67 kDa laminin receptor: structure, function and role in cancer and infection

Il recettore per la laminina di 67 kDa: struttura, funzione e ruolo nel cancro e nell'infezione

Vincenza Elena Anna Rea[1], Francesca W. Rossi[2], Amato De Paulis[2], Pia Ragno[3], Carmine Selleri[4], Nunzia Montuori[1]

[1]Department of Cellular and Molecular Biology and Pathology and Center for Basic and Clinical Immunology (CISI);
[2]Department of Clinical Immunology and Allergy and CISI, "Federico II" University, Naples, Italy;
[3]Department of Chemistry and Biology;
[4]Hematology Branch, Department of Medicine, University of Salerno, Salerno, Italy

THE 67 KDA LAMININ RECEPTOR

A 67 kDa m.w. membrane protein able to bind laminin with high affinity was isolated in 1983, in 3 different laboratories, and named 67kDa laminin receptor (67LR).
In 1988 and 1989 cDNA clones coding for human and murine 67 LR were isolated. Unexpectedly, none of the isolated human and murine cDNA clones coded for a 67 kDa protein but for a peptide only 295 amino acid long, with a calculated m.w. of 32 kDa and an apparent electrophoretic mobility in SDS-polyacrylamide gel of about 37 kDa [1]. This peptide was considered to be the precursor of 67LR and named according to its molecular weight, 37 kDa laminin receptor precursor (37 LRP) [2].
Although the 37LRP has a transmembrane domain (amino acid residues 86-101), it is abundantly localized in the cytoplasm. Interestingly, 37LRP appears to be a multifunctional protein involved in the translational machinery and has also been identified as the p40 ribosome-associated protein. In addition, 37LRP has been found in the nucleus, where it is tightly associated with nuclear structures [1].
The mechanism by which 37LRP is incorporated into the mature 67LR is incompletely understood. Recent evidences demonstrate that 67 LR is acylated by fatty acids, suggesting that 37 LRP can dimerize with itself or with another peptide by strong hydrophobic bonds mediated by fatty acids. However, the amino-acid compositions of 67LR and 37LRP are identical, suggesting homodimer formation [3]. 67LR also interacts with integrins, dimeric cell membrane proteins that mediate cell adhesion to the ECM and signal transduction to the nucleus. Recent data indicate that 67LR and the α6β4 integrin may interact; the 67LR-α6β4 complex is able to recognize different sites on laminin, increasing the affinity of the bond itself. The interaction between 67LR and the α6 integrin subunit seems to occur in the cytoplasm, and is followed by cotranslocation to the cell surface [4]. Laminin conformation changes upon binding 67LR, thus interacting more efficiently with integrins [5] and becoming more sensitive to the action of proteolytic enzymes, with the release of motility fragments [6]. In mammalian cells 67LR also acts as a membrane receptor for the cellular prion protein (PrPc), with a role in its binding and endocytosis [7, 8].

LAMININ

Laminin-1, the primary 67LR ligand, is a component of the ECM and is the major glycoprotein of the basement membrane from all types of tissues. Laminin is a high-molecular-mass basement membrane glycoprotein, with a molecular weight of approx. 1 MDa, shaped as an asymmetric cross, with one long arm and three short arms. Laminin is a heterotrimer of three subunits: α (<400 kDa), β (<200 kDa) and γ (<200 kDa). Five different human α chains, three β chains and three γ chains have been identified,

and they can assemble in various combinations to form at least 15 laminin isoforms that have different tissue distributions and appear at different developmental stages [9].

67LR INTERACTION WITH LAMININ AND PRION PROTEINS

37LRP/67LR belongs to the group of Type II membrane proteins, spanning the plasma membrane once (aa 86-101) with its C-terminus exposed to the extracellular space [1].
At least three regions in the C-terminal region of the receptor are involved in the interaction with laminin: repeated sequences (TWEDS) at the C-terminal, a direct laminin binding region (amino acids 205-229) [10] and a heparan sulfate dependent laminin binding region (amino acids 161-180). The best characterized laminin binding domain is the region comprising residues 161-180, the so-called PEPTIDE G, containing the palindromic LMWWML sequence [1].
Rotary-shadowing electron microscopy showed that 67LR predominantly bound to the long arm of whole laminin, just below the cross intersection. The minimum sequence needed to displace 67LR binding to radiolabelled laminin is YIGSR. Both 205-229 and 161-180 sequences of 67LR can bind to the same minimal YIGSR region of the β1 chain of laminin-1 [1, 10].
However, peptide G (residues 161-180) also binds directly to the sulfated polysaccharide heparin. Heparin and laminin compete for binding to peptide G and this may represent a further level of regulation of the interaction between 67LR and the basement membrane [10].
Finally, phage display also revealed that the C-terminal TEDWS repeats of 67LR can also bind to the YIGSR region of laminin-1 [10].
67LR also acts as a receptor of the prion protein (PrP), which is essential for the development of transmissible spongiform encephalopathies. An abnormal form of the prion protein, named PrPsc, accumulates in the brains of affected individuals. Yeast two-hybrid analysis has allowed to identify the 37LRP, precursor of 67LR, as a receptor of cellular prion protein (PrPc) [7, 8] and responsible for the accumulation of PrPsc in infected neuronal cells [11].
A direct PrPc-binding domain on 67LR is localized between amino acid residues 161 and 180 (i.e.peptide G). A second PrPc binding site dependent on heparan-sulfated proteoglycans (HSPG) might be located between amino acid 180 and 285. Two binding domains for 37LRP on PrP have been discovered: a direct binding domain (aa 144-179) and an indirect one (aa 53-93), which depends on the presence of HSPGs that function as co-factors or coreceptors for the binding of PrPc to the 67LR [12].

67LR FUNCTION AS A LAMININ RECEPTOR

67LR overexpression promotes tumor cell adhesion and migration to laminin [1] and is considered a molecular marker of metastatic aggressiveness in cancers of many tissues, including including breast, lung, ovary, prostate, thyroid, as well as in leukaemia and lymphomas [13-15].
In vascular endothelial cells 67LR may have an important role in angiogenesis [16]. Recently, 67LR has been shown to be involved in the mobilization of HSCs (haemopoietic stem cells) by granulocyte-colony-stimulating factor, and the expression levels of 67LR positively correlate with mobilization efficiency of HSCs [17].
Interestingly, 67LR is involved in the regulation of cell proliferation and survival. Indeed, reduction of 67LR expression results in apoptosis; on the contrary, 67LR dependent cell signalling pathways are important for cell survival [18].
Moreover 67LR was identified as an interacting partner of phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes (PED/PEA-15), an anti-apoptotic protein whose expression is increased in several human cancers, enabling cell proliferation and resistance to apoptosis [19].

67LR FUNCTION AS A RECEPTOR OF BACTERIA, VIRUSES AND PRIONS

Some pathogens must necessarily infect host cells during their life cycle. To achieve this result, they often use cell membrane receptors for the extracellular matrix. The 67LR/37LRP protein is used for this purpose by many pathogens. Moreover, PrP, the agent responsible for spongiform encephalopathies, such as the bovine spongiform encephalopathy (BSE), scrapie and kuru is internalized through 67LR engagement [20]. The 67LR also acts as a receptor for some alphaviruses, such as the Sindbis virus, and it is responsible for the infection of mammalian cells by many other viruses. Among them, the 67LR/37LRP is reported to specifically interact with major serotypes of Dengue virus [20].

The protein may also be important in bacterial infections, such as bacterial meningitis development and the internalization of the E. coli K1 strain into brain endothelial cells.
Furthermore, 67LR promotes the internalization of cytotoxic necrotizing factor 1 (CNF1)-positive E. coli [21].

PRP INTERACTIONS

Human PrP (a 235-residue glycoprotein) is anchored to the cell membrane by a C-terminal GPI (glycosylphosphatidylinositol) moiety. PrP acts as a copper-binding protein and is at found at particularly high concentrations at synapse junctions where it is required for synaptic copper ion uptake.
This copper may then be made available to cuproenzymes, such as the copper/zinc superoxide dismutase. PrP functions as a laminin receptor and binds to the C-terminal domain of the γ-1 chain. This interaction is crucial for the process of neuritogenesis. An interaction between 37LRP/67LR and PrP was first established in a two-hybrid screening of a HeLa cDNA expression library for PrP-interacting proteins [7, 8, 12].
The pathogenic relevance of 37LRP/67LR and PrP interaction was revealed in tissues and cells of scrapie compared with non-scrapie-infected mice. Tissues with high levels of PrPsc (the modified pathogenic form of PrP) accumulation displayed correspondingly high levels of 37LRP, in particular in the brain tissue [7]. Both 37LRP and 67LR are expressed on the surface of mouse cortical cells and both forms may act as PrP receptors [8].
The surface expression of functional 37LRP/67LR appears to be a prerequisite for both binding and internalization of PrP [8]. Indeed, using a cell-binding assay with recombinant PrP, a LRP/LR dependent binding of PrP has been shown. Furthermore, it has been demonstrated, that PrP internalization process represents an active receptor mediated event [22]. By immunohistochemistry of adult rat brain, it has been shown that the 67LR is the major receptor form, which is expressed within the cytoplasm and at the plasma membrane in most neurons and in a subset of glia cells [23]. In contrast, 37LRP is much less abundant in adult than in postnatal central nervous system and its expression is restricted to a subclass of cortical interneurons known to be particularly sensitive to abnormal prion accumulation and rapidly degenerate during early stages of Creutzfeldt-Jakob Disease [20]. In addition, recent studies showed that 37LRP/67LR is not only involved in the PrPc metabolism, but fullfills also a crucial role in prion propagation. Using antisense LRP RNA or small interfering RNAs specific for LRP mRNA, PrPSc levels in scrapie-infected neuronal cells were reduced indicating a necessity for the laminin receptor LRP/LR for PrPSc propagation in cultured cells [11]. Very recently it was reported that microinjection of lentiviral vectors expressing siRNAs directed against LRP mRNA into the brain prolongs the pre-clinical phase in scrapie-infected mice [24].
Due to the facts that a (natural) infection with prions mostly occur via an oral route and that 37LRP/67LR act as receptor for prions, potential binding sites for PrP in the intestinal mucosa were examined. Interestingly, it has been demonstrated that bovine PrPSc is internalized by human enterocytes via an LRP/LR-mediated endocytosis [25]. In summary, an important role of the 37LRP/67LR in mediating binding and internalization of the prion protein and its involvement in pathological mechanisms has been clearly demonstrated.

CONCLUSIONS AND PERSPECTIVES

The 67LR represents the receptor for the cellular prion protein PrPc and a receptor for the infectious PrPsc, implicating that LRP/LR might represent a valuable and alternative target in prion disease therapy [26].
Understanding the biochemistry of this molecule is difficult and a proper understanding of 67LR interactions can only be achieved once we know both the molecular composition of the active species along with the three-dimensional structures of both 37LRP and 67LR. Possession of this information would also permit structure-based design of drugs to inhibit or enhance this interaction (or the interaction between 67LR and pathogens) [27].

Keywords: 67 laminin receptor, extracellular matrix, prion protein.

Conflict of interest disclosure
The authors declare that the article has not been sponsored, that no financial support has been given and finally that there is no conflict of interest.

SUMMARY

The 67 kDa high affinity laminin receptor (67LR) is a non integrin cell surface receptor for the extracellular matrix whose expression is increased in neoplastic cells and directly correlates with an enhanced invasive and metastatic potential. 67LR derives from homo- or hetero-dimerization of a 37 kDa cytosolic precursor (37LRP), by fatty acid acylation.
Interestingly, 37LRP is a multifunctional protein involved in the translational machinery and has also been found in the nucleus, where it is tightly associated with nuclear structures.
Acting as a receptor for laminin is not the only function of this protein; indeed, 67LR also acts as a receptor for viruses, such as Sindbis virus and Dengue virus, and is involved in the internalization of the prion protein.
Here, we review the current understanding of the structure and function of this molecule, highlighting its role in cancer and infectious diseases.

RIASSUNTO

Il recettore ad alta affinità per la laminina di 67 kDa (67LR) è un recettore di membrana di tipo non integrinico per la matrice extracellulare, la cui espressione è aumentata nelle cellule neoplastiche e correla direttamente con un aumentato potenziale invasivo e metastatico. Il 67LR deriva dall'omo-o etero-dimerizzazione di un precursore citoplasmatico con peso molecolare di 37 kDa (37LRP), mediante acilazione con acidi grassi. È interessante notare che il 37LRP è una proteina multifunzionale, coinvolta anche nell'apparato traduzionale e localizzata nel nucleo, dove è strettamente associata a strutture nucleari.
Agire come recettore per la laminina non è l'unica funzione di questa proteina; infatti, il 67LR è anche un recettore per virus, come Sindbis virus e virus Dengue, ed è coinvolto nell'internalizzazione della proteina prionica.
In questo lavoro, passiamo in rassegna le attuali conoscenze della struttura e del funzionamento di questa molecola, evidenziando il suo ruolo nel cancro e nelle malattie infettive.

REFERENCES

[1] Montuori N., Sobel M.E. The 67-kDa laminin receptor and tumor progression. *Curr. Top. Microbiol. Immunol.* 213, 205-214, 1996.
[2] Rao C.N., Castronovo V., Schmitt M.C., et al. Evidence for a precursor of the high-affinity metastasis-associated murine laminin receptor. *Biochemistry* 28, 7476-7486, 1989.
[3] Landowski T.H., Dratz E.A., Starkey J.R. Studies of the structure of the metastasis-associated 67 kDa laminin binding protein: fatty acid acylation and evidence supporting dimerization of the 32 kDa gene product to form the mature protein. *Biochemistry* 34, 11276-11287, 1995.
[4] Ardini E., Tagliabue E., Magnifico A., et al. Co-regulation and physical association of the 67 kD monomeric laminin receptor and the ⊠6⊠4 integrin. *J Biol. Chem.* 272, 2342-2345, 1997.
[5] Berno V., Porrini D., Castiglioni F., et al. The 67 kDa laminin receptor increases tumor aggressiveness by remodeling laminin-1. *Endocr. Relat. Cancer* 12, 393-406, 2005.
[6] Ardini E., Sporchia B., Pollegioni L., et al. Identification of a novel function for 67-kDa laminin receptor: increase in laminin degradation rate and release of motility fragments. *Cancer Res.* 62, 1321-1325, 2002.
[7] Rieger R., Edenhofer F., Lasmézas C.I., Weiss S. The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells. *Nat. Med.* 3, 1383-1388, 1997.
[8] Gauczynski S., Peyrin J.M., Haïk S., et al. The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. *EMBO J.* 20, 5863-5875, 2001.
[9] Patarroyo, M., Tryggvascon, K., Virtanen, I. Laminin isoforms in tumour invasion, angiogenesis and metastasis. *Semin. Cancer Biol.* 12, 197-207, 2002.
[10] Kazmin D.A., Hoyt T.R., Taubner L., et al. Phage display mapping for peptide 11 sensitive sequences binding to laminin-1. *J. Mol. Biol.* 298, 431-45, 2000.
[11] Leucht C., Simoneau S., Rey C., Vana K., Rieger R., Lasmézas C.I., Weiss S. The 37kDa/67 kDa laminin receptor is required for PrP(Sc) propagation in scrapie-infected neuronal cells. *EMBO Rep.* 4, 290-295, 2003.
[12] Hundt C., Peyrin J.M., Haïk S., et al. Identification of interaction domains of the prion protein with its 37-kDa/67-kDa laminin receptor. *EMBO J.* 20, 5876-5886, 2001.
[13] Menard S., Tagliabue E., Colnaghi M.I. The 67 kDa laminin receptor as a prognostic factor in human cancer. *Breast Cancer Res. Treat.* 52, 137-145, 1998.

[14] Montuori N., Selleri C., Risitano A.M., et al. Expression of the 67-kDa Laminin Receptor in acute myeloid leukemia cells mediates adhesion to laminin and is frequently associated with monocytic differentiation. *Clin. Cancer Res.* 5, 1465-1472, 1999.
[15] Montuori N., Müller F., De Riu S., et al. Laminin receptors in differentiated thyroid tumors: restricted expression of the 67-kilodalton laminin receptor in follicular carcinoma cells. *J. Clin. Endocrinol. Metab.* 84, 2086-2092, 1999.
[16] Stitt A.W., McKenna D., Simpson D.A.C., et al. The 67 kDa laminin receptor is preferentially expressed by proliferating retinal vessels in a murine model of ischemic retinopathy. *Am. J. Pathol.* 152, 1359-1365, 1998.
[17] Selleri C., Ragno P., Ricci P., et al. The metastasis-associated 67-kDa laminin receptor is involved in G-CSF-induced hematopoietic stem cell mobilization. *Blood*. 108, 2476-2484, 2006.
[18] Scheiman J., Tseng J.C., Zheng Y., Meruelo D. Multiple functions of the 37/67-kd laminin receptor make it a suitable target for novel cancer gene therapy. *Mol. Ther.* 18, 63-74, 2010.
[19] Formisano P., Ragno P., Pesapane A., et al. PED/PEA-15 interacts with the 67 kDa laminin receptor and regulates cell adhesion, migration, proliferation and apoptosis. *J. Cell. Mol. Med.* 16: 1435-1446, 2012.
[20] Vana K., Zuber C., Pflanz H., et al. LRP/LR as an alternative promising target in therapy of prion diseases, Alzheimer's disease and cancer. *Infect. Disord. Drug Targets.* 9, 69-80, 2009.
[21] Kim K.J., Chung J.W., Kim K. S. 67-kDa laminin receptor promotes internalisation of cytotoxic necrotizing factor 1-expressing *E. coli* K1 into human brain microvascular endothelial cells. *J. Biol. Chem.* 280, 1360-1368, 2005.
[22] Nelson J., McFerran N.V., Pivato G., et al. The 67 kDa laminin receptor: structure, function and role in disease. *Biosci. Rep.*, 28, 33-48, 2008.
[23] Baloui H., von Boxberg Y., Vinh J., et al. Cellular prion protein/laminin receptor: distribution in adult central nervous system and characterization of an isoform associated with a subtype of cortical neurons. *Eur. J. Neurosci.* 20, 2605-2616, 2004.
[24] Pflanz H., Vana K., Mitteregger G., et al. Microinjection of lentiviral vectors expressing small interfering RNAs directed against laminin receptor precursor mRNA prolongs the pre-clinical phase in scrapie-infected mice. *J. Gen. Virol.* 90, 269-274, 2009.
[25] Kolodziejczak D., Da Costa Dias B., Zuber C., et al. Prion interaction with the 37-kDa/67-kDa laminin receptor on enterocytes as a cellular model for intestinal uptake of prions. *J. Mol. Biol.* 402, 293-300, 2010.
[26] Omar A., Jovanovic K., Da Costa Dias B., et al. Patented biological approaches for the therapeutic modulation of the 37 kDa/67 kDa laminin receptor. *Expert Opin. Ther. Pat.* 21, 35-53, 2011.
[27] Lavecchia A., Di Giovanni C., Pesapane A., et al. Discovery of new inhibitors of Cdc25B dual specificity phosphatases by structure-based virtual screening. *J Med Chem.* 55, 4142-4158, 2012.