Laminin Binding Conveys Mechanosensing in Endothelial Cells

Torsten Gloe and Ulrich Pohl

Institute of Physiology, Ludwig Maximilians University, 80336 Munich, Germany

In endothelial cells, forces like shear stress are transferred to focal adhesion sites and activate in concert with matrix receptor kinases, leading to an initiation of signaling cascades. The laminin binding protein is one of these matrix receptors and is critically involved in sensing and quantification of shear stress.

Endothelial cells are specifically located on the border between the bloodstream and the underlying extracellular matrix, called the basement membrane. The cellular adhesion to this matrix occurs via certain receptors and is a prerequisite for the maintenance of an intact vascular wall. However, if the main duty of matrix attachment were only pure adhesion to preserve the overall structure of the tissue, the matrix composition would consequently be poor and its distribution simple. The opposite is found in nature: the matrix composition appears to be very complex, and the list of new components is still growing. But more importantly, its architecture remains to be identified and described in detail. This complex overall structure indicates that the matrix could contain additional information for the endothelial cells that, under certain conditions such as elevated shear stress, may be translated into cellular signals.

Endothelial cells sense shear stress with focal adhesion sites to the matrix

Because of their special position, endothelial cells are ideally suited for sensing changes in blood flow velocity, which cause differences in shear stress. These changes in shear stress are directly responsible for numerous cellular responses, like release of autacoids such as nitric oxide, prostaglandins, and the endothelial-derived hyperpolarizing factor, which are all necessary for rapid adaptations in vessel diameter. Beside these fast and acute reactions, there are also known long-term responses that result, for example, in altered gene expression. According to a widely accepted hypothesis, shear stress is sensed by endothelial cells in so-called focal adhesion sites (Fig. 1) (9). These are structures in which clustered matrix receptors meet the cytoskeleton via certain linker molecules. Changes in their composition and/or cellular tension lead subsequently to the activation of kinases, which in turn initiate appropriate signaling cascades for altered gene expression.

The matrix receptors can be roughly divided into two classes: the integrin and nonintegrin groups, both of which critically participate in the translation of matrix-borne information into cellular signals. This leads, for example, to new vessel formation and remodeling not only during embryonic development but also in response to chronic changes in shear stress and low oxygen tensions and even in pathophysiological situations like tumor development and metastasis.

The complicated network of signaling cascades is not only regulated by the recruitment of various matrix receptors within the cell but also by the composition of the matrix itself and by the fact that, because of extracellular proteases, this composition could further be altered after synthesis by proteolytic cleavage. As a consequence, hidden or cryptic new binding sites to matrix components are exposed and lead via specific receptors to an additional signal generation within the cell (19).

Cellular responses to changes in shear stress are (in part) laminin dependent

During exposure to changes in laminar flow, endothelial cells alter their morphology and orientation from a cobblestone formation to a more elongated growth pattern, which is aligned to the direction of the flow. This is the first hint that extracellular mechanical forces like shear stress are recorded and quantified by the endothelial cell and translated into cellular responses. Following the hypothesis that binding to the extracellular matrix serves as an initiator for those intracellular signals, cell-matrix interactions are predestined to sense shear stress, helping the cells to respond appropriately to changes in their mechanical load.

One example is the gene expression of endothelial nitric oxide synthase (eNOS), a protein that appears, among other things, to be upregulated by nearly twofold during shear stress. To narrow down possible matrix receptors that might be responsible for this increased expression, we used different matrix components such as adhesion substrate for endothelial cells during shear stress experiments. Surprisingly, coating the culture vessels with collagen type I or fibronectin or the use of noncoated glass plates had no inductive effects on eNOS expression under shear stress. Only in the case of a laminin-rich matrix was the expected twofold increased gene expression detected, both on the level of transcription and translation, indicating that laminin might play a key role in shear stress-dependent eNOS expression (10).

Laminins are glycoproteins and are known to be a major component of the basement membrane. They are directly responsible for numerous cellular responses, like release of autacoids such as nitric oxide, prostaglandins, and the endothelial-derived hyperpolarizing factor, which are all necessary for rapid adaptations in vessel diameter. Beside these fast and acute reactions, there are also known long-term responses that result, for example, in altered gene expression. According to a widely accepted hypothesis, shear stress is sensed by endothelial cells in so-called focal adhesion sites (Fig. 1) (9). These are structures in which clustered matrix receptors meet the cytoskeleton via certain linker molecules. Changes in their composition and/or cellular tension lead subsequently to the activation of kinases, which in turn initiate appropriate signaling cascades for altered gene expression.

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Laminin consists of three polypeptide chains (α, β, and γ) that are joined together in a cross shape. By a combination of five α-chain isoforms, three β-chain isoforms, and two γ-chain isoforms in total, 11 different laminin isoforms are generated (Table 1) (16).

Most of these isoforms are known to be differently expressed during embryogenesis, indicating their importance in tissue development. Since it can easily be isolated from the EHS tumor, the availability of laminin I is very high; therefore, it is by far the best-studied laminin, and numerous cellular binding proteins to this isoform have been described.

### Laminin receptors and their binding sites

Figure 2 summarizes some of these binding sites, which were mostly identified by in vitro binding studies. Several integrins are described to bind to laminin I, whose binding sites are located at the lower stem of the cross-shaped molecule (3). However, it has also been reported that, after limited proteolytic digestion, other integrins are also enabled to bind to sites in the upper part of the α-chain (6). As a prototype of nonintegrin receptors, the 67-kDa laminin-binding protein (LBP) is one of the best studied. Its binding site to laminin is located at the amino acid subsequence YIGSR of the γ1-chain (12). Usually, binding sites are verified by cell adhesion assays to intact laminin in the presence of soluble peptides, which represent the binding subsequences and compete with the receptors. However, not much is known about the recruitment and interplay between these distinct receptor types in complex and mature matrices. In summary, adhesion studies showed that integrins are serving as the principal receptors and are responsible for cellular adhesion to laminin, whereas the nonintegrin receptor proteins like LBP may have different accessory functions in the initiation of signaling cascades.

### Nature and structure of LBP

The gene for LBP, which in the literature is also termed the 67-kDa LBP, p40, and 37-kDa LBP, is located on chromosome 3 at the locus 3p21.3, and it was shown by Jackers et al. (13) that several processed pseudogenes could be found. All of the cells studied in vitro so far express LBP, and interestingly, expression can be correlated to the metastatic potency of certain tumor cells. Since LBP is also proposed as a member of the translational machinery (18), one might speculate that LBP belongs to the so-called housekeeping gene family of con-

### TABLE 1. Laminin isoforms

<table>
<thead>
<tr>
<th>Name</th>
<th>Other/Trivial Name</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN-1</td>
<td>EHS-laminin</td>
<td>α1β1γ1</td>
</tr>
<tr>
<td>LN-2</td>
<td>Merosin</td>
<td>α2β1γ1</td>
</tr>
<tr>
<td>LN-3</td>
<td>S-laminin</td>
<td>α1β2γ1</td>
</tr>
<tr>
<td>LN-4</td>
<td>S-merosin</td>
<td>α2β2γ1</td>
</tr>
<tr>
<td>LN-5</td>
<td>Kalinin</td>
<td>α3β3γ2*</td>
</tr>
<tr>
<td>LN-6</td>
<td>K-laminin</td>
<td>α3β1γ1*</td>
</tr>
<tr>
<td>LN-7</td>
<td>KS-laminin</td>
<td>α3β2γ1*</td>
</tr>
<tr>
<td>LN-8</td>
<td></td>
<td>α4β1γ1</td>
</tr>
<tr>
<td>LN-9</td>
<td></td>
<td>α4β2γ1</td>
</tr>
<tr>
<td>LN-10</td>
<td>Drosophila-like laminin</td>
<td>α5β1γ1</td>
</tr>
<tr>
<td>LN-11</td>
<td></td>
<td>α5β2γ1</td>
</tr>
</tbody>
</table>

*The α3 chains of these isoforms may be full length or truncated.
stantly expressed genes (1).

In the past, several groups of investigators studied the structure of the LBP protein independently. Castronovo et al. (7) used specific antibodies raised to subsequences of LBP to monitor its distribution within and at the cell surface. By this method, they mapped a region around amino acids 86–101, which most probably represents the transmembrane region. The terminal region faces the extracellular space, and the first part is exposed to the cytoplasm. The laminin binding site was mapped to amino acids 161–180. From its mRNA sequence, the estimated size of LBP would be ~37 kDa. Using antibodies against peptides deduced from the cDNA sequence, most investigators found an additional band in SDS-PAGE of 67 kDa in size. This posttranslational modification could be due to homo- or heterodimerization via fatty acids; however, the exact nature of this modification needs to be further characterized. Interestingly, this modification is very stable and cannot be reversed by denaturing with SDS nor by reducing agents like mercaptoethanol (5, 14).

The most intensively studied feature of LBP is its ability to bind to laminin. Iwamoto et al. (12) were screening proteins that bind laminin by using affinity chromatographic techniques and first described the 37/67-kDa laminin-binding protein as LBP. This binding was very strong and could be inhibited with the peptide YIGSR, a subsequence of the short arm of the laminin β1-chain. In later studies, this peptide was used to prevent cellular binding to laminin, and it was found that application of YIGSR greatly inhibited new vessel formation not only in vitro but also in vivo. These inhibitory effects are mainly due to an impaired angiogenesis, leading to insufficient oxygen and nutrient supply to the tumors. The latter fact led to the hypothesis that YIGSR would be an attractive therapeutic agent against tumor growth. Numerous papers exist describing the inhibitory effect of the peptide YIGSR in angiogenesis, tumor growth, and metastasis. In addition, it was shown for certain tumors that the LBP expression correlates well with its aggressiveness and therefore could probably be used as a prognostic marker (17). However, the exact mechanism and function of LBP as a matrix receptor is not fully understood.

The nonintegrin laminin receptor LBP is involved in mechanosensing

One of the functions of LBP could be mechanosensing. Extending studies that showed that shear-stress-induced eNOS gene expression is critically dependent on the existence of laminin I in the extracellular matrix, we repeated shear stress experiments in the presence of the LBP inhibitory peptide YIGSR, supposing that this subsequence of the laminin β1-chain represents the binding site of LBP and could function as a competitive inhibitor for the laminin LBP interaction. With this peptide, no changes in cellular adhesion during shear stress were found, verifying that LBP is not a matrix receptor for firm physical adhesion. However, the increased gene expression of eNOS was completely abolished by YIGSR, whereas the incubation of a control peptide (YIGSK, with a conservative exchange in the last position) had no inhibitory effect. These data indicate that LBP is more likely involved in the generation of signal cascades leading to altered gene expression (10).

These results fit well to findings showing that LBP is co-localized with vinculin and α-actinin, which are members of the focal adhesion points where changes of shear stress seem to be recorded and translated into cellular signals (15). Furthermore, it was shown by Ardini et al. that LBP is co-, up-, and down-regulated at the translational level with certain integrins (2), and is involved in protein tyrosine phosphorylation (4). This leads to the hypothesis that one single matrix component, like laminin, might be bound simultaneously by two distinct types of receptors, integrins and LBP. By this the architecture of the matrix would be mirrored into the cell. This process was studied by Colognato et al. (8), who could impressively demonstrate that the extracellular pattern of polymerized laminin induces a corresponding receptor-cytoskeleton network within the cell. Consequently, changes in the composition and/or overall structure of the matrix would be recognized and translated into intracellular signals via changes in the fine structure of focal adhesion sites (Fig. 3). It is attractive and reasonable to
assume that binding to one single laminin molecule by both integrins and LBP leads to a clustering and fixation of these receptors and, even more importantly, of their intracellular binding partners as well. Changes in the mechanical load of these cells would then alter this fine structure and could enable focal adhesion site-associated kinases to phosphorylate their targets and initiate intracellular signaling cascades.

Summary

Considering all of this information together, it is very likely that the cellular laminin binding via LBP serves more as a regulatory than as a cell adhesion element. This fact can be supported by our own observations as well by others. Cellular attachment to laminin I could only be inhibited by 50% when the LBP-antagonizing peptide YIGSR was used in very high doses, whereas the application of RGD peptides, which interfere with integrin binding, are much more potent and almost completely inhibit adhesion at low doses.

To fully understand the function of LBP in the generation of intracellular signal cascades, future work must study the intracellular linkers by which LBP is connected to signaling components, such as kinases, and the exact mechanisms in which LBP is involved in the activation of signal cascades leading to adaptations to the mechanical load of endothelial cells, such as increased angiogenesis and arteriogenesis due to elevated shear stress.

References