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PROSPECT

New Insights Into the Metastasis-Associated 67 kD Laminin Receptor

Sylvie Ménard,[1]* Vincent Castronovo,[2] Elda Tagliabue,[1] and Mark E. Sobel[3]

[1]Division of Experimental Oncology E, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy
[2]Metastasis Research Laboratory, University of Liege, Liege, Belgium
[3]Molecular Pathology Section, National Cancer Institute, Bethesda, Maryland 20814

Abstract The interactions between tumor cells and laminin or other components of the extracellular matrix have been shown to play an important role in tumor invasion and metastasis. These interactions are mediated by different cell surface molecules, including the monomeric 67 kD laminin receptor. This molecule appears to be very peculiar since so far only a full-length gene encoding a 37 kD precursor protein has been isolated and the mechanism by which the precursor reaches the mature form is not understood. Based on clinical data, which clearly demonstrate the importance of the receptor in tumor progression, studies were conducted to define the structure, expression, and function of this laminin receptor as a step toward developing therapeutic strategies that target this molecule. The data suggest that acylation of the precursor is the key mechanism in maturation of the 67 kD form. The function of the membrane receptor is to stabilize the binding of laminin to cell surface integrins, acting as an integrin-accessory molecule, although homology of the gene encoding the receptor precursor with other genes suggests additional functions. Downregulation of the receptor expression on tumor cells might open new therapeutic approaches to decrease tumor aggressiveness. J. Cell. Biochem. 67:155–165, 1997.

Key words: adhesion receptors; tumor progression; ribosomal proteins; laminin; cancer

The ability of cancer cells to invade the surrounding tissues and disseminate to distant sites defines the invasive and metastatic phenotype. Metastases are the major causes of morbidity and mortality in patients with solid malignant tumors and are the result of successive interactions between cancer cells and host tissues. In the early 1980s, it was postulated that blocking tumor invasion and metastases might be a promising therapeutic alternative to the classical antimitotic arsenal, which affected all types of proliferating cells, including normal cells. Thus, understanding the molecular mechanisms that determine tumor invasion and metastasis became a major challenge of cancer research.

Invasion of the basement membrane (BM) by cancer cells was identified as a critical step in the metastatic process and therefore as an ideal target to block malignant progression. BM is a specialized extracellular matrix which separates tissue compartments from each other and acts as a physical barrier against passive cell diffusion. Invasion of BM is an active process involving multiple interactions between malignant cells and BM components. To study the molecular mechanisms of BM invasion, a model was proposed consisting of the cyclic repetition of three major processes: 1) attachment of cancer cells to BM components through specific cell surface receptors, 2) degradation of BM following local secretion of proteolytic enzymes, and 3) migration of the cells into the adjacent tissue compartments [Castronovo, 1993; Flug et al., 1995]. Studies of these processes became possible after the identification of the major components of BM through the use of the Engelbreth-Holm-Swarm tumor, a murine cancer that produces BM molecules in large excess. At that time, laminin was identified as a high molecular weight glycoprotein of 10^7 daltons composed of three chains that form a cross-shape structure. Laminin has multiple biological functions [Yamada et al., 1992]. Its interaction with cancer cells was identified as a key event in tumor invasion and metastasis [Barsky et al., 1984].

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*Correspondence to: Sylvie Ménard, Division of Experimental Oncology E, Istituto Nazionale Tumori, Via Venezian 1, 20133 Milan, Italy. E-mail: menard@istitutotumori.mi.it

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Laminin serves as a major adhesion substrate for invasive cancer cells, and there is a direct correlation between the ability of malignant cells to attach to laminin and their metastatic potential. The observation that laminin bound to the cancer cell surface in a saturable fashion and with high affinity suggested the presence of a cell surface laminin receptor or laminin-binding proteins. This hypothesis led several groups to attempt to purify the putative laminin receptor(s) by chromatographic procedures, and in 1983 three independent laboratories reported the identification of an apparently unique 67 kD protein isolated from cell membranes of cancer cells [Rao et al., 1983; Malinoff et al., 1983] and of normal muscle cells [Lesot et al., 1983]. This molecule was called the 67 kD laminin receptor (67LR). The use of polyclonal and monoclonal antibodies raised against the 67LR showed that this receptor was responsible for the high binding affinity of laminin to the cancer cell surface (Kd = 2 nM). Immunoperoxidase and immunoblotting studies clearly demonstrated that the 67LR was overexpressed on the cancer cell surface and that this overexpression correlated with the invasive and metastatic capacity of these cells. Thus, it was postulated that the 67LR plays a significant role in the acquisition of the metastatic phenotype.

ORGANIZATION AND LOCATION OF THE 67LR GENE

The corresponding cDNA was isolated to better understand the structure, function, and molecular mechanisms responsible for upregulation of the 67LR in cancer cells. Screening of a human cDNA expression library using monoclonal antibody LR1, which recognizes the 67LR on immunoblots and inhibits laminin binding to the cell surface [Liotta et al., 1985], identified six clones that share a common domain (i.e., the epitope identified by LR1) [Wewer et al., 1986]. Confirmation of the cDNA was obtained by the perfect match of the amino acid sequence predicted from the cDNA with the amino acid sequence revealed by microsequencing of peptides obtained from the cyanogen bromide digest of authentic 67LR purified by laminin chromatography. Antibodies generated against synthetic peptides whose sequences were derived from the cDNA recognized a 67 kD protein and interfered with the chemotactic response of cancer cells to laminin in a Boyden-modified chamber assay [Wewer et al., 1987]. Full-length cDNAs of the 67LR were obtained in both human [Yow et al., 1988] and mouse [Rao et al., 1989]. The human cDNA was obtained during the identification of a gene specifically expressed in malignant colon carcinoma and isolated by differential screening methods. The murine full-length clone was isolated by screening a cDNA library using the partial human 67LR cDNA as a probe. Primer extension experiments unequivocally demonstrated that the clones contained the complete 5′ sequence of the mRNA. Unexpectedly, the polypeptide predicted from the 67LR cDNA sequences was only 295 amino acids long, with a calculated molecular mass of 32 kD [Rao et al., 1989]. In vitro translation of selectively hybridized mRNA indicated a product with an apparent molecular mass of 37 kD on SDS-PAGE. Interestingly, this polypeptide is identical to the ribosome-associated protein p40. Today, cDNAs encoding the 37 kD protein have been identified and characterized in at least 17 species. Remarkable homology of the 37LRP/p40 sequences between species suggests an essential biological role for this protein.

The association between increased expression of the 37LRP/p40 polypeptide and acquisition of the invasive and metastatic phenotype raised great interest in the molecular mechanisms regulating expression of the polypeptide. Initial Southern hybridization experiments using genomic DNA showed that the mammalian genome contains several copies of the 37LRP/p40 gene. Mice and humans retain six [Fernández et al., 1991] and 26 copies [Jackers et al., 1996a], respectively, of the 37LRP/p40 gene. Most of these copies were found to be pseudogenes generated by retropositional events [Jackers et al., 1996a; Bignon et al., 1991], which has made it difficult to isolate the active corresponding gene, especially in humans. Interestingly, the chicken genome contains a single copy of the 37LRP/p40 gene [Clausse et al., in press], with a predicted high homology to the human gene that enabled a screen of a chicken genomic library using the human 37LRP/p40 cDNA probe. The 37LRP/p40 active chicken gene that was isolated is composed of seven exons and six introns. It does not contain a classical TATA box, and RNAse protection assay and primer extension experiments demonstrated the presence of several transcription initiation sites. Parallel efforts to isolate the corresponding human gene using PCR amplification of a putative

intron-containing fragment of the active 37LRP/p40 gene were successful only with a PCR primer designed from domains of the 37LRP/p40 cDNA sequence that were highly affected by base substitutions in the corresponding pseudogenes. Isolation and characterization of the full-length active human 37LRP/p40 gene and its upstream and downstream flanking region showed that the gene is also composed of seven exons and six introns and shared some intriguing similarities with housekeeping genes, more particularly with genes encoding ribosomal proteins. Indeed, the 5′ end of the 37LRP/p40 active gene contains a very small first exon, a cap site located in a pyrimidine-rich tract, and no TATA box [Jackers et al., 1996b]. Four Sp1 sites were identified in the promoter and six in intron 1. These features are similar to those of the chicken gene. Interestingly, the human 37LRP/p40 gene contains the full sequence of the small nuclear RNA E2 in intron 4 and two Alu sequences in intron 3. Further studies are needed to determine the nature of cis-regulatory elements responsible for the upregulation of the 37LRP/p40 gene in cancer.

Fluorescent in situ hybridization assays served to map the human 37LRP/p40 gene to 3p21.3, a chromosomal locus frequently involved in karyotypic rearrangements associated with cancers [Jackers et al., 1996b]. Large deletions and losses involving this chromosomal band are found in virtually all small cell carcinomas of the lung and in most non-small cell lung carcinomas [Moscow et al., 1994; Hibi et al., 1992; Heppell-Parton et al., 1992; Carritt et al., 1992]. Alterations in the 3p21 region have been also reported in renal cancer [Lubinski et al., 1994], head and neck cancers [Li et al., 1994], breast carcinoma [Chen et al., 1994], and colon carcinoma [Bronner et al., 1994]. The search for a potential tumor suppressor gene at 3p21.3 by positional cloning led to the identification of a novel integrin α subunit gene whose expression is upregulated in small cell lung carcinoma. This aberrant upregulation might have been caused by an as yet unidentified mutation or by deletion of other genes in the 3p21 region. Increased expression of the 37LRP/p40 gene is a consistent feature of aggressive carcinoma. The 37LRP/p40 molecule is therefore the second gene involved in cell-matrix interactions whose chromosomal location is in 3p21 and whose expression is upregulated in cancer [Hibi et al., 1994]. It seems possible that alterations affecting this chromosome locus lead to activation of genes involved in tumor invasion and metastases.

HOMOLOGY OF THE 37LRP/P40 WITH OTHER MOLECULES AND OTHER FUNCTIONS

cDNA corresponding to the 37LRP/p40 polypeptide has been cloned by several groups and in several species as a sequence corresponding to (1) the precursor of the 67 kD laminin receptor [Castronovo et al., 1991a; Rao et al., 1989a], (2) an antigen differentially expressed at the surface of invasive cancer cells [van de Ouweland et al., 1989; Yow et al., 1988], (3) the Sindbis virus receptor [Wang et al., 1992], (4) the ribosome-associated protein p40 [Rosenthal et al., 1995; Tohgo et al., 1994; Makrides et al., 1988], (5) a positional marker in the development of the embryonic eye [Rabacchi et al., 1990], and (6) a mitotic phosphoprotein [Westendorf et al., 1990]. The amino acid sequence corresponding to this cDNA is extremely well conserved through evolution, with at least 98.3% homology among mouse, bovine, and human sequences and 99% homology between rat and human sequences. In eukaryotes, homologies in the amino-terminal portion of the protein have been found with a yeast mitochondrial protein [Davis et al., 1992], an intermediate filament protein in the human cytoskeleton [Siianova et al., 1991], a protein of Hydra [Keppel et al., 1991], and a plant protein [Garcia-Hernandez et al., 1994; Axelos et al., 1993]. This high conservation of the sequence suggests a key biological function(s). A putative important function is supported by the recent finding that one open reading frame present in the genome of the archaen *Haloarcula marismortui* encodes a protein 40% identical to the 37LRP/p40 cDNA [Ouzonis et al., 1995].

Sequence comparison of the chicken 37LRP/p40 cDNA with the mammalian cDNA has revealed the striking conservation of this gene since birds and mammals diverged in evolution (i.e., 300 million years ago). By contrast, the amino acid sequence of the polypeptide in invetebrates and eukaryotes is highly variable in the carboxy-terminal region. Interestingly, this region corresponds exactly to exons 6 and 7. Thus, these two highly conserved exons in vertebrates appear to have been the target of a strong evolutionary pressure and might represent the locus of a specific vertebrate function.

A 67LR molecule related to the 37LRP has been clearly described in vertebrates only. The mechanism(s) through which the 37LRP is processed into the mature 67LR remains to be elucidated. It has been suggested that the 67LR could result from homo- or heterodimerization. The remarkable conservation of the carboxy-terminal part of the 37LRP/p40 protein among vertebrates raises the possibility that this region might contain the element(s) responsible for the biosynthesis of the 67LR. The central part of the polypeptide, conserved through all species, might be associated with the putative translational function of the 37LRP/p40 protein. In fact, this central region confers the 37LRP/p40 protein with a parental relationship to RS2 prokaryotic ribosomal protein [Ouzonis et al., 1995; Kromer et al., 1991].

An evolutionary scenario for the 37LRP/p40 protein can be envisioned that is an original vital function involved in translation processes and the progressive acquisition of new roles (e.g., as a basic building block of a receptor to accommodate the new extracellular matrix protein, laminin). The 37LRP/p40 gene encodes a single polypeptide which might be involved in two different cell functions: 1) as a 37–40 kD ribosome-associated protein and 2) when incorporated into a 67 kD molecule, as a laminin receptor (Fig. 1). Such a dual fate for a single gene product might constitute a parsimonious means of protein function diversification during evolution.

REGULATION OF 37LRP GENE EXPRESSION AND 67LR EXPRESSION

Initial observations of the pattern of expression of the 67LR by immunohistochemistry using antibodies raised against 37LRP synthetic peptides suggested that undifferentiated tumors expressed more 67LR than did cancers with more differentiated phenotypes [Wewer et al., 1987]. In addition, 37LRP mRNA levels were dramatically increased in fetal rat intestine [Anilkumar et al., 1993] and in undifferentiated, mitotically active crypt cells compared to more differentiated villus cells [Rao et al., 1994]. In vitro studies have, in general, supported those observations. For example, Rao et al. [1989] showed that undifferentiated murine

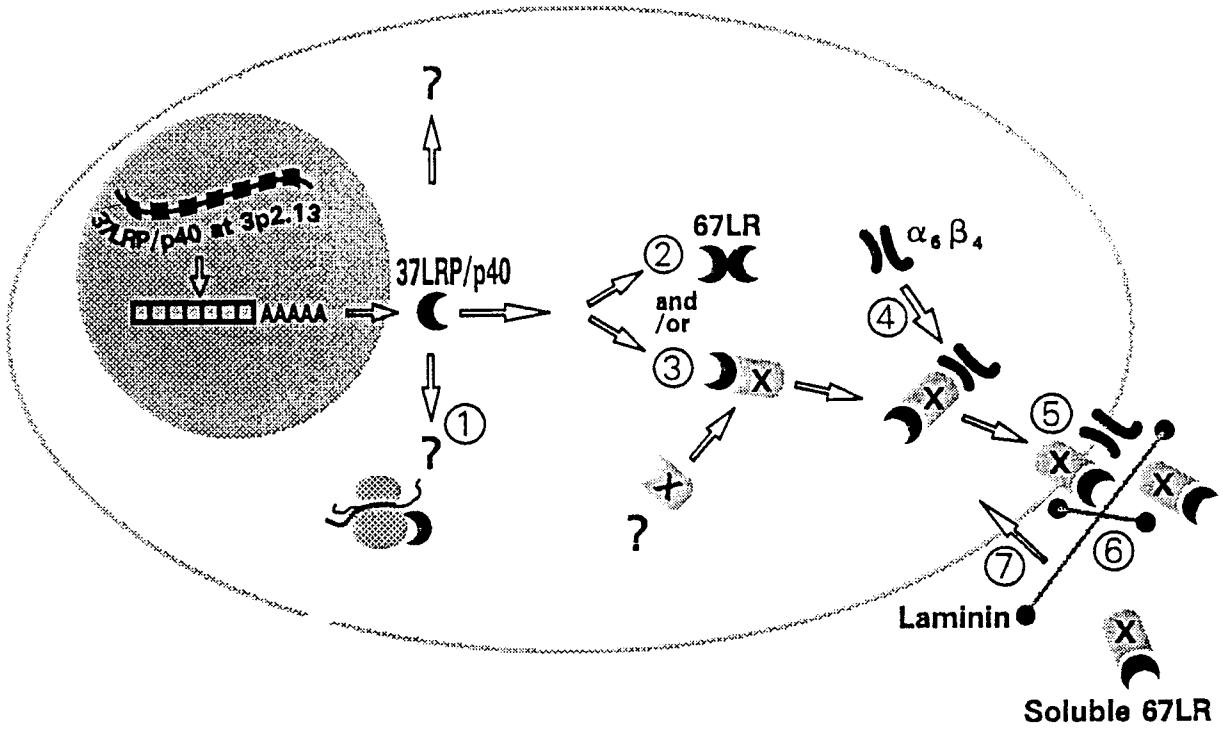

Fig. 1. Biosynthesis and function of the 37LRP and the 67LR. **1:** The 37LRP/p40 becomes a ribosomal protein. **2:** Homodimerization of the acylated 37LRP forms the 67LR. **3:** Heterodimerization of the 37LRP with another unknown protein forms the 67LR. **4:** The 67LR associates with laminin-specific integrins in the cytoplasm. **5:** The complex is translocated to the cell membrane. **6:** The 67LR is shed and binds to laminin coated on the cell membrane. **7:** Laminin coated on the membrane increases the translocation of the cytoplasmic receptor complex.

F9 cells induced to differentiate to parietal endoderm by treatment with retinoic acid and dibutyryl cAMP have markedly increased expression of laminin and type IV collagen but less 37LRP mRNA. Note, however, that CE44 teratocarcinoma embryoid bodies synthesize a 67 kD laminin receptor as well as the B1 and B2 chains of laminin during differentiation into parietal endoderm [Unda et al., 1994]. Moreover, a 67 kD YIGSR-binding protein identified in human neuroblastoma LA-N1 cells by affinity chromatography was expressed at lower levels upon dibutyryl cAMP–induced differentiation of these cells and 37LRP mRNA levels were also decreased [Bushkin-Harav et al., 1995].

The laminin receptor was found to be upregulated also by cytokines and inflammatory agents. Immunocytological evaluation of cancer lesions using antibodies raised against 37LRP synthetic peptides demonstrated high expression of antigen in mononuclear inflammatory cells [Castronovo et al., 1990a]. Consistent with this finding, silica induction of murine macrophages resulted in increased levels of 37LRP mRNA [Segade et al., 1995]. Cytokines such as interferon increased immunofluorescence staining for 37LRP antigen in human umbilical vein endothelial cells [Raghunath et al., 1993].

Upregulation of 37LRP was observed after the interaction of cells with extracellular matrix proteins. Human cell lines derived from breast cancer or melanoma have increased levels of 37LRP mRNA after exposure to extracellular matrix proteins such as laminin and fibronectin [Castronovo and Sobel, 1990]. Human melanoma A2058 cells in suspension also respond to laminin [Romanov et al., 1994]. Immunogold studies using anti-67LR monoclonal antibody MLuC5, as well as anti-37LRP synthetic peptide antibodies, demonstrated increased antigen expression as well as a migration of antigen toward the membrane [Romanov et al., 1994]. Cycloheximide inhibited the response to laminin [Romanov et al., 1995], suggesting that protein synthesis is involved in the cellular response to laminin. Thus, laminin treatment of melanoma cells results in increased 37LRP mRNA and protein levels, suggesting that a positive feedback loop links the presence of ligand with increased expression of the binding protein.

When grown in the absence of steroids, estrogen receptor- and progesterone receptor-positive human breast cancer cell lines T47D and MCF7 express less 37LRP mRNA and protein than do steroid receptor-negative breast cancer cells such as MDA-MB231. However, when estrogen and/or progesterone are restored to steroid hormone–depleted growth medium, 37LRP mRNA and protein levels in the steroid receptor-positive cell lines increase to those found in the steroid receptor-negative cells [Van den Brûle et al., 1992; Castronovo et al., 1989]. Variants of the T47D breast cancer cell line that are more tumorigenic than the parent cell line and that express more progesterone receptor demonstrate a fourfold increase in 37LRP mRNA and protein in response to progestin treatment [Shi et al., 1993].

BIOSYNTHESIS

The first indication of a relationship between the 37LRP and the 67LR was the isolation of multiple clones from an expression cDNA library that was screened with a monoclonal antibody to authentic purified 67 kD laminin receptor [Wewer et al., 1986].

The isolated cDNA clones, however, encoded only a computer-predicted polypeptide of 32 kD, which on SDS polyacrylamide gels has a relative mobility of a 37 kD protein. The cDNA-predicted sequence does not contain a consensus site for N-linked glycosylation, and, although there are multiple serine and threonine residues, there is no evidence of O-linked glycosylation. Indeed, the 67LR is not stained by periodic acid-Schiff (PAS) [Cioce et al., 1993]. Treatment of 67LR by neuraminidase, O-glycanase, or Endo-F glycosidase did not alter molecular mass [Landowski et al., 1995a]. Thus, the mechanism by which a 37 kD precursor might be posttranslationally modified into a 67 kD protein is not obvious. Significantly, with the exception of multiple mRNA sizes observed during spermatogenesis [Fulcher et al., 1993], only a single species mRNA is recognized by 37LRP cDNA on Northern blots of RNA from many sources. Recent evidence suggests that the 67LR is acylated by the fatty acids palmitate, oleate, and stearate [Landowski et al., 1995a]. Apparently these fatty acids are covalently associated with the protein via ester linkages, suggesting that the 37LRP can dimerize either with itself or with another peptide to form the 67LR.

Immunoblot studies utilizing antibodies directed against synthetic peptides derived from different domains of the 37LRP cDNA-predicted protein recognized varying patterns of 37 kD and 67 kD proteins, suggesting that some epitopes of the 67LR may be masked and unavailable for detection by antibodies depending on conformation of a 37 kD precursor or a mature 67 kD membrane-associated protein [Castronovo et al., 1992b; Rao et al., 1989b].

Both 37 kD and 67 kD polypeptides were precipitated from metabolically labeled human melanoma cells [Castronovo et al., 1991a] using an antibody directed against an amino-terminal domain of the 37LRP. Significantly, in pulse-chase studies, the metabolic label was present exclusively in the 37 kD protein at early time points and gradually was chased into the 67 kD polypeptide, suggesting a precursor-product relationship. Antibody directed against a carboxy-terminal domain of the 37LRP can also immunoprecipitate both 37 kD and 67 kD polypeptides, as can polyclonal antibodies directed against recombinant 37LRP [Montuori et al., submitted].

Cotransfection of hamster 37LRP cDNA into Chinese hamster ovary (CHO) cells with a dihydrofolate reductase (DHFR) gene under selective growth conditions favoring expression of DHFR resulted in overexpression of 67LR [Landowski et al., 1995a]. Transfection of his-tagged 37LRP into human HeLa cells resulted in the production of tagged 67LR with laminin-binding properties [Montuori et al., submitted]. Significantly, cell surface biotinylation studies detected the his-tagged 67LR, demonstrating that the 37LRP is a precursor molecule that becomes a 67 kD laminin-binding protein on the cell surface. The final mechanisms by which the 37LRP is modified into a 67LR are yet to be elucidated but may very well involve acylation of the precursor protein.

EXPRESSION OF THE 67LR IN TUMORS

When it was observed that the molecule is overexpressed in the majority of tumors tested, 67LR quickly became a major focus of research. Indeed, a large body of evidence, obtained by diverse methodologies (i.e., northern blot mRNA analysis and immunohistochemistry [Castronovo and Sobel, 1990; Martignone et al., 1992), pointed to a role for the 67LR in tumor progression [Sobel, 1993]. In breast carcinoma, 67LR overexpression is associated with a poor prognosis [Martignone et al., 1993], mainly in laminin-producing tumors [Pellegrini et al., 1995]. This observation demonstrated the importance of the interaction between the 67LR and its ligand. Overexpression of 67LR was also found to provide prognostic information in addition to that given by the microvessel density [Gasparini et al., 1995]. Moreover, the 67LR marker has been used successfully for diagnosis in cytology [Castronovo et al., 1990a]. The strong correlation ($P < 0.01$) between 67LR expression and early dissemination of tumor cells at the bone marrow level further emphasizes the role of the receptor in the metastatic process [Ménard et al., 1994].

Colorectal, gastric, and cervical carcinomas also display increased expression of the 67LR [Castronovo et al., 1992a; Kondoh et al., 1992; Cioce et al., 1991; Campo et al., 1992], which is correlated with the clinical stage and/or prognosis. The increase in 67LR expression is often paralleled by the decreased expression of another nonintegrin laminin-binding protein, galectin-3 [Van den Brule et al., 1994], suggesting an inverse regulation of the expression of the two molecules that likely accounts for changes in laminin-binding affinity. Overexpression of 67LR is not restricted to epithelial tumors. Indeed, melanomas and lymphomas also display increased expression of the receptor [Carbone et al., 1995; Vacca et al., 1993].

ROLE OF THE 67LR AS A LAMININ RECEPTOR AND RELATIONSHIP WITH INTEGRINS AND LAMININ

A few years after the initial description of the 67 kD molecule as a laminin receptor, it became clear that integrins were the primary mediators of cell adhesion and of signal transduction to the nucleus upon cell-cell or cell–extracellular matrix interactions [Lafrenie et al., 1996; Mainiero et al., 1995]. In most of the studies on the role of the 67LR in adhesion, the involvement of integrins in this process was not taken into account. Thus, it is difficult to establish whether the 67LR per se mediates adhesion under physiological conditions. Indeed, in small cell lung carcinoma (SCLC) cell lines, 67LR expression levels were found to be proportional to VLA6 expression levels, indicating that the expression of the two receptors is likely controlled by the same mechanism [Pellegrini et al., 1994]. This coordinate expression was associated with adhesion to laminin [Pellegrini et

al., 1994], suggesting that both receptors are necessary for adhesiveness. Recent data also indicate that the 67LR and α6β4 receptor are coregulated, since treatment with α6 antisense oligonucleotides, which downmodulate integrin expression on the membrane, also proportionally reduced 67LR membrane expression [Ardini et al., 1997]. This regulation occurs at the level of translocation from the cytoplasm to the membrane, since the total amount of 67LR after treatment remained unchanged. Treatment with laminin, which increases 67LR expression, also increased the membrane expression of laminin-specific integrins [Ardini et al., 1997]. Both 67LR and α6β4 receptor are also physically associated, as shown by their coprecipitation with specific monoclonal antibodies [Ardini et al., 1997]. These findings suggest a possible synergistic role of the complex formed by the two receptors in laminin recognition. The α7β1 integrin, another laminin receptor, has been shown to bind to galectin-1 [Gu et al., 1994], which is a laminin-binding protein itself. By analogy, the 67LR and α6β4 receptor might interact, perhaps via the lectin activity of the 67LR and some carbohydrate determinants of the integrin. Such a complex might then recognize different binding sites on laminin and in turn dramatically increase the overall binding affinity.

LAMININ AND RECEPTOR BINDING SITES

Based on the amino acid sequence of the 37 kD precursor of the 67LR, two different domains have been identified as possible laminin-binding sites. The most well studied is peptide G, which contains the palindromic sequence LMWWML. This peptide was shown to bind laminin [Castronovo et al., 1991b], to inhibit the binding of tumor cells to endothelial cells [Castronovo et al., 1991c], and, quite unexpectedly, to increase the metastatic spread of human melanoma in nude mice [Taraboletti et al., 1993]. In fact, peptide G increases and stabilizes the binding of laminin on tumor cells [Magnifico et al., 1996], possibly by changing the conformation of laminin to favor membrane binding. This increase in membrane binding might, in turn, increase the metastatic potential, in analogy with the metastatic enhancement observed after treatment of tumor cells with exogenous laminin [Hunt, 1989]. A possible second laminin-binding domain has been hypothesized based on the hydrophobicity of the C-terminal sequence of the 67LR precursor [Landowski and Uthayakumar, 1995], suggesting a multivalent recognition of laminin by the receptor. Although the biochemistry of the entire 67LR is still unknown, some functional information suggests that a second polypeptide is present in the mature form. Indeed, laminin recognition of the entire receptor is lactose-dependent, suggesting the presence of a lectin domain that is absent in the precursor molecule [Castronovo et al., 1991a].

One of the sequences recognized by the 67LR on laminin is presumed to be the pentapeptide YIGSR, based on the recovery of the 67LR by affinity chromatography on YIGSR columns and the inhibition of cell binding on YIGSR substrates by antiserum that functionally blocks the 67LR [Massia et al., 1993]. However, other data for example, the absence of direct binding between peptide G and YIGSR argue against the involvement of YIGSR in 67LR recognition. The previous studies indicating a role for the peptide in 67LR function did not account for the presence of 67LR-integrin complexes in laminin binding and thus might be misleading. In fact, if binding to laminin requires the presence of a 67LR-integrin complex, even peptides interfering with integrin recognition are expected to elute the complex from affinity chromatography columns. Another laminin-derived peptide, IKVAV, recognized by a laminin-binding protein of 110 kD [Kibbey et al., 1993], appears to be associated with the 67LR recognition of laminin. Indeed, this peptide, involved in the binding of laminin to the yeast *Histoplasma capsulatum*, is recognized by a 50 kD laminin-binding protein immunologically related to the 67LR [McMahon et al., 1995]. The two peptides, YIGSR and IKVAV, have opposite functions, with the former inhibiting [Iwamoto et al., 1996] and the latter stimulating metastasis [Bresalier et al., 1995]. This finding underlines the complexity of the role played by cell-cell and cell-substratum interactions in the metastatic process.

Although the 67LR has been studied mostly as a receptor for laminin, it is now clear that it also recognizes other molecules. Indeed, elastin [Grosso et al., 1991], collagen [Iwabuchi et al., 1996; Minafra et al., 1992], and heparin [Guo et al., 1992] have been described as possible ligands of this receptor, although at different binding sites. For example, the recognition of collagen V by the 67LR appears to depend on

carbohydrates since it is competed by lactose [Minafra et al., 1992], whereas the recognition of collagen IV by the 67LR expressed on neutrophils is sialic acid residue–dependent [Iwabuchi et al., 1996].

RELEASE OF SOLUBLE 67LR

In vitro studies of 67LR-positive tumor cells have shown that the 67LR is shed into the culture medium. The released receptor, which maintains the same molecular weight as the membrane-anchored molecule, also retains its laminin-binding capacity. The shed receptor is recovered in part as a complex with soluble laminin and in part as free receptor still able to bind cell membranes coated with laminin [Karpatová et al., 1996]. Because the complete structure of the 67LR is not yet known, the mechanism by which the 67LR is associated to the cell surface remains unclear. The 37 kD laminin receptor precursor (37LRP) contains a stretch of 16 hydrophobic amino acids that could span the cell membrane [Rao et al., 1989]. Immunofluorescence experiments have demonstrated that epitopes which are amino-terminal to this domain are inaccessible in nonpermeabilized cells, whereas epitopes on the carboxy-terminus bind their corresponding antibodies [Wewer et al., 1987]. This could be due to the association of those epitopes with the other putative polypeptide associated with the 37LRP rather than to the existence of an authentic transmembrane domain.

ROLE OF THE 67LR IN TUMOR PROGRESSION

The original hypothesis on the role of the 67LR in tumor formation was based on the adhesion properties mediated by this receptor [Stallmach et al., 1992]. Indeed, stabilization of laminin binding on tumor cells would in itself explain the increased metastatic potential of 67LR-positive cells, since coating of tumor cells with laminin is known to increase the number of experimental metastases in nude mice [Hunt, 1989]. However, other functions have been associated with 67LR expression in tumors. The demonstration that 67LR mRNA levels are inversely proportional to the population doubling time in SCLC cell lines [Sakai et al., 1992] strongly suggests a role in proliferation. Moreover, in human papillomavirus-associated cervical neoplasms, increased 67LR expression appears to correlate with the proliferative rather than with the invasive properties of the tumor cells since induction occurs in in situ lesions without any evidence of invasion [Demeter et al., 1992]. In accord with this observation, an increase in 67LR expression was found in regenerating rat liver [Anilkumar et al., 1993]. Finally, laminin treatment was also shown to stimulate proliferation of melanoma cells [Mortarini et al., 1995].

As with the free peptide G containing the LMWWML motif, the released 67LR also strongly increases laminin binding to tumor cells, so the released receptor rather than the membrane molecule might be responsible for the increase in aggressiveness.

CONCLUSIONS

The homology of the gene encoding the 67LR precursor, 37LRP, with ribosomal protein genes, which are highly conserved during evolution, suggests that the 37LRP plays multiple roles in the cell. During evolution, the gene appears to have gained a sequence encoding a laminin-binding domain that is responsible for new functions such as cell-matrix interactions. Such interactions, which play a fundamental role in development, are also relevant in cancer progression. Detailed information on how laminin recognition mediates tumor cell-matrix interactions will open new possibilities in efforts to interfere with this process for therapeutic purposes.

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