MICROSCOPY RESEARCH AND TECHNIQUE 51:238–246 (2000)

Proteolytic Modification of Laminins: Functional Consequences

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ABSTRACT The laminin family contains a number of complex, multi-domain proteins that participate in a large variety of biologic processes. Limited proteolysis has been utilized extensively as a tool with which to determine laminin structure/function relationships. In addition, proteolytic modification of laminins may occur as a component of heterotrimer assembly and secretion, or may follow incorporation of mature laminin into the extracellular matrix. Conversely, laminin binding to cellular receptors may also influence proteinase expression. This review will highlight specific examples to demonstrate the functional interplay between laminins and proteinases in the regulation of laminin structure and function as well as in the subsequent control of proteinase expression. *Microsc. Res. Tech. 51:238–246, 2000.* © 2000 Wiley-Liss, Inc.

INTRODUCTION

The laminins are an adhesive glycoprotein family comprised of high molecular weight disulfide bonded heterotrimers with the chain composition of αβγ. To date five α, three β, and two γ chains have been described, forming at least twelve distinct laminin heterotrimers. Although laminin isoforms are distinguished by both subunit composition and tissue distribution, studies employing amino acid sequence comparisons and electron microscopy suggest that the overall domain structure is well conserved. Elucidation of laminin structural domains and determination of structure/function relationships have relied extensively on the technique of limited proteolysis. More recent studies have demonstrated that limited proteolytic modification of laminins can modify heterotrimer assembly and regulate the bioactivity of the secreted, matrix-associated molecule. Conversely, binding of laminins to cellular receptors may influence proteinase expression, suggesting that enzyme levels may be regulated in part by substrate availability. The purpose of this review is to highlight specific examples wherein proteinases have been utilized to study laminin structure or have been shown to alter laminin function. These examples will focus primarily on laminin-1 (α1β1γ1), the first isoform to be discovered and the most extensively studied (Timpl et al., 1979), and laminin-5 (α3β3γ2), a truncated isoform prevalent in epithelial tissues (Verrando et al., 1987; reviewed in Jones et al., 1998).

Rotary shadowing electron microscopy and proteolytic fragmentation studies (summarized below) utilizing laminin-1 purified from the murine EHS tumor have demonstrated that the prototypical laminin-1 is an 820 kDa α1β1γ1 heterotrimer. Both covalent and non-covalent interactions contribute to the basic structure of most laminins, a cruciform with three short arms and one long arm (Sasaki et al., 1988) (Fig. 1A). The short arms represent the amino termini of each individual subunit whereas the long arm is a rod-like coiled coil comprised of the carboxyl termini of all three subunits. The individual laminin subunits share a common structural motif composed of repeating EGF-like domains interrupted by globular domains (Fig. 1A). The amino terminal region of the α1 chain, for example, contains three globular (G) domains separated by EGF repeats making up the short arm, followed by a central domain of about 600 residues in an alpha helical structure, and a unique carboxyl-terminal globular domain (about 1,000 residues) containing five globular sub-domains (G1–G5) of approximately 180–200 amino acids each (Beck et al., 1990; Sasaki et al., 1988). The β1 and γ1 subunits (220 and 200 kDa, respectively) are smaller than the α1 subunit, containing only two globular domains at the amino terminus followed by the central alpha helical rod domain and lacking a carboxyl-terminal globular domain. The long arm of the cross is created by covalent association of the carboxyl-terminal and central domains of the α1, β1 and γ1 chains forming the triple helical coiled-coil structure, whereas the amino termini of all three subunits remain free, resulting in the characteristic cruciform shape of laminin-1 (Beck et al., 1993; Engel, 1993). Three disulfide bonds link all three chains near the center of the cross and another disulfide bond links the β1 and γ1 chains at their carboxy termini at the end of the rod domain (reviewed in Engvall and Wewer, 1996; Malinda and Kleinman, 1996; Timpl and Brown, 1994).

Relative to laminin-1, laminin-5 is a smaller (460 kDa) isoform comprised of three subunits, α3β3γ2 (Marinkovich et al., 1992). All three subunits are truncated at their amino termini, resulting in a Y-shaped, rather than cruciform, structure (Fig. 1B). Nonetheless, the laminin-5 α3 subunit retains the large carboxyl-terminal globular domain. A more detailed description of laminin structural properties can be found elsewhere in this issue. However, in addition to structural distinctions, laminin-1 and laminin-5 differ in tissue

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Received 20 March 2000; accepted in revised form 3 April 2000

This work was supported by NIDCR grant P01 DE12328 (M.S.S.) and pilot funding from NCI/NIDCR grant DE/CA11921 (M.S.S.)

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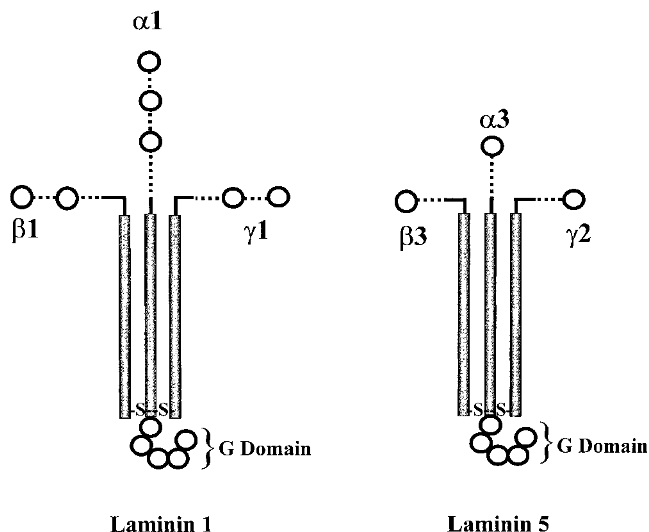

Fig. 1. Schematic representation of laminins 1 and 5. Each laminin heterotrimer is made up of an α, β, and γ subunit. Globular domains are drawn as open circles, and the EGF like repeats are represented by the dotted lines. The helical segment is represented by the shaded reactangles. Laminin 5 is considerably shorter than the laminin 1 due to its truncated α, β, and γ subunits that contain only one globular domain each at the amino terminal end.

distribution. Whereas laminin-1 is expressed ubiquitously in epithelium and endothelium, laminin-5 is found in the basement membrane of specialized squamous epithelia with protective and secretory functions such as skin and mucous membranes (Aberdam et al., 1994; Rouselle et al., 1991; Verrando et al., 1987).

PROTEOLYSIS DURING LAMININ-1 ASSEMBLY AND MATURATION

Although laminin-1 subunits are structurally homologous, they represent unique gene products that assemble intracellularly. Assembly is mediated by interaction of the carboxyl terminal ends of the individual subunits. This region is disulfide bonded in the mature protein; however, disulfide bond formation is not a requirement for assembly but serves to connect and stabilize the three chains (Beck et al., 1993; Engel, 1993). More recent studies with recombinant fragments and peptides demonstrate that the C terminal 100 amino acids of domain I (carboxy terminal portion of the long arm coiled coil structure) contains all the required information for chain selection (allowing formation of only βγ or β β but not α β or γγ dimers) and assembly (Nomizu et al., 1994; Utani et al., 1994; Yurchenco et al., 1997). Formation of the long-arm coiled-coil region is an early event in heterotrimer assembly, and maturation is limited by the rate of incorporation of the α1 subunit. Incompletely assembled β1γ1 dimers are formed initially, but not secreted, followed by incorporation of the α1 subunit into the complex. Although individual α1 subunits may be secreted from the cell, secretion of β and γ chains is dependent on α1 (Yurchenco et al., 1997). Similar data have been reported for laminin-5, wherein formation of β3γ2 heterodimers is followed by the addition of the α3 subunit to complete the assembly of mature laminin 5 α3β3γ2 heterotrimer prior to secretion (Matsui et al., 1995).

In some laminin isoforms, the assembled heterotrimer may be subjected to extracellular proteolytic modification to produce the mature protein. For example, laminin-5 is assembled intracellularly and is secreted as a 460 kDa heterotrimeric precursor. Two of the subunits are subsequently modified to produce an approximately 400 kDa specie. Processing of the α3 subunit from a ~200 to a ~165 kDa form produces a ~440 kDa laminin-5 molecule. Subsequently, a ~400 kDa specie may also be generated as a result of γ2 chain processing from 140 to 80 kDa (Marinkovich et al., 1992; Matsui et al., 1995). In addition to altering laminin structural properties, these limited and distinct processing events may also function as a fine control mechanism for highly localized regulation of cellular behavior (discussed in Alteration of Laminin Function by Limited Proteolysis). Proteolytic processing of the α2 subunit of laminins-2 and -4 has also been reported. A proteolytic cleavage site (RRKRR*QT at residues 2,571–2,576; * designates the cleavage site) at the C terminus of a basic sequence BxBB (where B denotes a basic amino acid) of the recombinant α2 G3 domain has been recognized. This sequence resembles a typical furin-like cleavage site and is unique to the α2 chain. A similar cleavage site is often involved in the conversion of precursor forms of various extracellular proteins including collagen XVI (Steiner et al., 1992; Talts et al., 1998; Tillet et al., 1995). The 80-kDa cleavage product was also observed in tissue-derived laminin-2 and laminin-4, indicating the potential physiological relevance of α2 chain processing. Mutational experiments demonstrate that such modification is essential for α-dystroglycan binding activity of the globular domain of the α2 chain. However, the enzyme responsible for the cleavage is yet to be identified (Talts et al., 1998; Talts and Timpl, 1999).

LIMITED PROTEOLYSIS TO DELINEATE STRUCTURE/ACTIVITY RELATIONSHIPS

A combined approach using limited proteolysis and antibody mapping has proven to be useful for the study of laminin structure. In addition, isolation of proteolytic fragments obtained through controlled enzymatic digestion of laminin has been widely utilized to elucidate the influence of individual laminin domains (or groups of domains) on cellular behavior. This approach is based upon the assumption that compact protein domains are resistant to proteolysis, whereas the more flexible inter-domain boundaries are susceptible to cleavage. It should be noted that the enzymes used in these studies are unlikely to play a physiologic role in laminin processing or modification, but rather represent biochemical tools for cleavage of a complex, multidomain protein into smaller functional units. Two such examples are provided below, in which studies using the serine proteinase elastase and the aspartic proteinase pepsin are summarized.

Limited Elastase Digestion

Limited elastolysis of laminin-1 generates three major fragments E3 (~50 kDa), E8 (~280 kDa), and E9 (~80 kDa), as well as a number of small fragments (Paulsson et al., 1985) (Fig. 2A). Detailed studies based

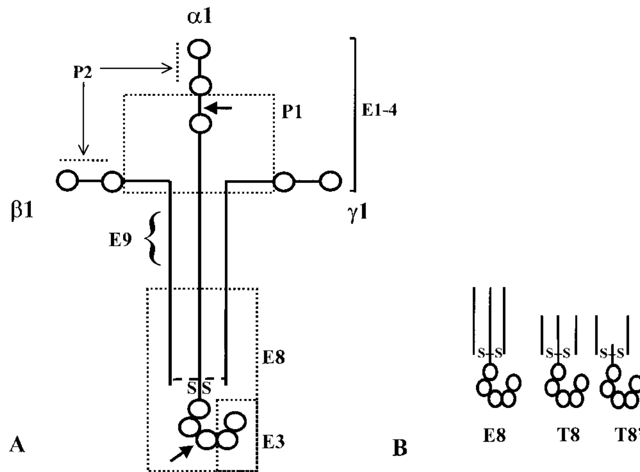

Fig. 2. Schematic diagram showing the various proteolytic fragments of laminin 1. Laminin 1 can be cleaved by limited elastase or pepsin digestion to give rise to a number of biologically active fragments. **A:** The letter E designates the elastase derived fragments while the letter P designates the pepsin derived fragments. The black arrows indicate the RGD sequences in the P1 and E8 fragments. **B:** Further tryptase digestion of the elastase derived E8 fragment gives rise to T8 and T8′.

on amino terminal sequence analysis, electron microscopy, and antibody mapping localized both the E8 and E9 fragments to the long arm of laminin-1, indicating that these elastase products contain portions of all three subunits. In contrast, E3 is comprised of the carboxy terminal fragment of the α1 chain containing the fourth and fifth globular subdomain of the α1 G domain (Fig. 2A).

Laminin-1 has been shown to influence diverse biological processes including cellular adhesion, proliferation, morphology, differentiation, and migration and can participate in extracellular matrix assembly (reviewed in Kleinman et al., 1985). In many cases, the sequences or structural domains that mediate many of these complex biologic effects have been further defined following isolation and characterization of laminin-1 proteinase digestion products. For example, the E3 subdomain has heparin binding activity similar to intact laminin-1, and may thus contribute to interaction of laminin-1 with heparin-containing proteoglycans in the extracellular matrix or on the cell surface (Ott et al., 1982; Yurchenco et al., 1990, 1993). Cellular adhesion to fragment E3 is likely mediated by non-integrin cell surface receptors such as alpha dystroglycan (Gee et al., 1994; Sonnenberg et al., 1990; Yurchenco et al., 1990). Laminin dependent survival and outgrowth of cultured chick sympathetic neurons is effectively blocked with anti-E3 antibodies, indicating that the E3 heparin binding domain mediates biological effects attributed to intact laminin (Edgar et al., 1984). Additional experiments utilizing purified E3 demonstrated that the activity responsible for the laminin-induced change in mammary epithelial cell shape was contained within the E3 segment (completely blocked by purified E3 segment). In contrast, transcriptional activation of the beta casein gene by laminin was only partially dependent on this fragment (Muschler et al., 1999), as purified E3 segment was not sufficient to mimic the regulatory effects of intact laminin (Streuli et al., 1995). Similar to E3, a recombinant α1G domain can also promote heparin binding as well as myoblast attachment and spreading (Yurchenco et al., 1993). Recently, the use of recombinant DNA technology has enabled precise mapping of the heparin and dystroglycan binding domain to amino acid residues 2,766–2,770, 2,791–2,793, and 2,819–2,820 of the fourth globular subdomain contained within the E3 fragment (Andac et al., 1999).

Many diverse functional effects have also been attributed to the E8 fragment (Fig. 2A). This region of laminin-1 has been shown to promote cellular attachment, spreading, and locomotion. The E8 fragment can mediate cellular attachment via the α3β1, α6β1, α6β4, and α7β1 integrins on the cell surface, making it the major cell binding domain of laminin (reviewed in Ziober et al., 1996). Additional studies demonstrated that the E8 fragment can effectively substitute for intact laminin to regulate establishment of cellular polarity in developing kidney cells via α6 containing integrins (Klein et al., 1988; Sorokin et al., 1990) and to promote myoblast attachment and locomotion in a β1 integrin-dependent manner (Goodman et al., 1989; von der Mark et al., 1991). Both intact laminin-1 and the E8 fragment also enhance the proliferation and differentiation of neuroepithelial cells (Drago et al., 1991) and promote neurite outgrowth (Begovac et al., 1991), while the short arm-containing fragments E1-4 are ineffective (Fig. 2A).

Tryptase digestion of E8 has been utilized to further delineate biologically active regions in this relatively large fragment. Two major tryptase cleavage products, designated T8 and T8′, result from the removal of approximately 450 residues from the N terminus (Fig. 2A) (Deutzmann et al., 1990). Although over half of the amino-terminal rod-like region of E8 is removed, many biologic activities are retained in the T8 product including the ability to influence cellular adhesion and spreading. However, the neurite outgrowth promoting activity of E8 is significantly reduced in the T8 subfragment, suggesting that necessary sequence or structural information is removed by tryptase (Deutzmann et al., 1990).

Limited Pepsin Digestion

Pepsin digestion of laminin-1 gives rise to two major fragments designated P1 (290 kDa) and P2 (45 kDa) and a mixture of smaller fragments (Rohde et al., 1980) (Fig. 2B). P1 and P2 together make up more than one third of the intact laminin molecule. The P1 fragment was originally visualized as three rods joined together at one end whereas the P2 fragment was seen as a 18-nm-long rod, arising from the end portions of the short arms (Engel et al., 1981; Ott et al., 1982) (Fig. 2B). The P1 fragment is comprised of the central cross-like portion derived from segments of all three subunits and contains multiple EGF-like repeats. P1 has mitogenic effects on cells and a number of recent reports have found the P1 fragment to be associated with various pathological conditions that involve basement membrane destruction. For example, serum or urinary P1 fragment has been used as a potential diagnostic and prognostic marker for transitional cell carcinoma

of the bladder (Mungan et al., 1996), renal thrombotic microangiopathy (Segarra et al., 2000), and diabetes (Nakajima et al., 1991). The specific function of the P2 fragment has not been extensively characterized.

Many studies have attempted to identify specific sequences that mediate cellular attachment to laminin, and evidence suggests that adhesion can be mediated by multiple sites on the molecule using distinct receptors. For example, the P1 fragment contains an RGD attachment sequence localized within the α1 subunit, but this epitope is not active in the intact molecule (Fig. 2B). Interestingly, this cryptic adhesion sequence can be unmasked by limited proteolysis (Aumailley et al., 1990a). The P1 RGD site has been reported to interact with the integrin αvβ3, but is unaffected by α6 or β1 function blocking antibodies (Aumailley et al.,1990a; Sonnenberg et al, 1990). A second RGD sequence has been localized in the E8 fragment (Fig. 2A), but evidence suggests that this RGD site is not active in adhesion (Aumailey et al., 1990a). Instead, RGD-independent adhesion of E8 to the α6β1 integrin has been reported, supporting the existence of multiple cell-matrix attachment sites in the molecule (Aumailey et al., 1990b). The ability of E8 to mediate establishment of cell polarity in developing kidney epithelium was shown to be α6 integrin-dependent (Sorokin et al., 1990). However, the E8-mediated promotion of neurite outgrowth was unaffected by α6 integrin function blocking antibodies, suggesting the potential for alternative active sequences within the E8 fragment (Deutzman et al., 1990). An additional low affinity, integrin-independent binding of cells to the E3 fragment has also been demonstrated and is likely mediated by interaction with cell-surface proteoglycans (Sonnenberg et al., 1990).

Bioactive Laminin Peptides

As described above, the use of limited proteolysis to generate laminin fragments for purification and characterization is a common strategy to elucidate structure/function relationships. The advantage of this approach is that functional regions identified within properly folded, three-dimensional structural domains can contain amino acid sequences from more than one laminin subunit or from more than one segment of the protein primary structure. An alternative strategy is to evaluate functional information contained within linear epitopes. This may be accomplished by purification of very low molecular weight polypeptide proteolysis products; however, this approach is cumbersome and often yields only small quantities of polypeptides. More commonly, functional domains identified using limited proteolysis are investigated further by generating a series of overlapping short (5–20 amino acids) synthetic peptides corresponding to the primary sequence of the laminin subunit(s) contained within the fragment. In recent years, a large number of studies have utilized this approach to identify specific sequences within the laminin primary structure that regulate distinct biologic processes. It should be noted, however, that the majority of these studies utilized synthetic peptides to mimic activities present in intact laminins. While it is tempting to speculate that these active sequences may also be released from laminin heterotrimers in vivo by proteinases prevalent in the microenvironment, data in direct support of this hypothesis are limited.

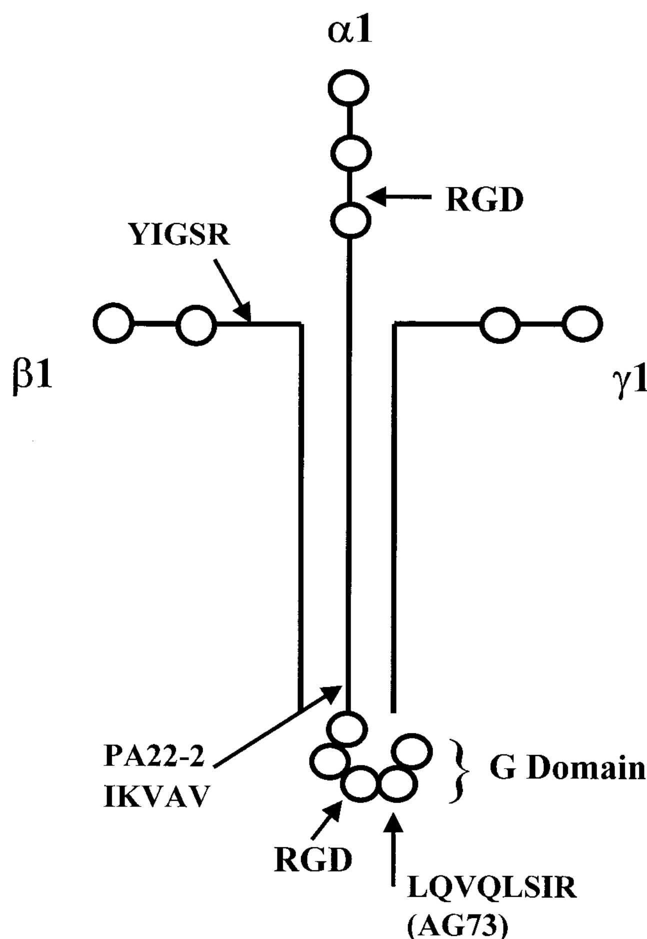

Fig. 3. Location of various bioactive peptides on laminin 1 molecule.

Laminin-1 has been studied extensively for its ability to influence cellular behavior and active sequences have been identified in both the α1 and β1 subunits. For example, a 19-mer peptide designated PA22-2 (CSRARKQAASIKVAVSADR-NH_2; amino acids 2,091–2,108) localized immediately adjacent to the globular domain at the carboxyl-terminus of the α1 subunit (Fig. 3) was shown to promote adhesion, spreading, and migration of B16-F10 melanoma cells and to enhance neurite outgrowth. Subsequent studies localized the cell adhesion and neurite outgrowth promoting activity to the pentapeptide IKVAV (Tashiro et al., 1989; Yamada and Kleinman, 1992). This sequence was demonstrated to be unique within the laminin molecule and highly conserved among laminin α subunits. Peptide PA22-2 stimulated the invasive and metastatic activity of murine B16F10 melanoma cells, resulting in enhanced lung colonization (Kanemoto et al., 1990; Royce et al., 1992). Treatment of melanoma cells with PA22-2 stimulated type IV collagenase activity (Kanemoto et al., 1990) and increased the activation of plasminogen by tissue-type plasminogen activator

(tPA) (Stack et al., 1993), suggesting that the mechanism for the pro-invasive effect likely involves promotion of tumor cell proteolytic activity. This is supported by studies showing binding of both tPA and plasminogen to the carboxyl-terminal region of the laminin-1 α1 subunit (Moser et al., 1993) and kinetic data demonstrating that the PA22-2 peptide enhanced the catalytic efficiency of tPA-catalyzed activation of plasminogen to plasmin (Stack and Pizzo, 1994). The α1-derived peptide was found to promote plasminogen activation by inducing a conformational change in the zymogen that increases its efficacy as a tPA substrate. Detailed structure/activity studies in which one or more charged amino acids were substituted with Asn and/or Ala demonstrated that all charged (positive or negative) residues are required for the stimulatory effect of PA22-2 (Stack and Pizzo, 1994). In addition to PA22-2, another α1-derived peptide designated AG73 (RKRLQVQLSIRT; Fig. 3) has also been shown to stimulate both neurite outgrowth (Richard et al., 1996) and proteinase secretion from PC12 cells (Weeks et al., 1998). Similar to PA22-2, this peptide also promotes tumor adhesion, migration, and invasion in vitro and lung colonization of B16F10 melanoma cells in vivo. The minimal active sequence has been localized to an octapeptide (LQVQLSIR) (Kim et al., 1998; Weeks et al., 1998). Similar sequences (RKRLQVQLSIRT and KNRLTIELEVRT) derived from the globular domains of α1 or α2 chains have also been shown to support neuronal cell attachment and neurite outgrowth (Richard et al., 1996).

The β1 subunit also contains active sequences, the most well-characterized of which is the nonapeptide CDPGYIGSR (amino acids 925–933; Fig. 3). This sequence, like that of PA22-2, is unique to laminin. However, in contrast to the pro-metastatic activities of PA22-2, the β1 chain peptide has anti-metastatic activity partly due to its anti-angiogenic effect on tumors (reviewed in Grant et al., 1994; Kleinman et al., 1993; Yamamura et al., 1993). The active sequence YIGSR binds to the 67-kDa laminin receptor (Graf et al., 1987a,b), blocks lung colonization by murine B16F10 melanoma cells (Iwamoto et al., 1987; Kleinman et al., 1989), and inhibits both growth and metastasis of several types of tumors including HT1080 fibrosarcoma, leukemia, and melanoma (Fridman et al., 1990; Iwamoto et al., 1996; Yoshida et al., 1999). Additional activities attributed to YIGSR include induction of apoptosis in HT1080 cells (Kim et al., 1994), differentiation of sertoli cells (Hadley et al., 1990; Papadopoulos and Dym 1994), and migration of neural crest cells (Bilozur and Hay, 1988). A recent report indicates that YIGSR can initiate signal transduction, resulting in tyrosine phosphorylation of a number of intracellular proteins, suggesting a potential biochemical mechanism for these diverse biologic effects (Bushkin-Harav and Littauer, 1998).

ALTERATION OF LAMININ FUNCTION BY LIMITED PROTEOLYSIS

Limited proteolytic modification of extracellular matrix macromolecules such as laminin can modify cellular recognition sites, thereby disrupting downstream signaling events and modulating the behavior of cells in contact with the laminin substratum. Altering the nature of cellular contact with laminin has been shown to have diverse functional consequences. The two examples provided below, demonstrating that laminin cleavage regulates neural cell death and epithelial cell motility, suggest that limited proteolytic modification of laminin in vivo may function as a fine regulatory mechanism for highly localized control of cellular behavior.

Laminin Proteolysis and Neuronal Cell Death

Experimental administration of excitotoxins can induce neurodegeneration in the murine hippocampus and co-administration of proteinase inhibitors provides a protective effect against neuronal cell death (Tsirka et al., 1996). Mice genetically deficient for either plasminogen or tPA expression ($Plg^{-/-}$ or $tPA^{-/-}$ mice) are resistant to excitotoxin-induced neurodegeneration, suggesting that tPA-catalyzed plasminogen activation may play a role in the death of hippocampal neurons (Tsirka et al, 1997a,b). Examination of extracellular matrix proteins present in the hippocampus demonstrated a loss of laminin expression following excitotoxin injection. Disappearance of laminin staining is temporally and spatially correlated with cell death and hippocampal laminin expression is maintained in $tPA^{-/-}$ mice or in animals treated with a plasmin inhibitor (Chen and Strickland, 1997). Together these data suggest that tPA/plasmin-mediated laminin degradation results in disruption of neuron-laminin interactions, thereby sensitizing hippocampal neurons to cell death. These in vivo results are supported by in vitro studies demonstrating that proteolytic processing of laminin by plasmin impairs long-term potentiation (LTP) in organotypic hippocampal cultures. Modification of cellular interaction with proteolyzed laminin mimics changes in hippocampal LTP observed during development or in various pathological conditions, suggesting that proteolytic modification of laminin may contribute to the control of synaptic plasticity (Nakagami et al., 2000).

Laminin-5 Proteolysis and Epithelial Cell Migration

The laminin-5 isoform (α3β3γ2) induces the formation of hemidesmosomes, which promote stable cell: matrix adhesion (Jones et al., 1998; Langhofer et al., 1994). However, laminin-5 has also been identified at the invasive front of tumor specimens where cells are actively migrating, suggesting that the protein also mediates motility (Galliano et al., 1995; Pyke et al., 1994; Ryan et al., 1994). Recent studies provide an explanation for these apparently contradictory functions of laminin-5 in promoting both adhesion and migration, and demonstrate that limited proteolytic modification of specific laminin-5 subunits alters the protein structure and thereby modulates its function. Intact human laminin-5 contains a 190 kDa α3 subunit and promotes cell motility. However, binding of both plasminogen and tPA to laminin-5 induces plasmin formation and subsequent proteolytic modification of the laminin-5 α3 G domain to a 160-kDa specie (Goldfinger et al., 1998) (Fig. 4). Efficient laminin-5 α3 G domain processing is observed at low plasmin:laminin-5 ratios (1:20), demonstrating that this cleavage occurs at physiologically relevant enzyme:substrate ra-

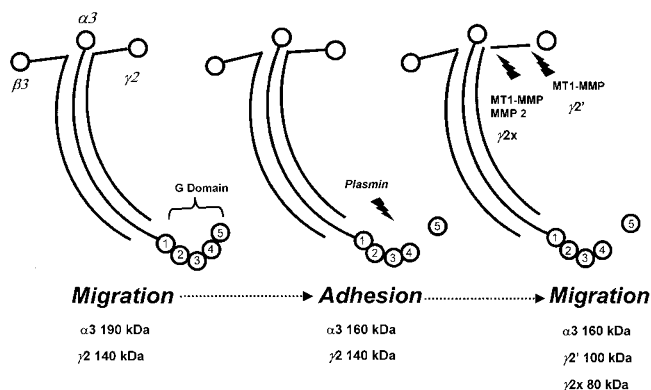

Fig. 4. Limited proteolysis of laminin 5. Laminin 5 secreted with an intact α3 G domain is present at the invasive front of tumors and promotes migration. Plasmin mediated cleavage forms the truncated α3 chain. Plasmin cleavage within the G4-G5 subdomain boundary results in a modified laminin 5 that now nucleates hemidesmosome formation, resulting in stable cell:matrix adhesion. Proteolytic cleavage of laminin 5 γ2 chain by MMP2 or MT1-MMP modifies the molecule further, rendering it promigratory.

tios. As a functional consequence of α3 G domain cleavage, cellular behavior is altered. Cells in contact with plasmin-modified laminin-5 exhibit a 3-fold decrease in motility and an 11-fold increase in hemidesmosome number, suggesting that limited plasmin cleavage induces stable cell-matrix contacts that prevent motility (Goldfinger et al., 1998) (Fig. 4). As discussed below (in Does Laminin Regulate Proteinase Expression), laminin-5-induced aggregation of α3β1 integrin on epithelial cells also upregulates urinary-type PA (uPA) expression and plasmin formation, resulting in proteolysis of laminin-5 present in the subcellular matrix, providing an additional mechanism for modulation of cell motility (Ghosh et al., 2000). Based on these data, it is interesting to speculate that migratory cells deposit intact laminin-5 into the matrix for proteolytic processing to an adhesive substratum. In support of this hypothesis, immunohistochemical examination of healing wounds demonstrates cross-reactivity with antibodies specific for full-length α3 subunit only at the migratory edge of the wound (Goldfinger et al., 1999). The plasmin-modified form of laminin-5 is susceptible to additional cleavages within the γ2 chain catalyzed by matrix metalloproteinase (MMP)-2 or membrane-type 1 MMP (MT1-MMP) (Giannelli et al., 1997; Koshikwa et al., 2000). The γ2 subunit is processed from a 140-kDa specie to 100 and 80 kDa forms, designated γ2′ and γ2x, respectively (Marinkovich et al., 1992; Giannelli et al., 1997) (Fig. 4). Although these cleavages were observed at non-physiologic enzyme:substrate ratios, MMP-mediated laminin-5 processing has been hypothesized to expose a cryptic epitope that promotes motility. This is supported by data demonstrating that induction of motility could be blocked using MMP inhibitors or by treatment of cells with MT1-MMP-specific antisense oligonucleotides (Koshikawa et al., 2000). Together these data suggest that multiple proteinases may modulate cellular behavior by modifying matrix-associated laminin-5 to support specific physiological functions.

DOES LAMININ REGULATE PROTEINASE EXPRESSION?

As detailed above, recent data demonstrate that proteolytic modification of specific laminin isoforms may provide for highly localized regulation of cellular behavior. Laminins associate with cells through a variety of mechanisms including binding to specific integrins such as α3β1, α6β1, and α6β4 (Ziober et al., 1996), as well as to non-integrin receptors including the 67-kDa laminin receptor (Graf et al., 1987a). Proteolysis may alter receptor recognition sites or expose cryptic epitopes with novel biologic functions. Furthermore, there is emerging evidence in numerous cell types that ligand engagement of integrins or other cellular receptors may regulate proteinase expression, suggesting the hypothesis that matrix binding may regulate matrix structure and subsequently modify cell behavior.

Early studies on laminin regulation of proteinase expression demonstrated that both intact laminin-1 and the P1 fragment enhanced type IV collagenase activity in melanoma and fibrosarcoma cells (Turpeenniemi-Hujanen et al., 1986). Metalloproteinase induction was blocked by an antibody directed against the 67-kDa laminin receptor, implicating the YIGSR sequence in proteinase regulation. Peptides containing the IKVAV sequence were also shown to alter type IV collagenase production in a variety of cell types including melanoma cells, macrophages, and neuronal PC12 cells (Corcoran et al. 1995; Royce et al., 1992; Weeks et al, 1998). Treatment of macrophages with IKVAV-containing peptides resulted in activation of the mitogen-activated protein kinase (MAPK) pathway via protein kinase C, suggesting a mechanism for altered proteinase expression (Khan and Falcone, 2000). Laminin-induced activation of phospholipase D and the subsequent generation of lyosphosphatidic acid was also shown to transduce signals resulting in upregulation of gelatinase A (MMP-2) expression in ras transformed 3T3 fibroblasts, suggesting multiple mechanisms for laminin-mediated control of metalloproteinase expression (Reich et al., 1995).

In addition to metalloproteinases, expression of the plasminogen activators (PAs) urinary-type PA (uPA) and tissue-type PA (tPA) has also been shown to be regulated by laminin-1. PAs function to convert the plasma zymogen plasminogen to the active, broad-spectrum proteinase plasmin, thereby mobilizing a large reservoir of potential matrix-degrading proteolytic activity. For example, incubation of metastatic murine B16F10 melanoma cells with the laminin-1 α1 chain fragment (LamA $^{2091\text{-}2108}$, PA22-2) induced the expression of tPA, resulting in a 10-fold increase in tPA activity in conditioned media and a significant increase in tPA-catalyzed plasminogen-dependent hydrolysis of laminin-1 (Stack et al., 1993). In addition to regulating tPA expression, the LamA $^{2091\text{-}2108}$ peptide stimulated plasminogen activation by inducing a conformational change in plasminogen, which increases its efficacy as a tPA substrate (Stack et al., 1991; Stack and Pizzo, 1994), suggesting that laminin-derived peptides may also participate in post-translational control of proteinase activity. Similarly, interaction of MCF-7 human breast carcinoma cells with laminin-1, mediated via the α2β1 integrin, had multiple effects on proteinase

regulation. Whereas tPA activity was increased by 5-fold, a 2-fold decrease in uPA activity was observed (Sonohara et al., 1998). Concomitant treatment with estradiol and laminin-1 had a synergistic effect on tPA expression, resulting in a 15-fold enhancement of activity.

A potential functional link between integrin-mediated adhesion and induction of proteolysis was demonstrated in premalignant gingival keratinocytes. In this study, laminin-5-induced clustering of the $\alpha3\beta1$ integrin upregulated the expression of uPA (Ghosh et al., 2000). Although expression of the cellular uPA receptor (uPAR) was unaffected, a dramatic re-distribution of uPAR to sites of clustered $\alpha3\beta1$ integrins was observed. These data suggest that uPA/uPAR/$\alpha3\beta1$/laminin-5 interactions may function to "focalize" uPA proteolytic activity to sites of cell-matrix contact. Aggregation of $\alpha3\beta1$ integrin induced uPA expression via signal transduction through a MEK/ERK-dependent pathway. This finding is supported by studies showing that ERK phosphorylation leads to uPA promoter activation (Lengyel et al., 1996) suggesting that $\alpha3\beta1$ integrin aggregation results in ERK phosphorylation and the subsequent activation of transcription factors that control uPA expression.

Together these data suggest that laminin ligation of cellular receptors can initiate signal transduction pathways that alter proteinase expression. The enzymes thus induced may then participate in limited proteolytic modification of laminins present in the pericellular matrix. It is interesting to speculate that proteolytic release of cryptic peptide epitopes, with functions biologically distinct from the parental laminin molecule, may ensue. Furthermore, proteolytic removal of specific functional domains may have dramatic effects on cellular interactions that control adhesion and motility. Exciting future directions in laminin research will likely involve additional studies to examine specific components of sub-cellular signaling pathways induced by intact laminin or its proteolytic fragments and to evaluate the mechanisms by which proteinase genes are transcriptionally activated by laminin binding. Further research into post-translational control of proteinase activity and elucidation of the biochemical details governing protein:protein interactions between individual proteinase:laminin pairs will also be informative. Such studies will undoubtedly provide a more detailed understanding of cellular mechanisms by which extracellular matrix proteins such as laminin may control their own structure and subsequent biological function via complex, multistep regulatory pathways.

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