and activity

MMPs are a family of secreted and membrane-bound zinc-dependent endopeptidases that have the capacity to degrade all components of the ECM. MMPs play central roles in morphogenesis, wound healing, tissue repair and remodeling in response to injury, and in the progression of diseases, such as inflammatory and neoplastic diseases (Nagase et al. 2006). The MMP family comprises 25 related but distinct vertebrate gene products, 24 of which are found in mammals and can be divided into six groups: collagenases (MMPs 1, 8, 13, 18), gelatinases (MMPs 2, 9), stromelysins (MMPs 3, 10, 11, 17), matrilysins (MMPs 7, 26), membrane-type MMPs (MT-MMPs; MMPs 14, 15, 16, 17, 24, 25), and other MMPs (MMPs 12, 19, 20). The expression of MMP genes is transcriptionally regulated by a variety of extracellular

factors including cytokines, growth factors, and ECM proteins (Gaffney et al. 2015). A number of studies have revealed that LMs or their proteolytic fragments can be involved in the regulation of MMP expression and/or activity. These subtle regulations that occur in a physiological context can be amplified in pathological situations such as cancer, contributing to tumorigenesis and invasion (Karamanos et al. 2021).

Early studies revealed that an interaction between LM-111 and the 67-kDa LM receptor promotes MDA-MB231 breast carcinoma cell aggressiveness through the upregulation of MMP-14 and enhanced gelatinolytic activity of MMP-2 (Berno et al. 2005). Subsequent studies have shown that LM-111 can increase MMP activity in human neural stem cells (Sypecka et al. 2009). Later, activation of MMP-9 expression was associated with an $\alpha 2\beta 1$ integrin-dependent migration process when the human cervical cancer SiHa cell line was cultured on an LM-111-coated surface (Maity et al. 2011). A mechanism involving focal adhesion kinase (FAK), integrin linked kinase, phosphatidylinositol-3-kinase (PI3K), and extracellular signal regulated kinase was followed by increased DNA-binding activity of NF- κ B and Ap1 and subsequent stimulation of MMP-9 gene expression (Maity et al. 2011). A similar signaling pathway was revealed when human breast cancer MCF-7 cells were plated on an LM-111-coated surface (Pal et al. 2014). In this study, the concomitant down-regulation of TIMP1, a negative regulator of MMP-9 activity, led to enhanced MMP-9 proteolytic activity. Another context in which a link between LM and MMPs was established is the kidney. The LM $\alpha 2$ chain was shown to contribute to pathogenesis in the Alport glomerular syndrome *via* activation of FAK on glomerular podocytes leading to the downstream activation of MMP-9, MMP-10, and MMP-12 gene expression (Delimont et al. 2014). A $\beta 1$ chain fragment released through MMP-2 processing, LN-LE1-LE4, was shown to modulate the behavior of pluripotent stem

cells and promote the epithelial-mesenchymal transition (EMT) in an $\alpha3\beta1$ -integrin–dependent fashion, whatever the fragment was delivered to the cells as a soluble or immobilized factor (Horejs et al. 2014). The $\beta1$ chain fragment triggered the down-regulation of MMP-2 in human and mouse cells, as well as the up-regulation of E-cadherin and MMP-9 in mouse cells during spontaneous stem cell differentiation.

The cryptic domains of LMs (*i.e.*, hidden in full-length LMs and exposed upon proteolysis) can elicit biological responses and be involved in the regulation of MMPs. For example, peptides containing the sequence S₂₀₉₉IKVAV, which is adjacent to the N-terminus the C-terminal LG1 domain of the LM $\alpha1$ chain, have been shown to induce the expression of MMP-9 by monocytes/macrophages (Corcoran et al. 1995; Khan and Falcone 1997), whereas intact LM-111 does not (Khan and Falcone 2000). A mouse $\alpha5$ LG1 domain-derived peptide was shown to induce macrophage and neutrophil chemotaxis both *in vitro* and *in vivo* and to enhance MMP-9 and MMP-14 activities (Adair-Kirk et al. 2003). Furthermore, human $\gamma2$ chain fragments and LG4-derived peptides from the $\alpha3$, $\alpha4$, and $\alpha5$ chains exhibit strong chemotactic activity towards leukocytes, neutrophils, and monocytes (Mydel et al. 2008; Kenne et al. 2010; Senyürek et al. 2014). Whether these activities are associated with activation of MMP expression or activity is an interesting question that remains to be answered. Another study revealed that LM-332 potentiates human monocyte differentiation to tumor-associated macrophage-like cells secreting a high level of MMP-9 (Kamoshida et al. 2014). Upregulation of MMP-1 and MMP-9 expression was also reported at both the transcriptional and protein levels in epidermal keratinocytes upon treatment with recombinant C-terminal LG4 or LG45 domains of the $\alpha3$ chain, an event that relies on an IL-1 β autocrine loop through the mitogen-activated protein kinase (MAPK) pathway (Momota et al. 2005; Utani et al. 2003; Michopoulou et al. 2020).

Several peptide sequences involved in this mechanism were identified as heparin binding sites (HBSs) and characterized as ligands for the proteoglycan-type cell surface receptors, the syndecans (Utani et al. 2003; Carulli et al. 2012; Rousselle and Beck 2013). However, the role of LM was not limited to this transcriptional activation as the $\alpha 3$ LG45 domain participated in establishing the gelatinolytic activity of epithelial podosomes in migrating keratinocytes during wound healing (Michopoulou et al. 2020). We showed that the $\alpha 3$ LG45 domain triggers the proteolytic activity of MMP-9 and MMP-14 in epithelial podosomes *via* a mechanism involving the cellular receptor syndecan-1 (Michopoulou et al. 2020).

4. Laminins are cell adhesion ligands for CD44

The cell surface glycoprotein CD44, a hyaluronic acid (HA) receptor, plays a role in the regulation of cell-cell interactions, cell adhesion, cell growth, migration, and invasion (Knudson et al. 1996; Ponta et al. 2003). CD44 is an 85-200 kDa transmembrane glycoprotein ubiquitously expressed throughout the body and is detected in both normal and tumor cells. CD44 is encoded by a single gene with 20 exons but has many different isoforms (**Figure 3A**). It is composed of a distal extracellular N-terminal domain, a stem region, a transmembrane domain, and an intracellular cytoplasmic C-terminal domain (**Figure 3B**). The standard form CD44s is the shortest and most commonly expressed isoform. It results from expression of the constant exons 1 to 10, which are found in all isoforms. A number of splicing variants (CD44v) result from the insertion of variable exons in the extracellular region near the membrane, called the stem region. There are 10 variant exons expressed in mice (designated v1–v10), whereas humans express only variant exons v2–v10 due to the

presence of an in-frame stop codon in exon v1 (Screaton et al. 1993). The N-terminal portion of the ectodomain is of a compact, ordered structure, stabilized by three disulfide bridges, that contains a HA binding region and glycosaminoglycan (GAG) chains (**Figure 4A**). The stem region varies according to the expression of the splicing domains in the CD44v isoforms, and the transmembrane and intracellular domains both play important roles in CD44 functionality and connection to the actin cytoskeleton through ezrin/radixin/moesin (ERM) proteins (Tsukita et al. 1994; Gal et al. 2003; Medrano-González et al. 2021). Importantly, upregulation of CD44 is often closely associated with abnormal tumor cell behavior (e.g., proliferation, survival, migration/invasion, and chemoresistance) (Bourguignon 2019; Medrano-González et al. 2021).

Understanding the various interactions of CD44 on a molecular structural level that would allow for reliable predictions of binding sites is difficult. The complex posttranslational modifications by N- and O-linked glycosylation greatly depends on cell types, developmental and environmental conditions. Large chondroitin and dermatan sulfate GAG chains attached to the stem region in CD44s, and heparan sulfate chains found within the variable regions are highly flexible making high-resolution structural analysis challenging. Except for the most N-terminal ca. 160 amino acids, even the unmodified ectodomain evades exploration by showing a high degree of intrinsic disorder with nearly 30% of residues being serine and threonine (**Figure 4B**). In contrast to the classical view of structure-function relation as a lock-and-key system, the resulting flexibility on the structural level allows the protein to adapt various conformations for optimal ligand binding (Habchi et al. 2014). Even the various structures solved at high resolution by x-ray crystallography may not be regarded as static. Molecular dynamics simulations suggest that Y42 can act as a

molecular switch that upon binding to HA changes the overall HA binding domain structure from a closed to open conformation that further increases affinity for HA (Guvench 2015).

CD44 is upregulated in a variety of cancers and can be expressed as CD44s or as a number of alternatively spliced CD44v isoforms. CD44 mediates its effects on the cancer cell by activating signaling pathways, such as protein kinases and transcription factors. The functional role of CD44 is pleiotropic, including induction of EMT and anti-apoptosis, alterations in the cellular cytoskeleton, and promotion of drug resistance. CD44 is a compelling marker of cancer stem cells from many solid malignancies (Takaishi et al. 2009; Du et al. 2008; Zöller 2011). In addition, interactions between HA and CD44 promote epidermal growth factor receptor-mediated pathways, consequently leading to tumor cell growth, tumor cell migration, and chemotherapy resistance in solid cancers (Thapa and Wilson 2016; Yaghibi et al. 2021).

In addition to its direct signaling function, CD44 forms a platform at the cell membrane for the assembly of various MMPs with their substrates, ultimately to modulate cell migration (Cauwe et al. 2007) (**Figure 3B**). CD44 is an important partner of MMP-9, playing a role in its anchorage to the cell membrane and preservation of its proteolytic activity (Yu and Stamenkovic 1999, 2000). Evidence suggests that the association of MMP-9 and CD44 in mouse and human tumor cells promotes invasion (Yu and Stamenkovic 1999; Gupta et al. 2013). Clustering of CD44 mediates MMP-9 relocation in breast tumor cells promoting its proteolytic activity on the membrane (Peng et al. 2007). The interaction of CD44 and the proteolytic form of MMP-9 is involved in the invasion activity of prostate cancer PC3 cells (Desai et al. 2007).

CD44 is also a molecular partner of MMP-14, playing a role in its translocation to the front of migrating cells (Mori et al. 2002). MMP-14 has been clearly characterized as a CD44 shedding enzyme with migration-promoting activity (Kajita et al. 2001). MMP-14 and CD44 are co-expressed at the lamellopodia of cells undergoing EMT (Cho et al. 2012). In normal mouse breast and uterine epithelium, the heparan sulfate (HS) chains of CD44v containing the exon v3 derived sequence bind active MMP-7 (Yu et al. 2002; Seiki 2002). CD44 also binds MMPs 2, 15, 16, 24, and 25 through their hemopexin domains in lamellipodia edges (Mori et al. 2002; Suenaga et al. 2005; Samanna et al. 2006) and can act indirectly by activating MMP-2 through MMP-14 cleavage (Zöller 2015) (**Figure 3B**).

CD44 has been identified as a component of podosomes, which are protrusive adhesion structures involved in ECM degradation and mechanosensing (Murphy and Courtneidge 2011; Linder and Wiesner 2016). It was shown to co-localize with the F-actin core of podosomes in osteoclasts (Chabadel et al. 2007), human primary epidermal keratinocytes (Michopoulou et al. 2020), and in 3D-cultured macrophages (Van Goethem et al. 2011). By demonstrating a direct interaction between the LM α 3 LG45 domain pair and the CD44 receptor expressed by human primary keratinocytes, we have revealed a determining role for this LM isoform in regulation of the proteolytic activity of MMP-14 and -9 in epithelial podosomes (Michopoulou et al. 2020). The 200-kDa CD44 isoform identified in our study is the long form v3–10 expressed by keratinocytes (Bourguignon and Bikle 2015). It is conceivable that the LM-332 LG45 domain concentrates active MMP-9 and -14 within epithelial podosomes through recruitment of CD44. In addition, the combination of LG45 and CD44 could facilitate binding of adaptor molecules to the cytoplasmic region in CD44 and activate multiple pathways involved in cell adhesion, migration, and proliferation

including Ras, MAPK, and PI3K (Xu et al. 2020). Although this mechanism has been demonstrated in the physiological context of skin repair, it could undoubtedly be implemented in cancer cells in which the expression of LM-332 is frequently upregulated (Rousselle and Scoazec 2020). In cancer cells, the α 3LG45 domain could regulate the expression and concentrate active MMP-9 and MMP-14 in invadopodia, the adhesive actin-rich membrane protrusions with high proteolytic activity found in cancer cells (Augoff et al. 2020). An argument in favor of this hypothesis is based on our study showing that Ras/I κ B α -transformed keratinocytes lacking the LG45 domain exhibit a deficiency in MMP-9 and MMP-1 expression and a decreased invasive capacity (Tran et al. 2008). This deficiency was reversed by replacing LG45 through retroviral transduction. Whether the CD44 isoforms expressed in cancer cells can bind to α 3LG45 is an important question (Chen et al. 2018). We already know that, in addition to the CD44v3-10 isoform, the 100-kDa standard CD44s expressed on melanoma A375 cells also binds the α 3LG45 domain (Michopoulou et al. 2020; Takahashi et al. 1999), and we may wonder whether the various CD44 isoforms will bind to α 3LG45 with different affinities. For example, the presence of variable exon 3, which encodes an HS chain carrying region, may impact the interaction (Bennett et al. 1995).

A link between LM-332 and the MMP-14/CD44 tandem was already revealed a few years earlier in a study showing that interactions between mature human thymocytes and LM-332 induces strong up-regulation of active MMP-14 leading to CD44 cleavage and thymocyte migration (Vivinus-Nebot et al. 2004). As the LG45 domain was cleaved off and removed from the LM-332 used in this study, we can evaluate the possible participation of the N-terminal end of the γ 2 chain, which also comprises an HBS, in this mechanism (Sasaki et al. 2001). One study revealed that the LN

domain of the LM $\gamma 2$ chain induces breast cancer MDA-MB-231 cell migration as a result of its binding to CD44 and phosphorylation of its cytoplasmic tail (Sato et al. 2015). Surprisingly, interaction studies uncovered a binding site for CD44 within the LEa2-LEa3 region, away from the HBS of the $\gamma 2$ chain identified within the L4 and LEa domains (Sasaki et al. 2001; Ogawa et al. 2007), suggesting the involvement of a LM binding domain distinct from the GAGs in the CD44 ectodomain (Sato et al. 2015). However, the study did not mention any MMP-9 or MMP-14 recruitment, though it clearly reported a $\gamma 2$ chain-dependent pro-migratory activation of CD44 reinforcing the hypothesis that LM-332 is a multi-site ligand for CD44. Based on these data we performed rigid body molecular docking simulations using a model of the LM $\gamma 2$ LEa2-LEa3 pair and the coordinates of the HBS domain of CD44. The best result with a good global minimal energy conformation shows a good steric fit of CD44 within the groove of the LE2-LE3 connection and suggests four pairs of residues that come into hydrogen bonding distance (**Figure 4C**). Though this model is in agreement with the experimental data, as any glycosylation and the stem portion of CD44s were not considered, it should be viewed with caution.

LMs were identified as ligands for CD44 already nearly 30 years ago. The binding of CD44 to a commercial LM preparation was detected in an ELISA-based assay using CD44 purified from lymphocytes (Jalkanen and Jalkanen 1992). The epithelial isoform CD44v8-10 (Brown et al. 1991), which is expressed in carcinoma cells such as human colorectal carcinoma KM-12, CCL 188, and MIP-101 cells, was identified as an LM-111 receptor (Ishii et al. 1993). These studies already reported that the interaction is mediated by the proteoglycan-rich region of the extracellular domain of CD44, an interaction site distinct from that of HA, which is located in the disulfide-rich N-terminus of the molecule (Ponta et al. 2003). In a study screening the adhesive

properties of overlapping short peptides covering the entire LG1 to LG5 domains of the $\alpha 5$ chain toward B16-F10 mouse melanoma cells, peptide A5G27 R₂₈₉₂LVSYNGIIFFLK corresponding to one β strands of the LG2 module with the terminal basic residues in the surface exposed loop regions was identified as a ligand for CD44 *via* its GAG moieties (Hibino et al. 2004). In the human sequence, this peptide relates to R₂₉₇₁LVSYSGLVFFLK. When visualized within the solved terminal structure of LM 511 (Takizawa et al. 2017), it is evident that the basic side chains are surface exposed at opposite ends of the LG2 domain (**Figure 4D**). Further positively charged surface areas are generated by K3009, K3010, and K3025. These five residues form a track that could further the alignment of a GAG chain. A similar track of basic residues crucial for heparin binding has been previously reported for the LM LG4 domain (Yamashita et al. 2004). The LM $\alpha 5$ chain interaction with CD44 stimulates melanoma cell growth, angiogenesis, migration, and metastasis (Hibino et al. 2005). CD44 overexpression in neural precursor cells significantly improved their spreading over LMs and increased the formation and length of filopodia, suggesting a role in migration (Deboux et al. 2013). Using a protein database approach, a role for CD44 interactions with LM-111 was predicted to play an important role in the axon growth of retinal ganglion cells during nerve regeneration (Ries et al. 2007). As CD44 is overexpressed on a wide variety of neuronal, epithelial, and hematopoietic malignancies, CD44 may be involved in either the malignant transformation of cells or the migration of malignant cells from the primary site to sites of metastasis. The mode of interaction of CD44 with LMs is very complex and can take place in a number of ways. Further work is now required to better understand these interactions and apprehend their biological consequences.

5. Laminins are targets of MMPs activity

Like any other ECM protein, LMs are subject to total or partial proteolysis by MMPs, leading to the release of protein fragments, which may play a role in tumor progression. Over the years, MMPs have been found to play a remarkable number of regulatory roles at the cellular level, including pathways involved in apoptosis, immunity, cellular migration, and angiogenesis (Fingleton 2006; Itoh and Nagase 2002; Shay et al. 2015). Thus, MMPs play a major role in carcinogenesis. MMP functionality often complements classical tumor properties, leading to invasion, immune system avoidance, and metastasis (Winkler et al. 2020). The specific role of each MMP in cancer and their dysregulation in many cancer types have been accurately documented recently (Gobin et al. 2019; Piperigkou et al. 2021; Karamanos et al. 2021). An exhaustive gene expression study of 15 different types of cancers compared to normal tissue confirmed that MMP expression has a large degree of heterogeneity across cancers and revealed that the most prevalent gene expression changes were upregulation as opposed to downregulation in tumor tissue *versus* control tissue (Gobin et al. 2019). Facilitation of cell migration and invasion is thought to be the principal effect of the MMP-mediated breakdown of basement membranes surrounding cancer cells (Jayadev and Sherwood 2017; Hanahan and Weinberg 2011). All molecular networks, including LM networks, as well as ECM/cell adhesion complexes are targets of MMP-mediated proteolysis. Notably, even if cells can physically breach basement membrane barriers in the absence of MMPs (Kelley et al. 2019), the massive production of MMPs by cancer cells greatly enhances their invasive capacity. Another barrier to tumor cell extravasation and invasion is the basement membrane underlying the endothelium of the vessel wall, where LM-411 and LM-511 are expressed (Spessotto et al. 2001). LMs can be cleaved by MMPs 2,

3, 7, 9, 10 to 16, 19, and 20 (Okada et al. 1986; Bejarano et al. 1988; Wilson and Matrisian 1996; Chandler et al. 1996; Ohuchi et al. 1997; Pirilä et al. 2003; Sadowski et al. 2005; Itoh and Seiki 2006; Laronha and Caldeira 2020), but invasive tumor cells primarily use MMP-2, MMP-9, and MMP-14 within invadopodia to cleave or degrade ECM proteins including LMs (Jacob and Prekeris 2015). Expression of MMP-14 in cancer cells correlates with their metastatic potential (Poincloux et al. 2009) and their ability to degrade fibrillar collagen and LMs. MMP-14 has the ability to activate MMP-2, MMP-9, and MMP-13 (Nishida et al. 2008; Knäuper et al. 2002; Li et al. 2017). The destruction of LM-111 by MMP-9 is thought to play a detrimental role in the early stage of breast cancer as the basement membrane no longer provides the appropriate signals to restrain epithelial cell proliferation (Beliveau et al. 2010).

The effects of LM degradation on cancer cell behavior, however, are more complex in the end because proteolytic cleavage leads to the release or exposure of either ECM-sequestered cytokines or a functional ECM fragment. Some LMs can express cryptic biological functions after proteolysis thereby altering cellular behavior and phenotypes (Niland and Eble 2020). The combined elastase/MMP-9-mediated proteolytic remodeling of LM-111 has been shown to unmask an epitope that triggers proliferation of dormant breast cancer cells through an $\alpha 3\beta 1$ integrin signaling mechanism leading to aggressive lung metastases (Albregues et al. 2018). This fascinating study demonstrated that the proteases present in neutrophil extracellular traps, together with DNA, are delivered to LM-111 through a DNA/LM interaction. LM-211, LM-411, and LM-511 could be degraded in the same way (Albregues et al. 2018).

LM $\alpha 1$, $\beta 1$, and $\gamma 1$ chains are subject to MMP-2 and MMP-9 cleavage, releasing proteolytic fragments endowed with integrin- or syndecan-mediated cellular functions

(Kikkawa et al. 2013). MMP-2 cleavage of the short arm of the $\beta 1$ chain is predicted to disassemble the LM network, releasing a $\beta 1$ LN–LE1-4 fragment that mediates $\alpha 3\beta 1$ -integrin dependent embryonic stem cell adhesion (Horejs et al. 2014). Structural changes occur after LM-511 cleavage by MMP-9 (Gu et al. 2005), and fragments with activity toward inflammatory or cancer cells were identified within the LM $\alpha 5$ chain (Adair-Kirk et al. 2003; Hibino et al. 2004; Kusuma et al. 2011). An 80-kDa LM $\alpha 2$ C-terminal LG3-LG5 fragment possibly generated by MMP-9 is thought to play a role in reshaping the blood–testis barrier and spermatogenic function in a rat model (Gao et al. 2017). Analysis of the secretome from MDCK cells undergoing Ras-induced EMT resulted in the identification of two LM $\alpha 5$ internal and C-terminal fragments generated from MMP-1 cleavage with pro-migratory and pro-angiogenic activities (Gopal et al. 2016). LM $\alpha 5$ has also been reported to be cleaved by MMP-14, leading to prostate cancer cell migration (Bair et al. 2005).

LM-332 has been the focus of increasing interest over the last few years in the cancer research field, particularly the various proteolytic cleavages of its three subunits (Rousselle and Beck 2013). Many proteases degrade LM-332 and have been exhaustively documented (Rousselle and Scoazec 2020). To fulfill its essential function of anchoring proteins in epithelial basement membranes (Rousselle et al. 1991; Rousselle et al. 1997; Champliand et al. 1996), LM-332 undergoes several physiological post-translational modification events. This maturation process allows its supramolecular integration and promotion of cell adhesion (Rousselle and Beck 2013). In cancers, additional cleavage events occur, compromising both these adhesive and connecting functions and additionally delivering pro-migratory signals to cells (Rousselle and Scoazec 2020). Cleavage of the $\beta 3$ chain by MMP-7 or MMP-14 has been reported to enhance colon or prostate carcinoma cell migration

and invasion, respectively (Remy et al. 2006) (Udayakumar et al. 2003). MMP-2 or MMP-14 cleavage of the $\gamma 2$ chain is reported to be a pro-tumorigenic signaling event (Giannelli et al. 1997; Koshikawa et al. 2000; Gilles et al. 2001). The released N-terminal LE repeats have been shown to induce EGF signaling and downstream MAPK activity in cancer cells (Koshikawa et al. 2004). The involvement of MMPs 3, 12, 13, 19 and 20 in the cleavage of the $\gamma 2$ chain (Pirilä et al. 2003; Sadowski et al. 2005; Väänänen et al. 2001) reinforces the status of LM-332 and its $\gamma 2$ subunit as an ideal target for MMPs in tumorigenesis.

Studies of the testis have reported that MMP-2 cleavage of the LM $\gamma 3$ chain, part of the $\alpha 3\beta 3\gamma 3$ LM heterotrimer localized at the apical ectoplasmic specialization of Sertoli cells (Yan and Cheng 2006), induces dysfunction in the Sertoli cell tight junction permeability barrier through a 50-amino-acid residue peptide sequence in domain L4 (Siu and Cheng 2004; Yan et al. 2008; Su et al. 2012; Li et al. 2020). This process is thought to provide an efficient mechanism for modulating different aspects of spermatogenic function in response to changes in the epithelial cycle of spermatogenesis (Li et al. 2020). During zebrafish skeletal muscle development, LM was shown to act as an MMP-11 mediator and regulator of fibronectin levels at the myotendinous junction (Jenkins et al. 2016).

Conclusion

ECM remodeling is an important contributor to tumor progression and metastasis by contributing to the dissemination of cancer cells to distant tissues as well as angiogenesis. Among the many extracellular proteases expressed by human cells, MMPs are the most prominent enzymes supporting ECM proteolytic degradation and

remodeling, as well as the modulation of cellular and ECM interactions. Whether MMPs are soluble/secreted in the tumor microenvironment or anchored to the cell membrane, their dysregulated expression in tumor cells, cancer-associated fibroblasts, and infiltrating immune cells is a hallmark of cancer. Being ideal targets of MMPs, the cleavage or degradation of LMs has structural and biological impacts favoring tumor development and metastasis. The fragments or peptides that are generated can induce the production of more MMPs in various cell types, setting up a vicious cascade. The proteolytic activity of MMPs is often localized in adhesion structures, such as invadopodia, or occurs in proximity to the cell membrane where LMs are expressed. Cellular receptors, such as CD44, play an important role in addressing and regulating MMP activity. Their connection to LMs could promote the establishment of an active MMP-rich microenvironment prone to efficient degradation and remodeling of the ECM. Thus, LMs can be targets of MMPs, bioactive factors stimulating their expression, or even interacting platforms capable of recruiting and concentrating them.

Acknowledgments

Original work leading to this review was generously supported by research grants from the French Agence Nationale de la Recherche (grant number ANR-18-CE92-0035-01, awarded to PR) and Rhône-Auvergne Pack Ambition Recherche 2020.

Table 1

Laminin isoforms

Name	Chain assembly	Previous	Original name
Laminin-111	$\alpha 1\beta 1\gamma 1$	1	EHS laminin
Laminin-211	$\alpha 2\beta 1\gamma 1$	2	merosin
Laminin-121	$\alpha 1\beta 2\gamma 1$	3	S-laminin
Laminin-221	$\alpha 2\beta 2\gamma 1$	4	S-merosin
Laminin-3A32	$\alpha 3A\beta 3\gamma 2$	5 or 5A	kalinin, epilegrin, nicein, ladsin
Laminin-3B32	$\alpha 3B\beta 3\gamma 2$	5B	
Laminin-3A11	$\alpha 3A\beta 1\gamma 1$	6 or 6A	K-laminin
Laminin-3A21	$\alpha 3A\beta 2\gamma 1$	7 or 7A	KS-laminin
Laminin-411	$\alpha 4\beta 1\gamma 1$	8	
Laminin-421	$\alpha 4\beta 2\gamma 1$	9	
Laminin-511	$\alpha 5\beta 1\gamma 1$	10	drosophila-like laminin
Laminin-521	$\alpha 5\beta 2\gamma 1$	11	
Laminin-213	$\alpha 2\beta 1\gamma 3$	12	
Laminin-423	$\alpha 4\beta 2\gamma 3$	14	
Laminin-523	$\alpha 5\beta 2\gamma 3$	15	
Laminin-522 ^a	$\alpha 5\beta 2\gamma 2$		
Laminin-212 ^b	$\alpha 2\beta 1\gamma 2$		
Laminin-222 ^b	$\alpha 2\beta 2\gamma 2$		
Laminin-333 ^c	$\alpha 3\beta 3\gamma 3$		

^a Laminin-522 reported so far solely in bone marrow.

^b The existence of either LM-212 or LM-222 is proposed based on studies of peripheral nerves in wild-type and LM $\alpha 2$ chain-deficient mice.

^c Laminin-333 is expressed at the apical specialization of adult rat testes, rather than in the testicular basement membrane.

References

- Adair-Kirk TL, Atkinson JJ, Broekelmann TJ, Doi M, Tryggvason K, Miner JH, Mecham RP, Senior RM (2003) A site on laminin alpha 5, AQARSAASKVKVSMKF, induces inflammatory cell production of matrix metalloproteinase-9 and chemotaxis. *J Immunol* 171 (1):398-406. doi:10.4049/jimmunol.171.1.398
- Albregues J, Shields MA, Ng D, Park CG, Ambrico A, Poindexter ME, Upadhyay P, Uyeminami DL, Pommier A, Küttner V, Bružas E, Maiorino L, Bautista C, Carmona EM, Gimotty PA, Fearon DT, Chang K, Lyons SK, Pinkerton KE, Trotman LC, Goldberg MS, Yeh JTH, Egeblad M (2018) Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. *Science* 361 (6409):eaao4227. doi:10.1126/science.aao4227
- Andrusier N, Nussinov R, Wolfson HJ (2007) FireDock: fast interaction refinement in molecular docking. *Proteins* 69 (1):139-159. doi:10.1002/prot.21495
- Augoff K, Hryniewicz-Jankowska A, Tabola R (2020) Invadopodia: clearing the way for cancer cell invasion. *Ann Transl Med* 8 (14):902. doi:10.21037/atm.2020.02.157
- Aumailley M, Bruckner-Tuderman L, Carter WG, Deutzmann R, Edgar D, Ekblom P, Engel J, Engvall E, Hohenester E, Jones JC, Kleinman HK, Marinkovich MP, Martin GR, Mayer U, Meneguzzi G, Miner JH, Miyazaki K, Patarroyo M, Paulsson M, Quaranta V, Sanes JR, Sasaki T, Sekiguchi K, Sorokin LM, Talts JF, Tryggvason K, Uitto J, Virtanen I, von der Mark K, Wewer UM, Yamada Y, Yurchenco PD (2005) A simplified laminin nomenclature. *Matrix Biol* 24 (5):326-332. doi:10.1016/j.matbio.2005.05.006
- Bair EL, Chen ML, McDaniel K, Sekiguchi K, Cress AE, Nagle RB, Bowden GT (2005) Membrane type 1 matrix metalloprotease cleaves laminin-10 and promotes prostate cancer cell migration. *Neoplasia* 7 (4):380-389. doi:10.1593/neo.04619
- Banerji S, Wright AJ, Noble M, Mahoney DJ, Campbell ID, Day AJ, Jackson DG (2007) Structures of the Cd44-hyaluronan complex provide insight into a fundamental carbohydrate-protein interaction. *Nature Structural & Molecular Biology* 14 (3):234-239. doi:10.1038/nsmb1201
- Beck K, Dixon TW, Engel J, Parry DA (1993) Ionic interactions in the coiled-coil domain of laminin determine the specificity of chain assembly. *J Mol Biol* 231 (2):311-323. doi:10.1006/jmbi.1993.1284
- Bejarano PA, Noelken ME, Suzuki K, Hudson BG, Nagase H (1988) Degradation of basement membranes by human matrix metalloproteinase 3 (stromelysin). *Biochem J* 256 (2):413-419. doi:10.1042/bj2560413
- Beliveau A, Mott JD, Lo A, Chen EI, Koller AA, Yaswen P, Muschler J, Bissell MJ (2010) Raf-induced MMP9 disrupts tissue architecture of human breast cells in three-dimensional culture and is necessary for tumor growth in vivo. *Genes Dev* 24 (24):2800-2811. doi:10.1101/gad.1990410
- Bennett KL, Jackson DG, Simon JC, Tanczos E, Peach R, Modrell B, Stamenkovic I, Plowman G, Aruffo A (1995) CD44 isoforms containing exon V3 are responsible for the presentation of heparin-binding growth factor. *The Journal of cell biology* 128 (4):687-698. doi:10.1083/jcb.128.4.687
- Berno V, Porrini D, Castiglioni F, Campiglio M, Casalini P, Pupa SM, Balsari A, Ménard S, Tagliabue E (2005) The 67 kDa laminin receptor increases tumor aggressiveness by remodeling laminin-1. *Endocr Relat Cancer* 12 (2):393-406. doi:10.1677/erc.1.00870

- Bourguignon LY, Bikle D (2015) Selective Hyaluronan-CD44 Signaling Promotes miRNA-21 Expression and Interacts with Vitamin D Function during Cutaneous Squamous Cell Carcinomas Progression Following UV Irradiation. *Front Immunol* 6:224. doi:10.3389/fimmu.2015.00224
- Bourguignon LYW (2019) Matrix Hyaluronan-CD44 Interaction Activates MicroRNA and LncRNA Signaling Associated With Chemoresistance, Invasion, and Tumor Progression. *Front Oncol* 9:492. doi:10.3389/fonc.2019.00492
- Brown TA, Bouchard T, St John T, Wayner E, Carter WG (1991) Human keratinocytes express a new CD44 core protein (CD44E) as a heparan-sulfate intrinsic membrane proteoglycan with additional exons. *J Cell Biol* 113 (1):207-221. doi:10.1083/jcb.113.1.207
- Carafoli F, Hussain SA, Hohenester E (2012) Crystal structures of the network-forming short-arm tips of the laminin β 1 and γ 1 chains. *PLoS One* 7 (7):e42473. doi:10.1371/journal.pone.0042473
- Carulli S, Beck K, Dayan G, Boulesteix S, Lortat-Jacob H, Rousselle P (2012) Cell surface proteoglycans syndecan-1 and -4 bind overlapping but distinct sites in laminin α 3 LG45 protein domain. *J Biol Chem* 287 (15):12204-12216. doi:10.1074/jbc.M111.300061
- Cauwe B, Van den Steen PE, Opdenakker G (2007) The biochemical, biological, and pathological kaleidoscope of cell surface substrates processed by matrix metalloproteinases. *Crit Rev Biochem Mol Biol* 42 (3):113-185. doi:10.1080/10409230701340019
- Chabadel A, Bañon-Rodríguez I, Cluet D, Rudkin BB, Wehrle-Haller B, Genot E, Jurdic P, Anton IM, Saltel F (2007) CD44 and beta3 integrin organize two functionally distinct actin-based domains in osteoclasts. *Mol Biol Cell* 18 (12):4899-4910. doi:10.1091/mbc.e07-04-0378
- Champlaud MF, Lunstrum GP, Rousselle P, Nishiyama T, Keene DR, Burgeson RE (1996) Human amnion contains a novel laminin variant, laminin 7, which like laminin 6, covalently associates with laminin 5 to promote stable epithelial-stromal attachment. *J Cell Biol* 132 (6):1189-1198. doi:10.1083/jcb.132.6.1189
- Chandler S, Cossins J, Lury J, Wells G (1996) Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. *Biochem Biophys Res Commun* 228 (2):421-429. doi:10.1006/bbrc.1996.1677
- Chen C, Zhao S, Karnad A, Freeman JW (2018) The biology and role of CD44 in cancer progression: therapeutic implications. *J Hematol Oncol* 11 (1):64. doi:10.1186/s13045-018-0605-5
- Cheng YS, Champlaud MF, Burgeson RE, Marinkovich MP, Yurchenco PD (1997) Self-assembly of laminin isoforms. *J Biol Chem* 272 (50):31525-31532. doi:10.1074/jbc.272.50.31525
- Cho SH, Park YS, Kim HJ, Kim CH, Lim SW, Huh JW, Lee JH, Kim HR (2012) CD44 enhances the epithelial-mesenchymal transition in association with colon cancer invasion. *Int J Oncol* 41 (1):211-218. doi:10.3892/ijo.2012.1453
- Colognato H, MacCarrick M, O'Rear JJ, Yurchenco PD (1997) The Laminin α 2-Chain Short Arm Mediates Cell Adhesion through Both the α 1 β 1 and α 2 β 1 Integrins*. *Journal of Biological Chemistry* 272 (46):29330-29336. doi:<https://doi.org/10.1074/jbc.272.46.29330>
- Colognato-Pyke H, O'Rear JJ, Yamada Y, Carbonetto S, Cheng YS, Yurchenco PD (1995) Mapping of network-forming, heparin-binding, and alpha 1 beta 1 integrin-

- recognition sites within the alpha-chain short arm of laminin-1. *J Biol Chem* 270 (16):9398-9406. doi:10.1074/jbc.270.16.9398
- Corcoran ML, Kibbey MC, Kleinman HK, Wahl LM (1995) Laminin SIKVAV peptide induction of monocyte/macrophage prostaglandin E2 and matrix metalloproteinases. *J Biol Chem* 270 (18):10365-10368. doi:10.1074/jbc.270.18.10365
- Deboux C, Ladraa S, Cazaubon S, Ghribi-Mallah S, Weiss N, Chaverot N, Couraud PO, Baron-Van Evercooren A (2013) Overexpression of CD44 in neural precursor cells improves trans-endothelial migration and facilitates their invasion of perivascular tissues in vivo. *PLoS One* 8 (2):e57430. doi:10.1371/journal.pone.0057430
- Delimont D, Dufek BM, Meehan DT, Zallocchi M, Gratton MA, Phillips G, Cosgrove D (2014) Laminin α 2-mediated focal adhesion kinase activation triggers Alport glomerular pathogenesis. *PLoS One* 9 (6):e99083. doi:10.1371/journal.pone.0099083
- Desai B, Rogers MJ, Chellaiah MA (2007) Mechanisms of osteopontin and CD44 as metastatic principles in prostate cancer cells. *Mol Cancer* 6:18. doi:10.1186/1476-4598-6-18
- Deutzmann R, Aumailley M, Wiedemann H, Pysny W, Timpl R, Edgar D (1990) Cell adhesion, spreading and neurite stimulation by laminin fragment E8 depends on maintenance of secondary and tertiary structure in its rod and globular domain. *Eur J Biochem* 191 (2):513-522. doi:10.1111/j.1432-1033.1990.tb19151.x
- Domogatskaya A, Rodin S, Tryggvason K (2012) Functional diversity of laminins. *Annu Rev Cell Dev Biol* 28:523-553. doi:10.1146/annurev-cellbio-101011-155750
- Dosztányi Z (2018) Prediction of protein disorder based on IUPred. *Protein Sci* 27 (1):331-340. doi:10.1002/pro.3334
- Du L, Wang H, He L, Zhang J, Ni B, Wang X, Jin H, Cahuzac N, Mehrpour M, Lu Y, Chen Q (2008) CD44 is of functional importance for colorectal cancer stem cells. *Clin Cancer Res* 14 (21):6751-6760. doi:10.1158/1078-0432.Ccr-08-1034
- Durbeej M (2009) Laminins. *Cell and Tissue Research* 339 (1):259. doi:10.1007/s00441-009-0838-2
- Fingleton B (2006) Matrix metalloproteinases: roles in cancer and metastasis. *Frontiers in Bioscience-Landmark* 11 (1):479-491. doi:10.2741/1811
- Gaffney J, Solomonov I, Zehorai E, Sagi I (2015) Multilevel regulation of matrix metalloproteinases in tissue homeostasis indicates their molecular specificity in vivo. *Matrix Biol* 44-46:191-199. doi:10.1016/j.matbio.2015.01.012
- Gal I, Lesley J, Ko W, Gonda A, Stoop R, Hyman R, Mikecz K (2003) Role of the extracellular and cytoplasmic domains of CD44 in the rolling interaction of lymphoid cells with hyaluronan under physiologic flow. *J Biol Chem* 278 (13):11150-11158. doi:10.1074/jbc.M210661200
- Gao Y, Mruk D, Chen H, Lui WY, Lee WM, Cheng CY (2017) Regulation of the blood-testis barrier by a local axis in the testis: role of laminin α 2 in the basement membrane. *Faseb j* 31 (2):584-597. doi:10.1096/fj.201600870R
- Gee SH, Blacher RW, Douville PJ, Provost PR, Yurchenco PD, Carbonetto S (1993) Laminin-binding protein 120 from brain is closely related to the dystrophin-associated glycoprotein, dystroglycan, and binds with high affinity to the major heparin binding domain of laminin. *J Biol Chem* 268 (20):14972-14980

- Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V (1997) Induction of Cell Migration by Matrix Metalloprotease-2 Cleavage of Laminin-5. *Science* 277 (5323):225. doi:10.1126/science.277.5323.225
- Gilles C, Polette M, Coraux C, Tournier JM, Meneguzzi G, Munaut C, Volders L, Rousselle P, Birembaut P, Foidart JM (2001) Contribution of MT1-MMP and of human laminin-5 gamma2 chain degradation to mammary epithelial cell migration. *J Cell Sci* 114 (Pt 16):2967-2976
- Gobin E, Bagwell K, Wagner J, Mysona D, Sandirasegarane S, Smith N, Bai S, Sharma A, Schleifer R, She J-X (2019) A pan-cancer perspective of matrix metalloproteases (MMP) gene expression profile and their diagnostic/prognostic potential. *BMC Cancer* 19 (1):581. doi:10.1186/s12885-019-5768-0
- Gopal SK, Greening DW, Zhu H-J, Simpson RJ, Mathias RA (2016) Transformed MDCK cells secrete elevated MMP1 that generates LAMA5 fragments promoting endothelial cell angiogenesis. *Scientific Reports* 6 (1):28321. doi:10.1038/srep28321
- Gu Z, Cui J, Brown S, Fridman R, Mobashery S, Strongin AY, Lipton SA (2005) A highly specific inhibitor of matrix metalloproteinase-9 rescues laminin from proteolysis and neurons from apoptosis in transient focal cerebral ischemia. *J Neurosci* 25 (27):6401-6408. doi:10.1523/jneurosci.1563-05.2005
- Gupta A, Cao W, Sadashivaiah K, Chen W, Schneider A, Chellaiah MA (2013) Promising Noninvasive Cellular Phenotype in Prostate Cancer Cells Knockdown of Matrix Metalloproteinase 9. *The Scientific World Journal* 2013:493689. doi:10.1155/2013/493689
- Guvench O (2015) Revealing the Mechanisms of Protein Disorder and N-Glycosylation in CD44-Hyaluronan Binding Using Molecular Simulation. *Front Immunol* 6:305. doi:10.3389/fimmu.2015.00305
- Habchi J, Tompa P, Longhi S, Uversky VN (2014) Introducing protein intrinsic disorder. *Chem Rev* 114 (13):6561-6588. doi:10.1021/cr400514h
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144 (5):646-674. doi:10.1016/j.cell.2011.02.013
- Hibino S, Shibuya M, Engbring JA, Mochizuki M, Nomizu M, Kleinman HK (2004) Identification of an active site on the laminin alpha5 chain globular domain that binds to CD44 and inhibits malignancy. *Cancer Res* 64 (14):4810-4816. doi:10.1158/0008-5472.Can-04-0129
- Hibino S, Shibuya M, Hoffman MP, Engbring JA, Hossain R, Mochizuki M, Kudoh S, Nomizu M, Kleinman HK (2005) Laminin alpha5 chain metastasis- and angiogenesis-inhibiting peptide blocks fibroblast growth factor 2 activity by binding to the heparan sulfate chains of CD44. *Cancer Res* 65 (22):10494-10501. doi:10.1158/0008-5472.Can-05-0314
- Hohenester E (2019) Structural biology of laminins. *Essays Biochem* 63 (3):285-295. doi:10.1042/ebc20180075
- Hohenester E, Yurchenco PD (2013) Laminins in basement membrane assembly. *Cell Adh Migr* 7 (1):56-63. doi:10.4161/cam.21831
- Horejs C-M, Serio A, Purvis A, Gormley AJ, Bertazzo S, Poliniewicz A, Wang AJ, DiMaggio P, Hohenester E, Stevens MM (2014) Biologically-active laminin-111 fragment that modulates the epithelial-to-mesenchymal transition in embryonic stem cells. *Proceedings of the National Academy of Sciences* 111 (16):5908-5913. doi:10.1073/pnas.1403139111

- Hozumi K, Suzuki N, Uchiyama Y, Katagiri F, Kikkawa Y, Nomizu M (2009) Chain-specific heparin-binding sequences in the laminin alpha chain LG45 modules. *Biochemistry* 48 (23):5375-5381. doi:10.1021/bi900542u
- Ido H, Harada K, Futaki S, Hayashi Y, Nishiuchi R, Natsuka Y, Li S, Wada Y, Combs AC, Ervasti JM, Sekiguchi K (2004) Molecular dissection of the alpha-dystroglycan- and integrin-binding sites within the globular domain of human laminin-10. *J Biol Chem* 279 (12):10946-10954. doi:10.1074/jbc.M313626200
- Ido H, Ito S, Taniguchi Y, Hayashi M, Sato-Nishiuchi R, Sanzen N, Hayashi Y, Futaki S, Sekiguchi K (2008) Laminin isoforms containing the gamma3 chain are unable to bind to integrins due to the absence of the glutamic acid residue conserved in the C-terminal regions of the gamma1 and gamma2 chains. *The Journal of biological chemistry* 283 (42):28149-28157. doi:10.1074/jbc.M803553200
- Ido H, Nakamura A, Kobayashi R, Ito S, Li S, Futaki S, Sekiguchi K (2007) The requirement of the glutamic acid residue at the third position from the carboxyl termini of the laminin gamma chains in integrin binding by laminins. *J Biol Chem* 282 (15):11144-11154. doi:10.1074/jbc.M609402200
- Inamori KI, Beedle AM, de Bernabé DB, Wright ME, Campbell KP (2016) LARGE2-dependent glycosylation confers laminin-binding ability on proteoglycans. *Glycobiology* 26 (12):1284-1296. doi:10.1093/glycob/cww075
- Ishii S, Ford R, Thomas P, Nachman A, Steele G, Jr., Jessup JM (1993) CD44 participates in the adhesion of human colorectal carcinoma cells to laminin and type IV collagen. *Surg Oncol* 2 (4):255-264. doi:10.1016/0960-7404(93)90015-q
- Itoh Y, Nagase H (2002) Matrix metalloproteinases in cancer. *Essays Biochem* 38:21-36. doi:10.1042/bse0380021
- Itoh Y, Seiki M (2006) MT1-MMP: a potent modifier of pericellular microenvironment. *J Cell Physiol* 206 (1):1-8. doi:10.1002/jcp.20431
- Jacob A, Prekeris R (2015) The regulation of MMP targeting to invadopodia during cancer metastasis. *Front Cell Dev Biol* 3:4-4. doi:10.3389/fcell.2015.00004
- Jalkanen S, Jalkanen M (1992) Lymphocyte CD44 binds the COOH-terminal heparin-binding domain of fibronectin. *J Cell Biol* 116 (3):817-825. doi:10.1083/jcb.116.3.817
- Jayadev R, Sherwood DR (2017) Basement membranes. *Current Biology* 27 (6):R207-R211. doi:<https://doi.org/10.1016/j.cub.2017.02.006>
- Jenkins MH, Alrowaished SS, Goody MF, Crawford BD, Henry CA (2016) Laminin and Matrix metalloproteinase 11 regulate Fibronectin levels in the zebrafish myotendinous junction. *Skeletal Muscle* 6 (1):18. doi:10.1186/s13395-016-0089-3
- Kajita M, Itoh Y, Chiba T, Mori H, Okada A, Kinoh H, Seiki M (2001) Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J Cell Biol* 153 (5):893-904. doi:10.1083/jcb.153.5.893
- Kamoshida G, Ogawa T, Oyanagi J, Sato H, Komiya E, Higashi S, Miyazaki K, Tsuji T (2014) Modulation of matrix metalloproteinase-9 secretion from tumor-associated macrophage-like cells by proteolytically processed laminin-332 (laminin-5). *Clin Exp Metastasis* 31 (3):285-291. doi:10.1007/s10585-013-9627-0
- Karamanos NK, Piperigkou Z, Passi A, Götte M, Rousselle P, Vlodavsky I (2021) Extracellular matrix-based cancer targeting. *Trends in Molecular Medicine*. doi:<https://doi.org/10.1016/j.molmed.2021.07.009>

- Kelley LC, Chi Q, Cáceres R, Hastie E, Schindler AJ, Jiang Y, Matus DQ, Plastino J, Sherwood DR (2019) Adaptive F-Actin Polymerization and Localized ATP Production Drive Basement Membrane Invasion in the Absence of MMPs. *Dev Cell* 48 (3):313-328.e318. doi:10.1016/j.devcel.2018.12.018
- Kenne E, Soehnlein O, Genové G, Rotzius P, Eriksson EE, Lindbom L (2010) Immune cell recruitment to inflammatory loci is impaired in mice deficient in basement membrane protein laminin alpha4. *J Leukoc Biol* 88 (3):523-528. doi:10.1189/jlb.0110043
- Khan KM, Falcone DJ (1997) Role of laminin in matrix induction of macrophage urokinase-type plasminogen activator and 92-kDa metalloproteinase expression. *J Biol Chem* 272 (13):8270-8275. doi:10.1074/jbc.272.13.8270
- Khan KM, Falcone DJ (2000) Selective activation of MAPK(erk1/2) by laminin-1 peptide alpha1:Ser(2091)-Arg(2108) regulates macrophage degradative phenotype. *J Biol Chem* 275 (6):4492-4498. doi:10.1074/jbc.275.6.4492
- Kikkawa Y, Hozumi K, Katagiri F, Nomizu M, Kleinman HK, Kobinski JE (2013) Laminin-111-derived peptides and cancer. *Cell Adh Migr* 7 (1):150-256. doi:10.4161/cam.22827
- Kikkawa Y, Sasaki T, Nguyen MT, Nomizu M, Mitaka T, Miner JH (2007) The LG1-3 tandem of laminin alpha5 harbors the binding sites of Lutheran/basal cell adhesion molecule and alpha3beta1/alpha6beta1 integrins. *J Biol Chem* 282 (20):14853-14860. doi:10.1074/jbc.M611706200
- Klausen MS, Jespersen MC, Nielsen H, Jensen KK, Jurtz VI, Sønderby CK, Sommer MOA, Winther O, Nielsen M, Petersen B, Marcatili P (2019) NetSurfP-2.0: Improved prediction of protein structural features by integrated deep learning. *Proteins* 87 (6):520-527. doi:10.1002/prot.25674
- Knäuper V, Bailey L, Worley JR, Soloway P, Patterson ML, Murphy G (2002) Cellular activation of proMMP-13 by MT1-MMP depends on the C-terminal domain of MMP-13. *FEBS Lett* 532 (1-2):127-130. doi:10.1016/s0014-5793(02)03654-2
- Knudson W, Aguiar DJ, Hua Q, Knudson CB (1996) CD44-anchored hyaluronan-rich pericellular matrices: an ultrastructural and biochemical analysis. *Exp Cell Res* 228 (2):216-228. doi:10.1006/excr.1996.0320
- Koshikawa N, Giannelli G, Cirulli V, Miyazaki K, Quaranta V (2000) Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. *The Journal of cell biology* 148 (3):615-624. doi:10.1083/jcb.148.3.615
- Koshikawa N, Schenk S, Moeckel G, Sharabi A, Miyazaki K, Gardner H, Zent R, Quaranta V (2004) Proteolytic processing of laminin-5 by MT1-MMP in tissues and its effects on epithelial cell morphology. *Faseb j* 18 (2):364-366. doi:10.1096/fj.03-0584fje
- Kusuma N, Anderson RL, Pouliot N (2011) Laminin α 5-derived peptides modulate the properties of metastatic breast tumour cells. *Clin Exp Metastasis* 28 (8):909-921. doi:10.1007/s10585-011-9422-8
- Laronha H, Caldeira J (2020) Structure and Function of Human Matrix Metalloproteinases. *Cells* 9 (5):1076. doi:10.3390/cells9051076
- Li H, Liu S, Wu S, Li L, Ge R, Cheng CY (2020) Bioactive fragments of laminin and collagen chains: lesson from the testis. *Reproduction* 159 (3):R111-r123. doi:10.1530/rep-19-0288
- Li S, Edgar D, Fässler R, Wadsworth W, Yurchenco PD (2003) The role of laminin in embryonic cell polarization and tissue organization. *Dev Cell* 4 (5):613-624. doi:10.1016/s1534-5807(03)00128-x

- Li Z, Takino T, Endo Y, Sato H (2017) Activation of MMP-9 by membrane type-1 MMP/MMP-2 axis stimulates tumor metastasis. *Cancer Sci* 108 (3):347-353. doi:10.1111/cas.13134
- Linder S, Wiesner C (2016) Feel the force: Podosomes in mechanosensing. *Experimental Cell Research* 343 (1):67-72. doi:<https://doi.org/10.1016/j.yexcr.2015.11.026>
- Liu L-K, Finzel B (2014) High-resolution crystal structures of alternate forms of the human CD44 hyaluronan-binding domain reveal a site for protein interaction. *Acta Crystallogr F Struct Biol Commun* 70 (Pt 9):1155-1161. doi:10.1107/S2053230X14015532
- Macdonald PR, Lustig A, Steinmetz MO, Kammerer RA (2010) Laminin chain assembly is regulated by specific coiled-coil interactions. *J Struct Biol* 170 (2):398-405. doi:10.1016/j.jsb.2010.02.004
- Maity G, Sen T, Chatterjee A (2011) Laminin induces matrix metalloproteinase-9 expression and activation in human cervical cancer cell line (SiHa). *J Cancer Res Clin Oncol* 137 (2):347-357. doi:10.1007/s00432-010-0892-x
- Maltseva DV, Rodin SA (2018) [Laminins in Metastatic Cancer]. *Mol Biol (Mosk)* 52 (3):411-434. doi:10.7868/s0026898418030059
- Matsui C, Wang CK, Nelson CF, Bauer EA, Hoeffler WK (1995) The assembly of laminin-5 subunits. *J Biol Chem* 270 (40):23496-23503. doi:10.1074/jbc.270.40.23496
- McKee KK, Harrison D, Capizzi S, Yurchenco PD (2007) Role of laminin terminal globular domains in basement membrane assembly. *J Biol Chem* 282 (29):21437-21447. doi:10.1074/jbc.M702963200
- Medrano-González PA, Rivera-Ramírez O, Montaña LF, Rendón-Huerta EP (2021) Proteolytic Processing of CD44 and Its Implications in Cancer. *Stem Cells International* 2021:6667735. doi:10.1155/2021/6667735
- Michopoulou A, Montmasson M, Garnier C, Lambert E, Dayan G, Rousselle P (2020) A novel mechanism in wound healing: Laminin 332 drives MMP9/14 activity by recruiting syndecan-1 and CD44. *Matrix Biol* 94:1-17. doi:10.1016/j.matbio.2020.06.004
- Miner JH, Patton BL, Lentz SI, Gilbert DJ, Snider WD, Jenkins NA, Copeland NG, Sanes JR (1997) 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* 137 (3):685-701. doi:10.1083/jcb.137.3.685
- Miner JH, Yurchenco PD (2004) Laminin functions in tissue morphogenesis. *Annu Rev Cell Dev Biol* 20:255-284. doi:10.1146/annurev.cellbio.20.010403.094555
- Momota Y, Suzuki N, Kasuya Y, Kobayashi T, Mizoguchi M, Yokoyama F, Nomizu M, Shinkai H, Iwasaki T, Utani A (2005) Laminin alpha3 LG4 module induces keratinocyte migration: involvement of matrix metalloproteinase-9. *J Recept Signal Transduct Res* 25 (1):1-17. doi:10.1081/rrs-200047870
- Mori H, Tomari T, Koshikawa N, Kajita M, Itoh Y, Sato H, Tojo H, Yana I, Seiki M (2002) CD44 directs membrane-type 1 matrix metalloproteinase to lamellipodia by associating with its hemopexin-like domain. *The EMBO journal* 21 (15):3949-3959. doi:10.1093/emboj/cdf411
- Morita A, Sugimoto E, Kitagawa Y (1985) Post-translational assembly and glycosylation of laminin subunits in parietal endoderm-like F9 cells. *Biochem J* 229 (1):259-264. doi:10.1042/bj2290259

- Murphy DA, Courtneidge SA (2011) The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. *Nature Reviews Molecular Cell Biology* 12 (7):413-426. doi:10.1038/nrm3141
- Mydel P, Shipley JM, Adair-Kirk TL, Kelley DG, Broekelmann TJ, Mecham RP, Senior RM (2008) Neutrophil elastase cleaves laminin-332 (laminin-5) generating peptides that are chemotactic for neutrophils. *J Biol Chem* 283 (15):9513-9522. doi:10.1074/jbc.M706239200
- Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 69 (3):562-573. doi:10.1016/j.cardiores.2005.12.002
- Navdaev A, Heitmann V, Desantana Evangelista K, Mörgelin M, Wegener J, Eble JA (2008) The C-terminus of the gamma 2 chain but not of the beta 3 chain of laminin-332 is indirectly but indispensably necessary for integrin-mediated cell reactions. *Exp Cell Res* 314 (3):489-497. doi:10.1016/j.yexcr.2007.10.027
- Nielsen PK, Yamada Y (2001) Identification of Cell-binding Sites on the Laminin α 5 N-terminal Domain by Site-directed Mutagenesis*. *Journal of Biological Chemistry* 276 (14):10906-10912. doi:<https://doi.org/10.1074/jbc.M008743200>
- Niland S, Eble JA (2020) Hold on or Cut? Integrin- and MMP-Mediated Cell-Matrix Interactions in the Tumor Microenvironment. *Int J Mol Sci* 22 (1). doi:10.3390/ijms22010238
- Nishida Y, Miyamori H, Thompson EW, Takino T, Endo Y, Sato H (2008) Activation of matrix metalloproteinase-2 (MMP-2) by membrane type 1 matrix metalloproteinase through an artificial receptor for proMMP-2 generates active MMP-2. *Cancer Res* 68 (21):9096-9104. doi:10.1158/0008-5472.Can-08-2522
- Nishiuchi R, Takagi J, Hayashi M, Ido H, Yagi Y, Sanzen N, Tsuji T, Yamada M, Sekiguchi K (2006) Ligand-binding specificities of laminin-binding integrins: a comprehensive survey of laminin-integrin interactions using recombinant alpha3beta1, alpha6beta1, alpha7beta1 and alpha6beta4 integrins. *Matrix Biol* 25 (3):189-197. doi:10.1016/j.matbio.2005.12.001
- Ogawa T, Tsubota Y, Hashimoto J, Kariya Y, Miyazaki K (2007) The Short Arm of Laminin γ 2 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* 18 (5):1621-1633. doi:10.1091/mbc.e06-09-0806
- Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y (1997) Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. *J Biol Chem* 272 (4):2446-2451. doi:10.1074/jbc.272.4.2446
- Okada Y, Nagase H, Harris ED (1986) A metalloproteinase from human rheumatoid synovial fibroblasts that digests connective tissue matrix components. Purification and characterization. *Journal of Biological Chemistry* 261 (30):14245-14255. doi:[https://doi.org/10.1016/S0021-9258\(18\)67011-5](https://doi.org/10.1016/S0021-9258(18)67011-5)
- Pal S, Moulik S, Dutta A, Chatterjee A (2014) Extracellular matrix protein laminin induces matrix metalloproteinase-9 in human breast cancer cell line mcf-7. *Cancer Microenviron* 7 (1-2):71-78. doi:10.1007/s12307-014-0146-6
- Paulsson M, Deutzmann R, Timpl R, Dalzoppo D, Odermatt E, Engel J (1985) Evidence for coiled-coil alpha-helical regions in the long arm of laminin. *Embo j* 4 (2):309-316
- Peng ST, Su CH, Kuo CC, Shaw CF, Wang HS (2007) CD44 crosslinking-mediated matrix metalloproteinase-9 relocation in breast tumor cells leads to enhanced metastasis. *Int J Oncol* 31 (5):1119-1126

- Piperigkou Z, Kyriakopoulou K, Koutsakis C, Mastronikolis S, Karamanos NK (2021) Key Matrix Remodeling Enzymes: Functions and Targeting in Cancer. *Cancers (Basel)* 13 (6). doi:10.3390/cancers13061441
- Pirilä E, Sharabi A, Salo T, Quaranta V, Tu H, Heljasvaara R, Koshikawa N, Sorsa T, Maisi P (2003) Matrix metalloproteinases process the laminin-5 gamma 2-chain and regulate epithelial cell migration. *Biochem Biophys Res Commun* 303 (4):1012-1017. doi:10.1016/s0006-291x(03)00452-2
- Poincloux R, Lizárraga F, Chavrier P (2009) Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia. *J Cell Sci* 122 (Pt 17):3015-3024. doi:10.1242/jcs.034561
- Ponta H, Sherman L, Herrlich PA (2003) CD44: From adhesion molecules to signalling regulators. *Nature Reviews Molecular Cell Biology* 4 (1):33-45. doi:10.1038/nrm1004
- Pulido D, Briggs DC, Hua J, Hohenester E (2017) Crystallographic analysis of the laminin β 2 short arm reveals how the LF domain is inserted into a regular array of LE domains. *Matrix Biol* 57-58:204-212. doi:10.1016/j.matbio.2016.06.006
- Qin Y, Rodin S, Simonson OE, Hollande F (2017) Laminins and cancer stem cells: Partners in crime? *Semin Cancer Biol* 45:3-12. doi:10.1016/j.semcancer.2016.07.004
- Ramovs V, Te Molder L, Sonnenberg A (2017) The opposing roles of laminin-binding integrins in cancer. *Matrix Biol* 57-58:213-243. doi:10.1016/j.matbio.2016.08.007
- Remy L, Trespeuch C, Bachy S, Scoazec JY, Rousselle P (2006) Matrilysin 1 influences colon carcinoma cell migration by cleavage of the laminin-5 beta3 chain. *Cancer Res* 66 (23):11228-11237. doi:10.1158/0008-5472.Can-06-1187
- Ries A, Goldberg JL, Grimpe B (2007) A novel biological function for CD44 in axon growth of retinal ganglion cells identified by a bioinformatics approach. *J Neurochem* 103 (4):1491-1505. doi:10.1111/j.1471-4159.2007.04858.x
- Rousselle P, Beck K (2013) Laminin 332 processing impacts cellular behavior. *Cell adhesion & migration* 7 (1):122-134. doi:10.4161/cam.23132
- Rousselle P, Golbik R, van der Rest M, Aumailley M (1995) Structural requirement for cell adhesion to kalinin (laminin-5). *J Biol Chem* 270 (23):13766-13770. doi:10.1074/jbc.270.23.13766
- Rousselle P, Keene DR, Ruggiero F, Champlaud MF, Rest M, Burgeson RE (1997) Laminin 5 binds the NC-1 domain of type VII collagen. *J Cell Biol* 138 (3):719-728. doi:10.1083/jcb.138.3.719
- Rousselle P, Lunstrum GP, Keene DR, Burgeson RE (1991) Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. *J Cell Biol* 114 (3):567-576. doi:10.1083/jcb.114.3.567
- Rousselle P, Scoazec JY (2020) Laminin 332 in cancer: When the extracellular matrix turns signals from cell anchorage to cell movement. *Seminars in Cancer Biology* 62:149-165. doi:<https://doi.org/10.1016/j.semcancer.2019.09.026>
- Sadowski T, Dietrich S, Koschinsky F, Ludwig A, Proksch E, Titz B, Sedlacek R (2005) Matrix metalloproteinase 19 processes the laminin 5 gamma 2 chain and induces epithelial cell migration. *Cellular and Molecular Life Sciences CMLS* 62 (7):870-880. doi:10.1007/s00018-005-4478-8
- Samanna V, Wei H, Ego-Osuala D, Chellaiah MA (2006) Alpha-V-dependent outside-in signaling is required for the regulation of CD44 surface expression, MMP-2

- secretion, and cell migration by osteopontin in human melanoma cells. *Exp Cell Res* 312 (12):2214-2230. doi:10.1016/j.yexcr.2006.03.022
- Sasaki T, Göhring W, Mann K, Brakebusch C, Yamada Y, Fässler R, Timpl R (2001) Short arm region of laminin-5 gamma2 chain: structure, mechanism of processing and binding to heparin and proteins. *J Mol Biol* 314 (4):751-763. doi:10.1006/jmbi.2001.5176
- Sasaki T, Timpl R (2001) Domain IVa of laminin alpha5 chain is cell-adhesive and binds beta1 and alphaVbeta3 integrins through Arg-Gly-Asp. *FEBS Lett* 509 (2):181-185. doi:10.1016/s0014-5793(01)03167-2
- Sato H, Higashi S, Miyazaki K (2015) Amino-terminal fragments of laminin γ 2 chain stimulate migration of metastatic breast cancer cells by interacting with CD44. *Clin Exp Metastasis* 32 (5):405-415. doi:10.1007/s10585-015-9705-6
- Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Res* 33 (Web Server issue):W363-367. doi:10.1093/nar/gki481
- Screaton GR, Bell MV, Bell JI, Jackson DG (1993) The identification of a new alternative exon with highly restricted tissue expression in transcripts encoding the mouse Pgp-1 (CD44) homing receptor. Comparison of all 10 variable exons between mouse, human, and rat. *J Biol Chem* 268 (17):12235-12238
- Seiki M (2002) The cell surface: the stage for matrix metalloproteinase regulation of migration. *Curr Opin Cell Biol* 14 (5):624-632. doi:10.1016/s0955-0674(02)00363-0
- Senyürek I, Kempf WE, Klein G, Maurer A, Kalbacher H, Schäfer L, Wanke I, Christ C, Stevanovic S, Schaller M, Rousselle P, Garbe C, Biedermann T, Schitteck B (2014) Processing of Laminin α Chains Generates Peptides Involved in Wound Healing and Host Defense. *Journal of Innate Immunity* 6 (4):467-484. doi:10.1159/000357032
- Shay G, Lynch CC, Fingleton B (2015) Moving targets: Emerging roles for MMPs in cancer progression and metastasis. *Matrix Biol* 44-46:200-206. doi:10.1016/j.matbio.2015.01.019
- Siu MKY, Cheng CY (2004) Interactions of Proteases, Protease Inhibitors, and the β 1 Integrin/Laminin γ 3 Protein Complex in the Regulation of Ectoplasmic Specialization Dynamics in the Rat Testis1. *Biology of Reproduction* 70 (4):945-964. doi:10.1095/biolreprod.103.023606
- Smirnov SP, McDearmon EL, Li S, Ervasti JM, Tryggvason K, Yurchenco PD (2002) Contributions of the LG Modules and Furin Processing to Laminin-2 Functions*. *Journal of Biological Chemistry* 277 (21):18928-18937. doi:<https://doi.org/10.1074/jbc.M201880200>
- Spessotto P, Yin Z, Magro G, Deutzmann R, Chiu A, Colombatti A, Perris R (2001) Laminin isoforms 8 and 10 are primary components of the subendothelial basement membrane promoting interaction with neoplastic lymphocytes. *Cancer Res* 61 (1):339-347
- Su L, Mruk DD, Lie PP, Silvestrini B, Cheng CY (2012) A peptide derived from laminin- γ 3 reversibly impairs spermatogenesis in rats. *Nat Commun* 3:1185. doi:10.1038/ncomms2171
- Suenaga N, Mori H, Itoh Y, Seiki M (2005) CD44 binding through the hemopexin-like domain is critical for its shedding by membrane-type 1 matrix metalloproteinase. *Oncogene* 24 (5):859-868. doi:10.1038/sj.onc.1208258

- Suzuki N, Yokoyama F, Nomizu M (2005) Functional sites in the laminin alpha chains. *Connect Tissue Res* 46 (3):142-152. doi:10.1080/03008200591008527
- Sypecka J, Dragun-Szymczak P, Zalewska T, Domańska-Janik K (2009) Laminin promotes oligogliogenesis and increases MMPs activity in human neural stem cells of HUCB-NSC line. *Acta Neurobiol Exp (Wars)* 69 (1):37-45
- Takahashi K, Eto H, Tanabe KK (1999) Involvement of CD44 in matrix metalloproteinase-2 regulation in human melanoma cells. *Int J Cancer* 80 (3):387-395. doi:10.1002/(sici)1097-0215(19990129)80:3<387::aid-ijc9>3.0.co;2-t
- Takaishi S, Okumura T, Tu S, Wang SSW, Shibata W, Vigneshwaran R, Gordon SAK, Shimada Y, Wang TC (2009) Identification of gastric cancer stem cells using the cell surface marker CD44. *Stem Cells* 27 (5):1006-1020. doi:10.1002/stem.30
- Takizawa M, Arimori T, Taniguchi Y, Kitago Y, Yamashita E, Takagi J, Sekiguchi K (2017) Mechanistic basis for the recognition of laminin-511 by $\alpha 6 \beta 1$ integrin. *Sci Adv* 3 (9):e1701497. doi:10.1126/sciadv.1701497
- Talts JF, Andac Z, Göhring W, Brancaccio A, Timpl R (1999) Binding of the G domains of laminin alpha1 and alpha2 chains and perlecan to heparin, sulfatides, alpha-dystroglycan and several extracellular matrix proteins. *Embo j* 18 (4):863-870. doi:10.1093/emboj/18.4.863
- Thapa R, Wilson GD (2016) The Importance of CD44 as a Stem Cell Biomarker and Therapeutic Target in Cancer. *Stem Cells Int* 2016:2087204. doi:10.1155/2016/2087204
- Timpl R, Tisi D, Talts JF, Andac Z, Sasaki T, Hohenester E (2000) Structure and function of laminin LG modules. *Matrix Biology* 19 (4):309-317. doi:[https://doi.org/10.1016/S0945-053X\(00\)00072-X](https://doi.org/10.1016/S0945-053X(00)00072-X)
- Tran M, Rousselle P, Nokelainen P, Tallapragada S, Nguyen NT, Fincher EF, Marinkovich MP (2008) Targeting a tumor-specific laminin domain critical for human carcinogenesis. *Cancer Res* 68 (8):2885-2894. doi:10.1158/0008-5472.Can-07-6160
- Tsukita S, Oishi K, Sato N, Sagara J, Kawai A, Tsukita S (1994) ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. *The Journal of cell biology* 126 (2):391-401. doi:10.1083/jcb.126.2.391
- Tzu J, Marinkovich MP (2008) Bridging structure with function: structural, regulatory, and developmental role of laminins. *Int J Biochem Cell Biol* 40 (2):199-214. doi:10.1016/j.biocel.2007.07.015
- Udani M, Zen Q, Cottman M, Leonard N, Jefferson S, Daymont C, Truskey G, Telen MJ (1998) Basal cell adhesion molecule/lutheran protein. The receptor critical for sickle cell adhesion to laminin. *J Clin Invest* 101 (11):2550-2558. doi:10.1172/JCI1204
- Udayakumar TS, Chen ML, Bair EL, Von Bredow DC, Cress AE, Nagle RB, Bowden GT (2003) Membrane type-1-matrix metalloproteinase expressed by prostate carcinoma cells cleaves human laminin-5 beta3 chain and induces cell migration. *Cancer Res* 63 (9):2292-2299
- Utani A, Momota Y, Endo H, Kasuya Y, Beck K, Suzuki N, Nomizu M, Shinkai H (2003) Laminin alpha 3 LG4 module induces matrix metalloproteinase-1 through mitogen-activated protein kinase signaling. *J Biol Chem* 278 (36):34483-34490. doi:10.1074/jbc.M304827200

- Väänänen A, Srinivas R, Parikka M, Palosaari H, Bartlett JD, Iwata K, Grenman R, Stenman UH, Sorsa T, Salo T (2001) Expression and regulation of MMP-20 in human tongue carcinoma cells. *J Dent Res* 80 (10):1884-1889. doi:10.1177/00220345010800100501
- Vainionpää N, Kikkawa Y, Lounatmaa K, Miner JH, Rousselle P, Virtanen I (2006) Laminin-10 and Lutheran blood group glycoproteins in adhesion of human endothelial cells. *Am J Physiol Cell Physiol* 290 (3):C764-775. doi:10.1152/ajpcell.00285.2005
- Van Goethem E, Guiet R, Balor S, Charrière GM, Poincloux R, Labrousse A, Maridonneau-Parini I, Le Cabec V (2011) Macrophage podosomes go 3D. *Eur J Cell Biol* 90 (2-3):224-236. doi:10.1016/j.ejcb.2010.07.011
- Vivinus-Nebot M, Rousselle P, Breittmayer JP, Cenciarini C, Berrih-Aknin S, Spong S, Nokelainen P, Cottrez F, Marinkovich MP, Bernard A (2004) Mature human thymocytes migrate on laminin-5 with activation of metalloproteinase-14 and cleavage of CD44. *J Immunol* 172 (3):1397-1406. doi:10.4049/jimmunol.172.3.1397
- Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R, Schwede T (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* 46 (W1):W296-w303. doi:10.1093/nar/gky427
- Wilson CL, Matrisian LM (1996) Matrilysin: An epithelial matrix metalloproteinase with potentially novel functions. *The International Journal of Biochemistry & Cell Biology* 28 (2):123-136. doi:[https://doi.org/10.1016/1357-2725\(95\)00121-2](https://doi.org/10.1016/1357-2725(95)00121-2)
- Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z (2020) Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nature Communications* 11 (1):5120. doi:10.1038/s41467-020-18794-x
- Wizemann H, Garbe JH, Friedrich MV, Timpl R, Sasaki T, Hohenester E (2003) Distinct requirements for heparin and alpha-dystroglycan binding revealed by structure-based mutagenesis of the laminin alpha2 LG4-LG5 domain pair. *J Mol Biol* 332 (3):635-642. doi:10.1016/s0022-2836(03)00848-9
- Xu H, Niu M, Yuan X, Wu K, Liu A (2020) CD44 as a tumor biomarker and therapeutic target. *Experimental Hematology & Oncology* 9 (1):36. doi:10.1186/s40164-020-00192-0
- Yaghobi Z, Movassaghpour A, Talebi M, Abdoli Shadbad M, Hajiasgharzadeh K, Pourvahdani S, Baradaran B (2021) The role of CD44 in cancer chemoresistance: A concise review. *European Journal of Pharmacology* 903:174147. doi:<https://doi.org/10.1016/j.ejphar.2021.174147>
- Yamashita H, Beck K, Kitagawa Y (2004) Heparin binds to the laminin alpha4 chain LG4 domain at a site different from that found for other laminins. *J Mol Biol* 335 (5):1145-1149. doi:10.1016/j.jmb.2003.11.047
- Yan HHN, Cheng CY (2006) Laminin α 3 Forms a Complex with β 3 and γ 3 Chains That Serves as the Ligand for α 6 β 1-Integrin at the Apical Ectoplasmic Specialization in Adult Rat Testes*. *Journal of Biological Chemistry* 281 (25):17286-17303. doi:<https://doi.org/10.1074/jbc.M513218200>
- Yan HHN, Mruk DD, Wong EWP, Lee WM, Cheng CY (2008) An autocrine axis in the testis that coordinates spermiation and blood-testis barrier restructuring during spermatogenesis. *Proceedings of the National Academy of Sciences* 105 (26):8950. doi:10.1073/pnas.0711264105

- Yao Y (2017) Laminin: loss-of-function studies. *Cell Mol Life Sci* 74 (6):1095-1115. doi:10.1007/s00018-016-2381-0
- Yap L, Tay HG, Nguyen MTX, Tjin MS, Tryggvason K (2019) Laminins in Cellular Differentiation. *Trends in Cell Biology* 29 (12):987-1000. doi:<https://doi.org/10.1016/j.tcb.2019.10.001>
- Yu Q, Stamenkovic I (1999) Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev* 13 (1):35-48. doi:10.1101/gad.13.1.35
- Yu Q, Stamenkovic I (2000) Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev* 14 (2):163-176
- Yu WH, Woessner JF, Jr., McNeish JD, Stamenkovic I (2002) CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. *Genes Dev* 16 (3):307-323. doi:10.1101/gad.925702
- Yurchenco PD, Cheng YS, Colognato H (1992) Laminin forms an independent network in basement membranes. *J Cell Biol* 117 (5):1119-1133. doi:10.1083/jcb.117.5.1119
- Zöller M (2011) CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? *Nat Rev Cancer* 11 (4):254-267. doi:10.1038/nrc3023
- Zöller M (2015) CD44, Hyaluronan, the Hematopoietic Stem Cell, and Leukemia-Initiating Cells. *Front Immunol* 6:235. doi:10.3389/fimmu.2015.00235

Figure legends

Figure 1

Schematical models of the different laminin chains

LM chains contain tandem arrays of globular and rod-like regions. Chains with identical order of domains are combined (α 1/2, β 1/2/4, γ 1/3). The N-terminal ends of all chains contain tandem arrays of a variable number of epidermal growth factor-like repeats LE with eight cysteine residues in short rod-like domains (LEa–LEc), as well as various globular domains (LN, L4, L4a, L4b, LFa, LFb, LFx), some of which can interact with integrins. Truncated LE domains containing less than 8 cysteines are depicted by half-circles. LE numbering follows the laminin nomenclature ((Ponta et al. 2003; Cauwe et al. 2007; Medrano-González et al. 2021) though frequently, including in data base entries, they are counted consecutively starting at the N-terminus. The α chains have five globular domains (LG1 to LG5) at the C-terminus. LG1–LG3 bind

mostly to integrins, with some requiring the presence of the γ chain C-terminal region. LG4 and LG5 contain binding sites for dystroglycan (α DG), syndecans, and sulfated glycolipids. The LG4–LG5 tandem is thought to be cleaved off extracellularly (arrow) in α 3, α 4, and α 5, though this is not the case for the α 1 and α 2 chains; LG domains of α 2 stay attached to the molecule after cleavage. A small six cysteine residues containing domain L β of yet unknown biological function is present in the coiled coil regions of the β chains; originally the regions N- and C-terminal of L β were denoted by roman numerals II and I, respectively, but as this is unique to the β chains, this has been abolished. The LAMB4 gene coding for the β 4 chain might be a pseudogene as no transcripts has yet been found.

Figure 2

Prototype assembly model of laminins

The α , β , and γ chains assemble to form a coiled coil in at least 18 combinations (**Table 1**). (A) LM-111 is a prototype heterotrimer able to self-assemble and form a network due to the presence of LN domains at the N-terminal ends of its three subunits. (B) LM-332 is a prototype heterotrimer unable to self-assemble as its α and β subunits are truncated. S-S at the termini of the coiled coil regions denote the presence of disulfide bridges. SH indicates the location of free cysteine residues.

Figure 3

CD44 as a platform for MMP binding

(A) Structure of the human CD44 gene, which consists of 10 constant exons expressed in all CD44 mRNAs and proteins (grey boxes) and 9 variant exons v (colored boxes) expressed in CD44 splice variants (CD44v). Other vertebrate genes

contain an additional exon v1. The examples shown are the smallest isoform (standard CD44s, expressed ubiquitously in vertebrates) and the larger variant isoforms expressed in some epithelial cells and cancers. (B) CD44 protein structural domains. The CD44 protein is composed of an extracellular link domain (green), a stem domain (red), where the variant exon products are inserted, the transmembrane region, and the intracellular cytoplasmic domain. The CD44 extracellular domain is a platform for MMP-2, MMP-7, MMP-9, and MMP-14 binding and can indirectly activate MMP-2 through MMP-14 cleavage. Figure adapted from references (Ponta et al. 2003; Cauwe et al. 2007; Medrano-González et al. 2021).

Figure 4

Molecular constraints of CD44 and laminin interactions.

(A) The protein backbone of the HA binding domain of human CD44 is shown on the left with α -helices in red and β -strands in cyan (PDB code: 4PZ4, ref. (Liu and Finzel 2014)). Terminal residues, disulfide bonds and putative acceptor sites for N-linked glycosylation are depicted as ball-and-sticks. Disulfide bonds follow a C1-C6, C2-C5, C3-C6 arrangement. Residues involved in HA binding are presented in CPK style as based on the similarity with the mouse sequence (R41, R78, Y79, Y105) (Banerji et al. 2007) Y42 (green) has been implicated in acting as a molecular switch inducing a conformational change upon interaction of R41 with HA to a higher affinity state (Guvench 2015). A solvent-accessible surface view of the same orientation is shown in the middle, and one obtained by rotation of 180° around the vertical axis on the right. The electrostatic potential is represented by red and blue for negative and positive charges, respectively. The position of various residues is indicated by dashed lines with those involved in HA binding highlighted and connected by blue

lines. (B) Prediction of the intrinsic disorder of full-length human CD44 was performed using the IUpred3 (black line) (Dosztányi 2018) (<https://iupred.elte.hu/>) and NetSurfP-3.0 (red line) (Klausen et al. 2019) (<https://biolib.com/dtu/nsp3>) algorithms. (C) Putative interactions between the CD44 HA binding domain (blue) and LM γ 2 LEa2-LEa3 domains are shown for the protein backbones on the left and the corresponding solvent-accessible surfaces on the right. Residues within hydrogen bonding distance between proteins are represented in CPK style, where the apostrophe denotes CD44 residues (R101-G'152, I146-Y'79, C157-S'109, R170-N'110). Simulations were performed using PatchDock (Schneidman-Duhovny et al. 2005) (<https://bioinfo3d.cs.tau.ac.il/PatchDock/>) and refined with FireDock (Andrusier et al. 2007) (<https://bioinfo3d.cs.tau.ac.il/FireDock/>). LM γ 2 LEa2-LEa3 was modelled with SWISS-MODEL (Waterhouse et al. 2018) (<https://swissmodel.expasy.org/>) using PDB structure 4AQS of the mouse LM β 1 LEa1-LEa4 as a template (Carafoli et al. 2012). (D) The protein backbone (left) and the corresponding solvent-accessible surface (right) of the C-terminal region of human LM 511 is shown (PDB code 5XAU, chains A, B, C) (Takizawa et al. 2017). The LM α 5 sequence R₂₉₇₁LVSYSGLFFLK within the LG2 domain corresponding to the mouse peptide A5G27 (R₂₈₉₂LVSYNGIIFFLK) shown to be involved in CD44 binding (Hibino et al. 2004) extends over one β -strand and is depicted in CPK style with the basic terminal residues highlighted in blue. The surface view indicates that further lysine residues (K3009, K3010, K3025) are arranged along a track that could facilitate interactions with GAG chains. Sequence numbering follows UniProtKB/Swiss-Prot data base entries P16070.3 (CD44), Q13753.2 (LM γ 2) and O15230.8 (LM α 5).

Figure 1

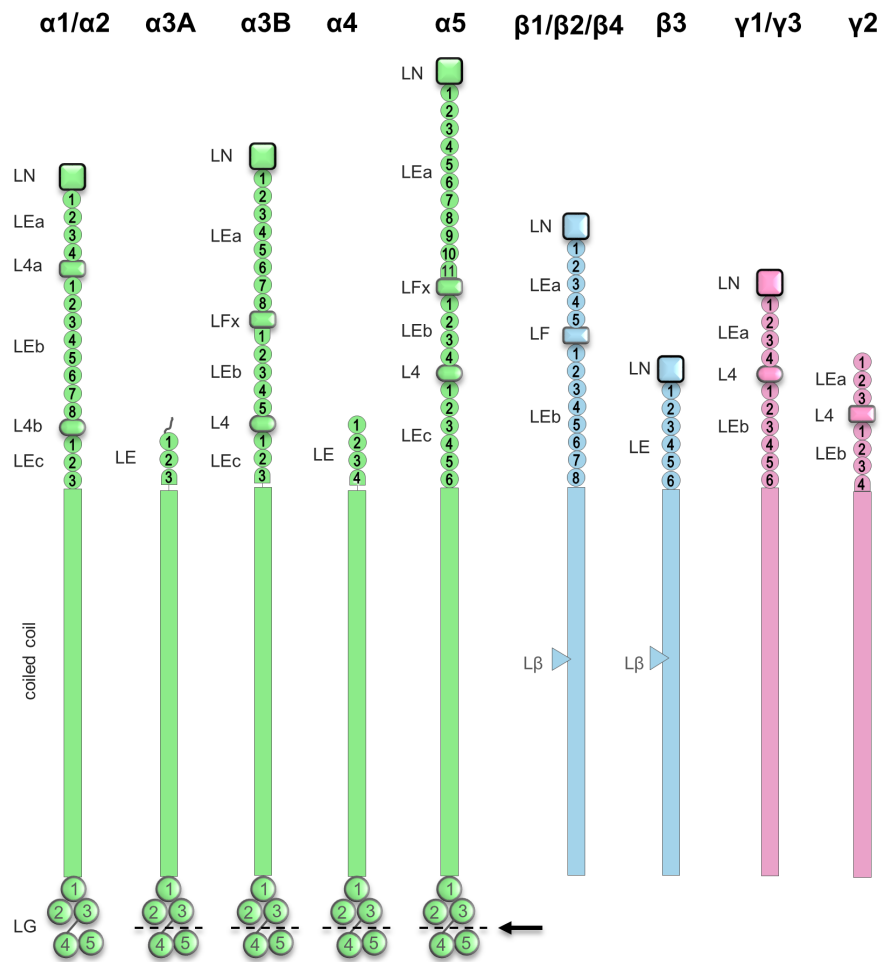


Figure 2

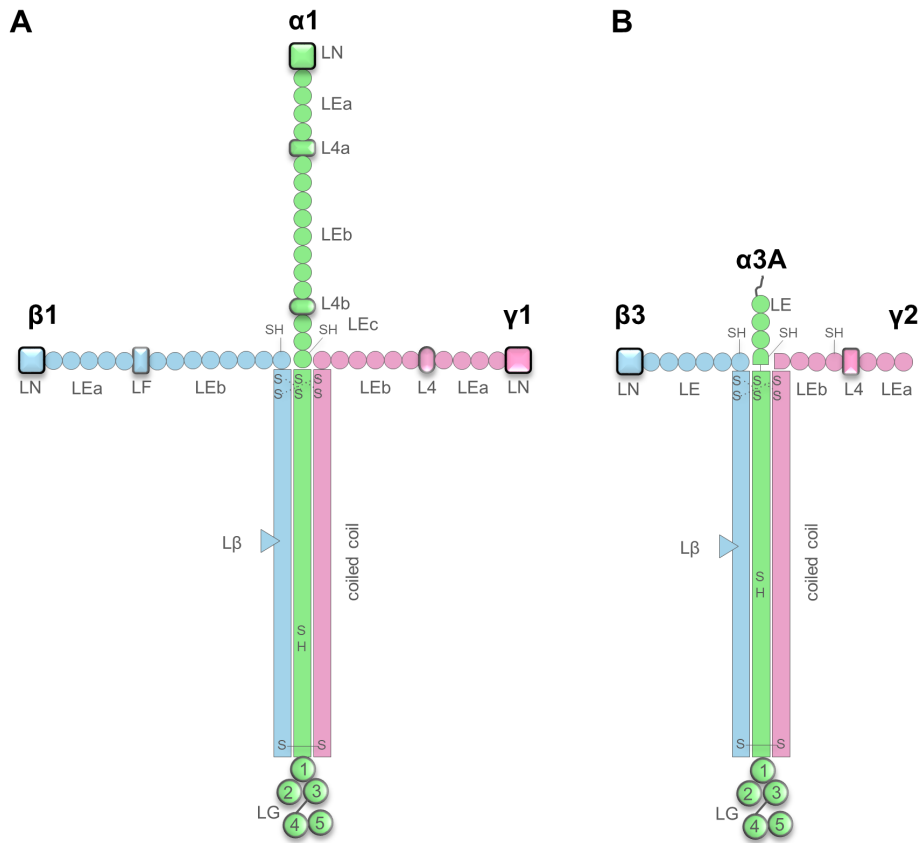


Figure 3

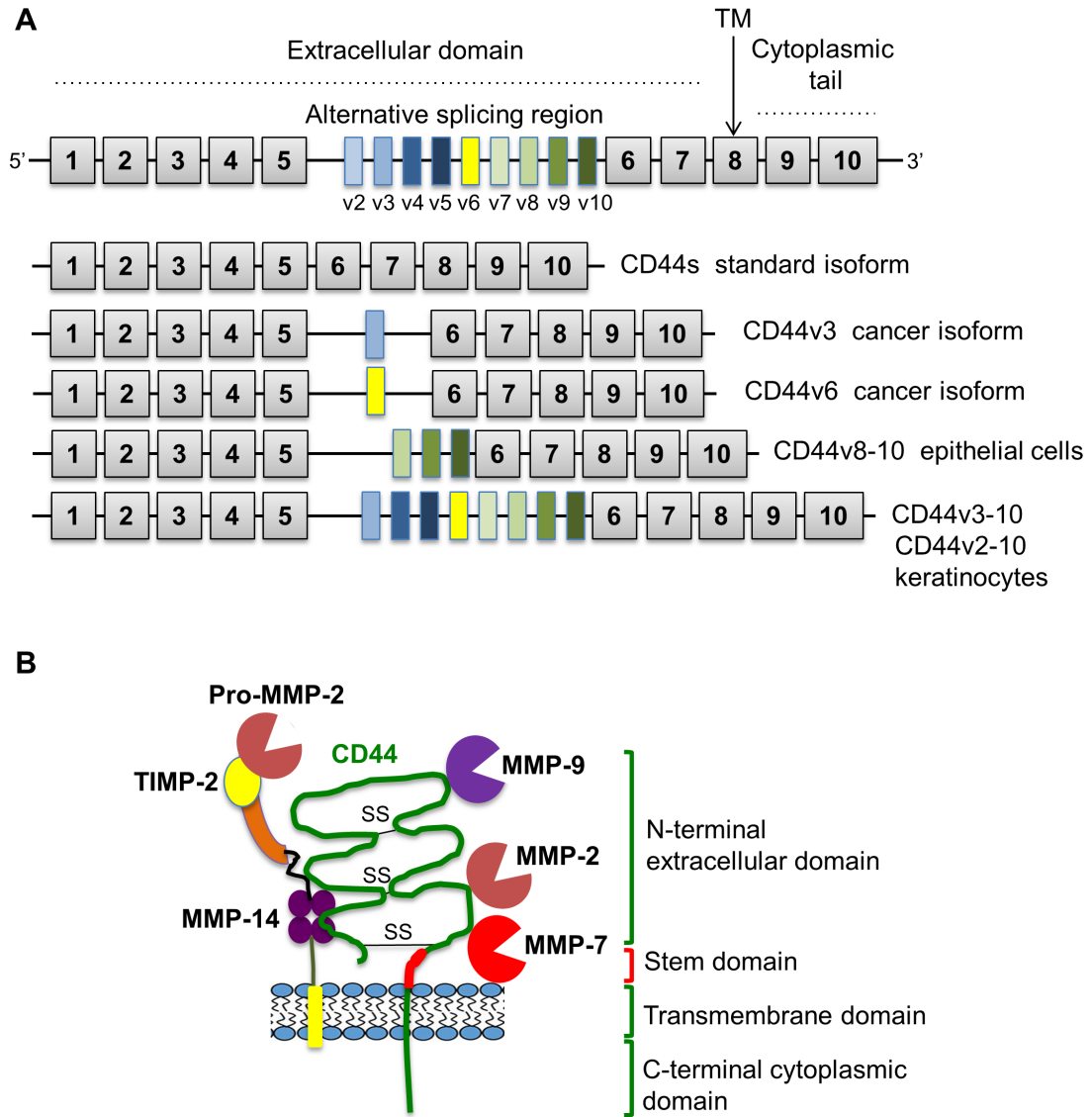


Figure 4

