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Defining the role of laminin-332 in carcinoma

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Abstract

The deadly feature of cancer, metastasis, requires invasion of cells through basement membranes (BM), which normally act as barriers between tissue compartments. In the case of many epithelially-derived cancers (carcinomas), laminin-332 (Ln-332) is a key component of the BM barrier. This review provides a historical examination of Ln-332 from its discovery through identification of its functions in BM and possible role in carcinomas. Current understanding points to distinct roles for the three Ln-332 subunits (α3, β3, γ2) in cell adhesion, extracellular matrix stability, and cell signaling processes in cancer. Given the large number of studies linking Ln-332 γ2 subunit with cancer prognosis, particular attention is given to the crucial role of this subunit in cancer invasion and to the unanswered questions in this area.

Keywords

Laminin-332; Cancer; Invasion; Prognosis; Extracellular matrix

1. Introduction

1.1. Cancer: the problem defined

The overwhelming complexity of the cancer field comes from the fact that cancer is not so much a specific disease as it is a cluster of many separate but related disorders. Ninety percent of cancer cases fall into the broad classification of carcinomas, or cancers of epithelially-derived cells, which make up the majority of the lining cells of the body. Along with cell-type, the tissue or organ of origin is used to define a specific cancer further multiplying the number of sub-classifications.

At its core, cancer is best described as unregulated cellular growth and invasion; however, the path to loss of growth regulation and the extent of dysregulation of other cellular traits can vary widely, especially with respect to the cell/tissue type(s) involved. This heterogeneity, even when subtle, can have enormous impact on treatment choice and clinical response. Even with such a wide array of individual characteristics, it has been concluded that most, if not all, cancers acquire changes affecting six particular areas, or hallmarks, of cancer: namely these are self-sufficiency in growth signals, sustained angiogenesis, apoptosis evasion, insensitivity

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to anti-growth signals, limitless replicative potential, and the ability for tissue invasion and metastasis (Hanahan and Weinberg, 2000). While increased potential to cripple any of these hallmarks would be a welcome addition to the therapeutic arsenal, it is the ability to colonize beyond the original site that makes a cancer potentially deadly by dramatically restricting means of treatment, such as surgical removal of a primary tumor. Many research efforts focus on understanding the molecular mechanisms underlying the metastatic proclivity of cancer cells. Yet, there is a dearth of suitable molecular targets specific for metastasis treatment.

1.2. Metastasis: distant spread requires local invasion

There is a popular school of thought known as the 'seed and soil hypothesis', which contends that the major determinant of success in growth at a secondary site is a hospitable environment ("soil") that matches the requirements of a metastasizing cell ("seed"). This idea was first articulated by Stephen Paget in his landmark 1889 *Lancet* paper to explain the observation that cancer cells (seeds) from certain organs were more likely to metastasize to particular distant sites (soil)(Paget, 1989). Several decades later, a new theory emerged from James Ewing (Ewing, 1928), who correlated these observations to the circulatory pattern from the original tumor site. In fact, it is now known that both of these theories are partially correct (see Chambers et al., 2002; Couzin, 2003; Fokas et al., 2007 for discussion), yet neither fully account for observed metastatic phenomena.

It is generally thought that the metastatic cascade requires several steps before clinical manifestations (reviewed in Fidler, 2002). Cells must escape from a primary tumor and enter into the blood or lymphatic system, survive in the circulation, extravasate from the vessel at a secondary site, and finally they must find a way to adapt and thrive at this new location (Chambers and Matrisian, 1997; Chambers et al., 2000). At several of these steps, crossing of BM must occur. For example, entry into and exit from the circulatory system absolutely require crossing of vessel BM. Furthermore, in the invasive spread to a neighboring organ, for which entering the circulation may not be an issue, the ability of a cancer cell to cross BM may be the sole obstacle for dissemination in and out of stroma. Thus, a comprehensive understanding of cancer metastasis inherently requires examination of local invasion and BM crossing by cancer cells (Fidler, 2002; Aznavoorian et al., 1993).

1.3. Local invasion: crossing the BM

Local invasion itself is primarily a composite of two main processes: enzymatic degradation of the BM that separates epithelial cells from underlying stroma, followed by migration through a newly cleared pathway (Liotta and Stetler-Stevenson, 1991). These processes can be accompanied by deregulation of normal adhesion between neighboring cells via decrease in cell–cell adhesion molecules such as E-cadherin (Bracke et al., 1996), though it has become increasingly clear that epithelially-derived cancer cells can also migrate collectively as sheets (reviewed in Friedl and Brocker, 2000). The invasion process involves metalloproteinases (MMPs) that break down the BM, the integrin family of cell receptors which function in attachment and migration, plus many additional protein families (Wittekind and Neid, 2005). MMP secretion is accompanied by formation of cell protrusions (pseudopodia, invadopodia, or other membrane extensions) and migration (reviewed in Adams, 2002; Weaver, 2006); in this way, invasive cells can bypass the normal tissue barriers confining them to one area of an organism.

1.4. BM: a barrier to invasion

The BM is a 50- to 100 nm layer of ECM components that separates all epithelial and endothelial cells from underlying stromal cells, and provides both structural support and vital signaling cues from the microenvironment (reviewed in Yurchenco and Schittny, 1990; Iozzo,

1998; LeBleu et al., 2007). BMs are vital for structural tissue integrity and can be found essentially in every organ of the body.

ECM molecules that make up the BM are secreted by surrounding stromal cells (e.g. epithelial cells or fibroblasts) and remodeled by the cells themselves to form sheets, upon which epithelial and endothelial cells rest. This serves as a physical barrier as well as a signal regulator by sequestering growth factors, chemokines, and other signaling factors, which are released upon matrix degradation (reviewed in Schenk and Quaranta, 2003).

ECM components are classified broadly as either proteoglycans or non-proteoglycans. Heparin-, chondroitin-, and keratan- sulfates make up the proteoglycans that have growth factor mimetic domains shown to stimulate fibroblast and chondrocyte proliferation; these molecules consist of a fibrous protein with long chains of unbranched polysaccharides called glycosaminoglycans (reviewed in Iozzo, 1998; Lander and Selleck, 2000).

BM non-proteoglycans come in many forms and have a wide variety of functions. Collagens are by far the most abundant and provide the bulk of structural support (reviewed in Hulmes, 1992). Fibronectins connect cells with collagens and other ECM components via cell-surface integrin receptors (reviewed in Teti, 1992). Laminins are glycoproteins that modulate adhesion and signaling through integrin binding; additionally, they adhere to other ECM molecules, including other laminins, to form a network that strengthens the membrane by resisting tensile force (reviewed in Aumailley and Smyth, 1998).

1.5. Laminins: BM structural molecules

Nearly 30 years ago, stroma from the Engelbreth–Holm–Swarm (EHS[1]) tumor was purified and found to contain large amounts of a novel non-collagenous glycoprotein the researchers termed laminin (Timpl et al., 1979). Now known as Ln-111, this molecule has previously been referred to as EHS laminin, Ln-1, or simply laminin in older literature (Aumailley et al., 2005). Since Ln-111, 15 other distinct laminins have been found in mammals (Aumailley et al., 2005). All members of the laminin family are heterotrimers of an α, β and γ chain, encoded in humans by 5α, 4β, and 3γ genes (Miner and Yurchenco, 2004). Structural similarity among all laminins has been proposed, based on sequence data and theoretical heterotrimer stability (Beck et al., 1993). This configuration was shown to contain the three chains wound together to form a triple helical coiled-coil domain stabilized by disulfide bonds, termed the long-arm. Portions of the β and γ chains, as well as a portion of α extending from the long arm make up three short arms, completing a cruciform structure (Beck et al., 1990).

The key structural role of laminins in BM is underscored by distinct phenotypes in laminin subunit null mice, including those lacking α2 (Kuang et al., 1998; Miyagoe et al., 1997), α3 (Ryan et al., 1999), α5 (Miner et al., 1998), β2 (Noakes et al., 1995), or γ1 (Smyth et al., 1999) laminin chains that have a lethal phenotype. Even though many details are still being elucidated, the general trend is that laminins are crucially involved in tissue organization, including the specification of key epithelial traits like cell polarity or protection from detachment-induced cell death (anoikis) (Miner and Yurchenco, 2004; Li et al., 2003). Cancer invasion can be seen as a failure to maintain tissue organization, making laminins important molecules to consider in cancer.

[1]The abbreviations used include: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EHS, Engelbreth–Holm–Swarm; EMT, epithelial to mesenchymal transition; HPV, human papillomavirus; IHC, immunohistochemistry; ISH, *in situ* hybridization; JEB, junctional epidermolysis bullosa; MS-PCR, methylation-specific PCR; MMP, metalloproteinase; PI3K, phosphoinositide 3-kinase; RT-PCR, reverse-transcription PCR.

Different cell types express different laminin types. Ln-332 is unique to and widespread among epithelia. Therefore, it is of high relevance for studies of carcinoma invasion.

1.6. Ln-332: the laminin formerly known as Ln-5

For the sake of clarity, especially in tracing early literature on Ln-332 and cancer, it is important to briefly recap the history of Ln-332 discovery and the evolution of its nomenclature. Ln-332 was referred to in the earlier literature as BM-600/nicein (Verrando et al., 1987; Verrando et al., 1988), GB3 antigen (Verrando et al., 1988), epiligrin (Carter et al., 1991), kalinin (Rousselle et al., 1991), and ladsin (Miyazaki et al., 1993); originally, these were all thought to be separate molecules. The common identity of some of these molecules was first suggested in the early 1990s, when strikingly similar characteristics began to emerge (Watt and Hotchin, 1992). Shortly thereafter, kalinin and nicein were found to share the same tissue distribution (Meneguzzi et al., 1992) and protease cleavage patterns (Marinkovich et al., 1993). Vailly and colleagues found that the 100-and 105-kDa subunits (γ chains) of nicein and kalinin have the same amino acid sequence, by comparing their respective cDNAs (Vailly et al., 1994). They concluded nicein and kalinin heterotrimers were identical and suggested that, based upon similar biochemical, immunological, and cell biology results from another group (Domloge-Hultsch et al., 1992), epiligrin was also the same molecule. Together with few other observations, it became accepted that all of these molecules were in fact one and the same, eventually leading to the Ln-5 unified nomenclature (Burgeson et al., 1994). Two years later, the issue was finally put to rest when Miyazaki's group released a report on the identification of their ladsin molecule as Ln-5 (Mizushima et al., 1996). A more logical and standardized nomenclature system was developed more recently, whereby current and future laminins would be named by their αβγ chain constituency regardless of discovery order (Aumailley et al., 2005), so the molecule formerly known as Ln-5 underwent a name change to Ln-332.

While the inconsistent nomenclature during the infancy of Ln-332 studies caused some confusion, there is no doubt over the identity of this laminin as an ECM protein unique to epithelial cells with crucial roles in cell adhesion, signaling and migration (reviewed in Colognato and Yurchenco, 2000). These heterotrimers consist of an α3, β3, and γ2 chain encoded by the LAMA3 (Ryan et al., 1994), LAMB3 (Gerecke et al., 1994), and LAMC2 genes (Vailly et al., 1994), respectively (Fig. 1). Due to inclusion of the truncated α3 chain, Ln-332 has 3 short arms instead of 2 (Rousselle et al., 1995).

1.7. Ln-332 subunits: human pathology and cancer

Although the emphasis of this review is cancer, the human pathology most commonly associated with Ln-332 is the autosomal recessive disease, junctional epidermolysis bullosa (JEB). Mutations in any of the three Ln-332 chains can result in JEB, which is characterized by severe skin blister formation (Aberdam et al., 1994; Pulkkinen et al., 1994a; Pulkkinen et al., 1994b; Pulkkinenm et al., 1995a; Vidal et al., 1995; Kivirikko et al., 1996). Clinical manifestations of JEB are due to an absence of Ln-332- containing adhesive structures known as hemidesmosomes (Pulkkinen and Uitto, 1999). Hemidesmosomes are vital for cell-matrix adhesion and involve interaction of Ln-332 with α6β4 integrin, collagen-VII, collagen-XVII, and other molecules (Borradori and Sonnenberg, 1999; Nievers et al., 1999). Knockout of α6β4 or collagen-VII in mice leads to similar blistering issues though the exact phenotype varies somewhat (van der Neut et al., 1996; Heinonen et al., 1999). Whether abnormalities in hemidesmosomes are also related to the role of Ln-332 in cancer remains an open issue. In the remainder of this review, we focus on each of the Ln-332 subunits separately, as they have each been reported to have distinct roles in cancer progression.

2. Laminin α3

The α3 chain is encoded by the LAMA3 gene, which has two transcript variants. The truncated LAMA3a variant is expressed and incorporated into heterotrimer (Ryan et al., 1994), while the other variant has thus far only been studied via overexpression of artificially produced cDNA (Kariya et al., 2004). Transcript regulation is achieved by alternative splicing and separate promoter sites (Ferrigno et al., 1997). Aside from Ln-332, α3 is also a part of Ln-311 (formerly Ln-6) and Ln-321 (formerly Ln-7) heterotrimers; all three of these molecules can be found covalently linked to each other in the stroma, strengthening adhesion of the attached epithelial cells (Champliaud et al., 1996). Integrin binding function was predicted for α3 chain once the LAMA3 gene was cloned and discovered to possess an RGD motif (residues 658–660) and an LDV motif (residues 313–315) in the α-helical domain (Ryan et al., 1994), since these sequences had been shown to mediate the binding of fibronectin to its integrin receptors (Pierschbacher and Ruoslahti, 1984; Wayner et al., 1989; Komoriya et al., 1991). However, the RGD motif is not exposed so any integrin binding function may involve other motifs.

The α3 chain is crucial for interaction with α3β1 integrin, which stimulates cell migration, adhesion, and spreading on Ln-332 (Shang et al., 2001). The α3β1 binding site was narrowed down to one of the large globular domains, LG3, using recombinant proteins. Integrin signaling, which also occurs through two other binding partners of Ln-332, α6β4 and α6β1, has critical roles in mediating actin protrusion, migration, and invasion (Mercurio et al., 2001). Recent work from the Marinkovich group (Tran et al., 2008) illustrates the vital role of the α3 chain in cancer by showing that its large globular domains 4–5, which are cleaved from normal skin cells, is highly expressed in carcinoma where it stimulates phosphoinositide 3-kinase (PI3K) and MMP activity, invasion, and tumor growth. Interestingly, these effects are countered *in vivo* by treatment with antibodies specific to this domain.

Interestingly, a region termed Domain IIIa in the α3 sequence contains two epidermal growth factor (EGF)-like repeats. This domain shares 46% sequence homology with the Domain IIIa sequence of Ln-111 and 48% with that of Ln-211 (formerly Ln-2) (Ryan et al. 1994). These EGF-like repeats can be found as part of each laminin chain in varying numbers, pointing to the possibility of nonintegrin-mediated signaling through growth factor receptor transactivation expanding the ability of Ln-332 to modulate cell behavior.

3. Laminin β3

The Ln-332 β3 chain was recently reported to also be a component of Ln-333 heterotrimer expressed in specialized adhesive membrane structures exclusive to germ cells in developing testis (Yan and Cheng, 2006). Like LAMA3, LAMB3 also encodes two transcript variants (Pulkkinen et al., 1995b); however, only the truncated LAMB3A form appears to be translated into protein (Hao et al., 2002). When this variant is expressed in prostate carcinoma cell lines naturally lacking its expression, cell migration and tumorigenicity are enhanced (Calaluce et al., 2004). It has previously been shown that MT1-MMP cleavage of this chain causes cell migration of prostate carcinoma cells *in vitro* (Udayakumar et al., 2003). Recent work has also highlighted the importance of β3 in prostate cancer by showing that hepsin, a protease overexpressed in 90% of human prostate tumors, induces cleavage of β3 chain in prostate cancer cells, leading to increased migration (Tripathi et al., 2008).

Anchoring fibrils, stalk-like structures that run orthogonal to the BM, are composed mainly of type VII collagen (Briggaman and Wheeler, 1975), while structurally similar thread-like projections called anchoring filaments are comprised of mostly Ln-332 (Sakai et al., 1986; Keene et al., 1987). One of the noncollagenous domains of type VII collagen NC1, contains a 285 amino acid subdomain, which binds Ln-332 β3, and to a lesser extent γ2, in a glycosylation-dependent manner (Rousselle et al., 1997; Chen et al., 1999). Chen and colleagues (Chen et

al., 1999) showed that serum from a patient with the autoimmune form of JEB contained NC1 auto-antibodies, which blocked Ln-332 binding, indicating a likely role for collagen-VII/Ln-322 interaction in this disease and underscoring the importance of this binding event in the ECM. The site of this interaction, determined to occur within the EGF-like domain of the β3 short arm by recombinant mutant mapping, is necessary for full adhesion activity of Ln-332, yet dispensable for motility (Nakashima et al., 2005). This interaction between β3 and collagen-VII was found to promote tumorigenesis in squamous cell carcinomas via activation of PI3K signaling, which led to apoptosis protection and increased tumor invasion (Waterman et al., 2007). The signaling through PI3K was apparently related to tumorigenic increase, rather than adhesive differences caused by Ln-332. Similarly, a recent report demonstrated that addition of purified Ln-332 to esophageal squamous carcinoma cells activates the PI3K pathway resulting in increased invasion. This process can be inhibited with Ln-322 blocking antibodies (Baba et al., 2008). These studies, along with others, begin to reveal the possible role of Ln-332 as a signaling molecule in the BM that may at times outweigh its structural BM functions.

The importance of β3 chain in providing structural stability should however not be downplayed. There are more JEB-causing mutations of Ln-332 structure linked to the β3 chain than the α3 and γ2 chains combined (Schneider et al., 2007). Transduction of wt β3 cDNA into primary keratinocytes from β3-mutated JEB patients results in the generation of tissue with restored function and expression including the ability to form hemidesmosomes (Robbins et al., 2001).

4. Laminin γ2

Unlike the other two chains, γ2 is unique to the Ln-332 trimer. It also has the distinction of being the only chain of the complex that can be secreted in monomeric form (Gagnoux-Palacios et al., 2001). Like the other chains, it has two transcript variants; the full-length mRNA is broadly expressed in epithelial cells of 17-week-old human embryonic tissues, while the truncated version seems to be restricted to cerebral cortex, lung, and distal tubules of the kidney (Airenne et al., 1996). Ln-332 γ2 protein can be detected in the BM of many normal human epithelial tissues including skin, lung, small intestine, stomach, kidney, and prostate. Surprisingly, γ2 has also been reported to occur in non-epithelial tissue, namely lymphatic areas encasing the small arteries of the thymus and spleen (Mizushima et al., 1998).

Many functions of γ2 rely on proteolysis [with some exceptions, such as the integrin α2β1-mediated binding required for keratinocyte migration (Decline and Rousselle, 2001)]. Our lab first reported the migration-inducing MMP-2 cleavage of breast epithelial cell Ln-332 γ2 (Giannelli et al., 1997), which was later confirmed to have a role in sex steroid-induced tissue reorganization and maturation *in vivo* (Giannelli et al., 1999). The γ2 cleavage can also be carried out by a related membrane-type MMP (MT1-MMP) in colon and breast carcinoma cells (Koshikawa et al., 2000). MT1-MMP cleavage also stimulated migration, as shown in the spontaneously immortalized normal breast epithelial cell line MCF10A (Gilles et al., 2001). Migration induction and the opposing well-known functions of Ln-332 in forming static adhesive structures (hemidesmosomes) were reconciled by the hypothesis that these functions may alternate to promote tissue homeostasis, perhaps in a MMP dependent manner. A seemingly conflicting report claims that MMP-2 and MT1-MMP have no role and instead suggest that mammalian tolloid metalloproteinase is responsible for γ2 cleavage in keratinocytes (Veitch et al., 2003); however, we showed that this apparent contradiction in experimental findings is likely due to tissue specific differences (Koshikawa et al., 2004; Koshikawa et al., 2005). Furthermore, other groups have also documented physiologically relevant MMP-2 and/or MT1-MMP cleavage of γ2 (Kiyoshima et al., 2005; Oku et al., 2006; Shen et al., 2007).

There are also reports of proteolytic cleavage of the γ2 chain by other proteases. Bone morphogenetic protein-1, important for maturation of procollagen types I (Wozney et al., 1988), II and III (Hojima et al., 1985) by cleavage, was shown to cleave human (Amano et al., 2000) and murine (Sasaki et al., 2001) γ2 chain. It has been reported that MMP-3, -8, -13, -14, -20 (Pirila et al., 2003) and −19 (Sadowski et al., 2005) process γ2, inducing varying degrees of epithelial cell migration. Outside of epithelium, Ln-332 was shown to serve as a migratory substrate for mature thymocytes following cleavage of CD-44 by MMP-14 (Vivinus-Nebot et al., 2004).

It is important to note that γ2 cleavage does not always stimulate migration (reviewed in Hintermann and Quaranta, 2004). To the contrary, by a series of experiments using naturally occurring γ2-null cells transfected with cDNA of wildtype or mutant γ2 chains, γ2 processing was required for incorporation of secreted Ln-332 into the BM, where it supports formation of static adhesive structures (hemidesmosomes) (Gagnoux-Palacios et al., 2001). This same report provided evidence that γ2 is secreted and incorporated into the BM both in monomeric form and as part of Ln-332 heterotrimers. Thus, the end result of γ2 cleavage may have at least two outcomes: either regulating structural integrity or serving as a migratory substrate.

It has been proposed that signaling initiation may be an additional consequence of γ2 cleavage. This is because all of the aforementioned cleavages occur in the short arm of the molecule, which harbors a repeat of EGF-like domains. Exposure of these domains by cleavage opens the possibility that they may become ligands for cell surface signaling receptors, i.e., of the EGF receptor family. Whether or not these EGF-domain cleavage products are readily available for binding and potential signaling remains to be seen. We reported evidence for γ2 EGF signaling initiated by the products of both MT1-MMP (Koshikawa et al., 2004) and MMP-2 cleavage (Schenk et al., 2003). In the case of MMP-2, this activity led to migration and survival through a divergent EGF receptor (EGFR) signaling pathway (Schenk et al., 2003). This is an example of a separable yet related function of γ2 chain in producing microenvironment cues and is especially relevant given the well-established role of increased EGFR expression and EGFR signaling in cancers of various types. In this respect, even more enticing is the increasing volume of literature reporting coexpression of γ2 and EGFR in cancer (Kiyoshima et al., 2005; Ono et al., 2002; Katoh et al., 2002; Fukai et al., 2005; Richter et al., 2005).

5. Ln-332 γ2 and cancer: a prognostic indicator

Numerous immunohistochemistry (IHC) and *in situ* hybridization (ISH) studies show Ln-332 γ2 chain to be at the leading edge of invading carcinomas, where it is positively correlated with invasiveness and patient survival (reviewed in Katayama and Sekiguchi, 2004). Several concrete examples are discussed below (see Table 1 for more inclusive list).

A study of 67 urothelial carcinomas revealed that γ2 over-expression is an effective independent prognostic indicator of intravesical recurrence (Kiyoshima et al., 2005). In hepatocellular cancers, γ2 is involved in epithelial to mesenchymal transition (Giannelli et al., 2005) and its detection is strongly associated with metastasis (Giannelli et al., 2003). Intriguingly, circulating γ2 fragments in serum are elevated in pancreatic ductal adenocarcinoma patients having liver metastases, particularly compared to those whose cancer has not spread, suggesting that circulating γ2 fragment may be useful as a prognostic indicator (Katayama et al., 2005).

Expression of Ln-332, particularly γ2, is implicated in progression of several types of head and neck cancer. MT1-MMP interaction with γ2 correlates with both metastasis and depth of invasion in esophageal squamous cell carcinomas (Shen et al., 2007). Transcrip-tome analysis unveiled LAMC2 among a panel of upregulated genes in oral tongue squamous cell carcinoma

specimens (Ye et al., 2008). In colorectal (Sordat et al., 1998), anal (Nilsson et al., 2005), pancreatic (Soini et al., 1996), and gastric cancers (Koshikawa et al., 1999), differential γ2 localization or expression levels have been shown to be of prognostic value.

Fascinating observations have also been made concerning the role of Ln-332 in "vascular or vasculogenic mimicry", defined as the ability of tumor cells (especially uveal melanoma) to take on endothelial-like morphology and produce functional vessels that anastomose with capillaries (Seftor et al., 2001; Hendrix et al., 2003). Seftor et al. previously showed that this phenomenon occurs in tumors that produce monomeric γ2 chain (Seftor et al., 2001), which has been shown to correlate with enhanced invasiveness *in vivo* (Koshikawa et al., 1999). Molecular and cellular mechanisms, however, remain to be elucidated.

Overall, these reports point to a possible role of Ln-332 γ2 as an indicator for prognosis in several types of cancer. In most cancer types, the general trend appears to be that overexpression of Ln-332 or one of its subunits, especially γ2, correlates with poor prognosis (Table 1). However, there are exceptions, such as breast and colon cancer, in which the presence of γ2 appears to predict better outcomes (Table 1). Clearly, more in-depth, perhaps standardized studies are necessary to solidify these trends.

6. Ln-332 γ2 and cancer: unanswered questions

A basic issue with reports of increases in Ln-332 γ2 staining in cancer specimens is that it is uncertain whether these are increases in protein level or simply reflect differential γ2 localization or cleavage within a tumor. Since γ2 is specific to Ln-332, it is frequently used as a surrogate marker for presence of the heterotrimer, thus examining expression of the other two chains is often neglected (see Table 1). The problem with this approach is that γ2 can be secreted as a monomer (Gagnoux-Palacios et al., 2001). Therefore, without verifying the presence of the other two chains, there remains ambiguity over the context of γ2 chain expression. This issue should be solved in future studies, since an antibody specific for the monomeric form of γ2 has been reported (Koshikawa et al., 2008).

Another area of concern is the wide variation in assessment techniques (see Table 1). For protein expression studies in human cancer tissue specimens, IHC has been widely used. It is well known that IHC methods are somewhat subjective, since various groups score staining intensity by differing protocols and criteria. For mRNA expression studies, ISH has been the most popular method, followed by northern blotting. The problem with both of these techniques is that there is no standard as to what probe is used. Taken together, these issues make it difficult to compare experimental results across studies from different laboratories.

Recently, we attempted to address this issue of standardizing studies by investigating the expression of γ2 chain in a collection of cancer specimens, analyzed by standardized cDNA microarray methods. While in the end only colorectal cancer specimen data were usable, nonetheless we were able to conclude that it is the decreased ratio of β3:γ2 mRNA expression that most closely correlates with progression to metastatic disease (Guess et al., 2009). We submit that the importance of our study is that it provides a standard approach to examine both heterotrimeric and monomeric γ2 chain expression, compatible for comparison across different studies.

7. Conclusions

Clearly, many additional investigations are necessary to clarify the role of Ln-332 and its subunits in carcinoma. In terms of expression, mining the cDNA expression microarray databases can be extremely valuable, as we reported (Guess et al., 2009). However, it will be necessary to complement these studies with protein expression methods, perhaps in tissue

microarrays. In this respect, appropriate antibodies, e.g., to various forms of Ln-332 and its distinct subunits and/or cleavage products, would be necessary. Furthermore, standardized ways to score intensity of staining are also desirable.

In terms of mechanism, the key issue remains to distinguish between BM structural and signaling roles of Ln-332 in cancer. This multiplicity of roles complicates analyses, because any changes in ECM caused by the over- or under- expression of Ln-332, will affect cells in several ways: adhesion through hemidesmosomes would be altered, as would crosslinkages with other ECM molecules normally associate with Ln-332; there would be a change of the normal that cell-ECM communication process; the aberrant signaling coupled with the adhesive changes could work to the benefit of the tumor cells and lead to increased invasion and other devastating effects. Deconvolution of these effects would also be exacerbated by any increase in γ2 monomer levels, which results in distinct signaling as well (e.g. EGFR, PI3K activation).

A novel avenue of possible investigation is provided by the recent discovery that human papillomavirus-16 E6 oncogene leads to microRNA (miR) regulation of the LAMB3 gene through miR-218 in cervical cancer (Martinez et al., 2008), underscoring the importance of gene-level regulation of Ln-332 subunits in cancer progression.

In summary, studies at various levels of complexity are necessary to clarify the extent to which Ln-332 is involved in cancer progression mechanisms. Nonetheless, the utility of Ln-332 and its subunits as cancer prognostic indicators should not be difficult to realize.

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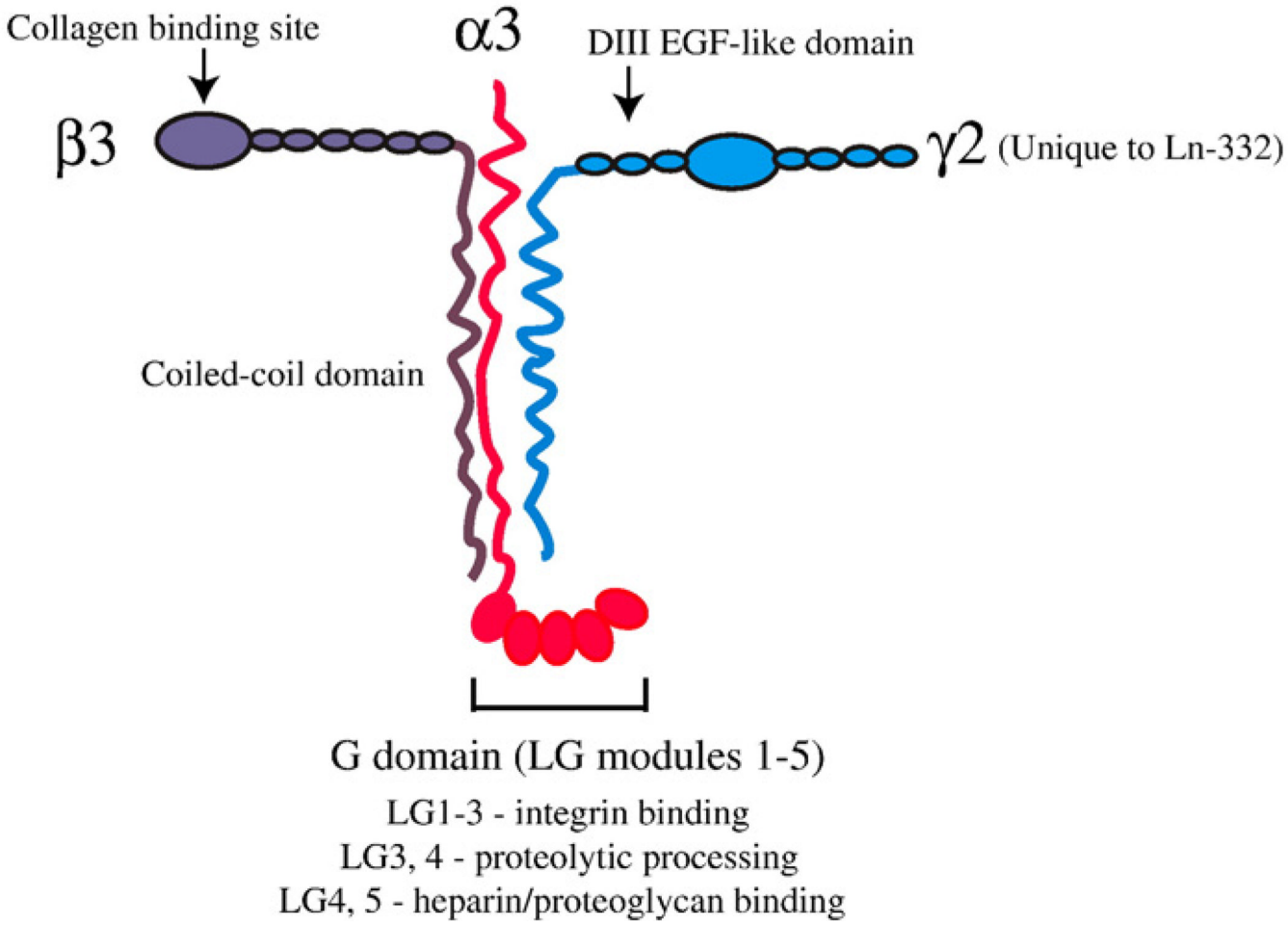

Fig. 1.

Laminin-332 structure and major roles. Representation of Ln-332 heterotrimer is shown. The long arm of the molecule contains all chains held in a coiled structure by disulfide linkage. The G domain, consisting of 5 LG modules (red circles beneath the long arm), of the α chain is responsible for various cellular functions. LG modules 1–3 are important for integrin binding important for cell signaling. LG modules 3 and 4 are important for proteolytic processing, and modules LG4 and LG5 interact and bind with heparin and proteoglycans (reviewed in Marinkovich, 2007). The β and γ short arms contain cleavable interaction domains with crucial ECM interaction and growth factor-like signaling functions.

Table 1

Ln-332 expression and cancer prognosis.

Tumor histology	Method	Chain(S)	Correlations	Reference
Bladder				
Urinary bladder carcinoma	MS-PCR	α3, β3	Invasion, stage, grade	Sathyanarayana et al., 2004
Urothelial carcinoma	IHC	γ2	Invasion, recurrence	Kiyoshima et al., 2005
Urinary bladder carcinoma	MS-PCR	γ2	Survival	Sathyanarayana et al., 2004
Breast				
Ductal carcinoma	ISH	γ2		Pyke et al., 1994
Ductal carcinoma	IHC, ISH	γ2		Pyke et al., 1995
Breast carcinoma	MS-PCR	α3	Size, stage	Sathyanarayana et al., 2003a
Breast carcinoma	MS-PCR	β3, γ2		Sathyanarayana et al., 2003a
Metaplastic breast Carcinoma	IHC	γ2	Initial diagnosis	Carpenter et al., 2008
Cervical				
Cervical carcinoma	IHC	γ2	Invasion	Noel et al., 2005
Cervical adenocarcinoma	IHC	γ2	Invasion	Negri et al., 2006
Cervical cancer	IHC	γ2	Progression	Wang et al., 2006
Cervical squamous carcinoma	IHC	γ2		Andersson et al., 2005
Cervical squamous adenocarcinoma	IHC	γ2		Andersson et al., 2005
Intraepithelial neoplastic lesion	IHC	γ2		Tringler et al., 2007
Cervical carcinoma	IHC	γ2	Invasion	Skyldberg et al., 1999
Colorectal				
Colon adenocarcinoma	ISH	γ2	Invasion	Pyke et al., 1994
Colorectal carcinoma	IHC	α3		Sordat et al., 1998
Colorectal carcinoma	IHC	β3, γ2	Invasion	Sordat et al., 1998
Colorectal carcinoma	IHC	α3	Invasion	Lohi et al., 2000
Colorectal carcinoma	IHC	β3, γ2, L n −332		Lohi et al., 2000
Colon adenocarcinoma	IHC, ISH	γ2	Invasion	Pyke et al., 1995
Colorectal carcinoma	IHC	α3		Sordat et al., 2000
Colorectal carcinoma	IHC, ISH	γ2	Invasion	Hlubek et al., 2001
Colorectal carcinoma	IHC	γ2		Habermann et al., 2001
Colon adenocarcinoma	IHC	γ2	Invasion	Lenander et al., 2001
Colorectal adenoma	IHC	γ2	Size, progression	Lenander et al., 2003
Colorectal carcinoma	IHC	γ2	Metstasis, survival	Aoki et al., 2002
Colorectal carcinoma	IHC	γ2		Masaki et al., 2003
Colorectal carcinoma	IHC	β3, γ2	Invasion	Akimoto et al., 2004
Colorectal cancer	IHC	γ2	Survival	Shinto et al., 2005
Colorectal cancer	IHC	γ2	Relapse	Lloyd et al., 2006
Colorectal cancer	IHC	γ2	Nodal involvement	Park et al., 2005
Colorectal carcinoma	IHC	β3, γ2, L n −332	invasion	Sordat et al., 2000
Gallbladder				
Gallbladder carcinoma	IHC	γ2	Invasion	Eguchi et al., 2008
Gastric				

Tumor histology	Method	Chain(S)	Correlations	Reference
Gastric carcinoma	IHC	α3	Invasion	Tani et al., 1996
Gastric carcinoma	IHC	α3		Tani et al., 1996
Gastric carcinoma	IHC	Ln −332		Tani et al., 1996
Gastric carcinoma	IHC	α3, β3		Koshikawa et al., 1999
Gastric carcinoma	IHC	γ2	Invasion	Koshikawa et al., 1999
Head and Neck				
Oral squamous cell carcinoma	IHC, ISH	γ2	Invasion	Kainulainen et al., 1997
Oral squamous cell carcinoma	IHC	γ2	Invasion	Thorup et al., 1998
Oral squamous cell carcinoma	IHC	α3	Invasion	Kosmehl et al., 1999
Oral squamous cell carcinoma	IHC	γ2	Invasion	Berndt et al., 2001
Laryngeal carcinoma	IHC	γ2	Invasion, progression	Nordemar et al., 2001
Laryngeal carcinoma	IHC	γ2		Hagedorn et al., 2001
Esophageal squamous cell carcinoma	IHC	γ2	Invasion, survival	Yamamoto et al., 2001
Squamous cell carcinoma of tongue	IHC	γ2	Invasion, survival	Katoh et al., 2002
Squamous cell carcinoma of tongue	IHC	β3, γ2	Invasion	Akimoto et al., 2004
Squamous cell carcinoma of tongue	IHC	γ2	Survival	Ono et al., 1999
Squamous cell carcinoma of tongue	IHC	γ2		Lim et al., 2004
Squamous cell carcinoma of tongue	IHC	γ2		Korpi et al., 2008
Hypopharyngeal cancer	IHC	γ2	Stage, survival	Nakayama et al., 2004
Esophageal squamous cell carcinoma	IHC	γ2	Survival	Baba et al., 2006
Esophageal squamous cell carcinoma	IHC	γ2	Stage, survival	Baba et al., 2008
Oral squamous cell carcinoma	IHC	γ2	Invasion, survival	Gasparoni et al., 2007
Oral squamous cell carcinoma	IHC	γ2	Initial diagnosis	Driemel et al., 2007
Esophageal squamous cell carcinoma	IHC	γ2	Invasion, met., survival	Fukai et al., 2005
Head and neck squamous cell carcinoma	IHC	γ2	Invasion	Anderson et al., 2001
Preneoplastic oral lesions	IHC	γ2	Invasion, progression	Nordemar et al., 2003
Follicular thyroid carcinoma	IHC	α3, β3, γ2		Lohi et al., 1998
Liver				
Hepatocellular carcinoma	IHC	γ2		Giannelli et al., 2001
Hepatocellular carcinoma	IHC, ISH, RT-PCR	α3, β3		Giannelli et al., 2003
Intrahepatic cholangiocarcinoma	IHC	γ2	Progression	Aishima et al., 2004
Hepatocellular carcinoma	IHC, ISH, RT-PCR	γ2	Metastasis	Giannelli et al., 2003
Lung				
Lung adenocarcinoma	IHC	γ2	Invasion	Akashi et al., 2001
Lung adenocarcinoma	IHC	α3, β3		Kagesato et al., 2001
Lung adenocarcinoma	IHC	γ2	Invasion, survival	Moriya et al., 2001
Lung adenocarcinoma	IHC	γ2	Invasion	Kagesato et al., 2001
Squamous cell carcinoma of lung	IHC, ISH	γ2		Maatta et al., 1999
Large cell lung carcinoma	ISH	γ2		Maatta et al., 1999
Small cell lung carcinoma	ISH	γ2		Maatta et al., 1999
Bronchio-aveolar adenocarcinoma	ISH	γ2		Maatta et al., 1999
Pancreas				

Tumor histology	Method	Chain(S)	Correlations	Reference
Pancreatic adenocarcinoma	IHC, ISH	γ2		Soini et al., 1996
Pancreatic ductal adenocarcinoma	IHC	γ2	Invasion	Fukushima et al., 2001
Pancreatic ductal adenocarcinoma	IHC	α3, β3, γ2		Tani et al., 1997
Pancreatic ductal adenocarcinoma	IHC	γ2	Invasion, survival	Takahashi et al., 2002
Prostate				
Prostate carcinoma	IHC, ISH	γ2	Invasion	Hao et al., 1996
Prostate carcinoma	IHC	α3	Invasion	Hao et al., 1996
Prostate carcinoma	IHC, ISH	α3, β3, γ2		Hao et al., 2001
Prostate carcinoma	MS-PCR	α3, β3, γ2	Stage	Sathyanarayana et al., 2003b
Prostate carcinoma	IHC	α3		Hao et al., 1996
Other tissues				
Basal cell carcinoma	IHC	Ln −332	Invasion	Schofield et al., 1998
Glioma	IHC	γ2		Fukushima et al., 1998
Leiomyosarcoma	ISH	γ2		Pyke et al., 1994
Leiomyosarcoma	IHC, ISH	γ2		Pyke et al., 1995
Melanoma	ISH	γ2		Pyke et al., 1994
Melanoma	IHC, ISH	γ2		Pyke et al., 1995
Papillary renal neoplasm	IHC	α3, β3		Lohi et al., 1996
Oncocytoma	IHC	α3, β3		Lohi et al., 1996
Squamous cell carcinoma	ISH	γ2		Pyke et al., 1994
Squamous cell carcinoma	IHC, ISH	γ2	Invasion	Pyke et al., 1995
Vaginal squamous cell carcinoma	IHC	γ2	Invasion, survival	Hellman et al., 2000
Cylindromatosis	IHC	α3, β3, γ2		Tunggal et al., 2002
Anogenital tract carcinoma	IHC	γ2	Invasion	Nordstrom et al., 2002
Ovarian clear cell carcinoma	IHC	γ2		Kato et al., 2007
Ovarian adenocarcinoma	IHC	Ln −332	Survival	Kohlberger et al., 2002