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Biological Activities of Laminin

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Laminin is a multifunctional protein with diverse biological activities. Like fibronectin, it can influence cell adhesion, growth, morphology, differentiation, migration, and agglutination as well as the assembly of the extracellular matrix. Laminin primarily affects cells of epithelial origin, and the response varies depending on the cell. Because most differentiated cells are difficult to maintain in culture, laminin may be an important supplement in studies on cell differentiation in vitro.

Key words: laminin, basement membrane, cell adhesion, extracellular matrix

Laminin is a large ($M_r = 10^6$) glycoprotein present in all basement membranes [1-7], where it is the most abundant constituent. It binds to various components of basement membrane and probably links these to one another [2,8-14] to form an integrated complex [7]. Laminin has been shown to have diverse biological activities (Table I), which are discussed in detail below in relation to information on the structure of laminin and its interaction with other molecules. A specific cell surface receptor [13-17] that may play a role in mediating the effects of laminin on cell adhesion [8,13,15-25], growth [26-31], morphology [32-35], and differentiation [36-41] has been identified for laminin. Laminin appears early in development [39] and has been identified in the four-cell stage embryo [36,37]. Most studies on laminin have utilized the protein isolated from various tumors [1,42,43] or produced by cell lines [44,45].

STRUCTURE AND BIOLOGICAL ACTIVITIES

Structure

When isolated in its native form, laminin was determined to be a glycoprotein composed of two or three chains of $M_r = 200{,}000$ and one chain of $M_r = 400{,}000$. These chains are held together by disulfide bonds [1]. Electron microscopy of rotary shadowed laminin revealed a cross-shaped structure [46] with rodlike segments and globular domains or regions. Laminin binds to matrix components, including type IV collagen, heparan sulfate proteoglycan, entactin, and nidogen [8-10,47], and to heparin. Using a combination of electron microscopy of rotary shadowed fragments

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TABLE I. Biological Activities of Laminin

Cell adhesion
Cell growth
Cell morphology
Cell differentiation
Matrix assembly
Cell migration

of laminin and chemical and biological assays, the domains on laminin for type IV collagen, heparin, and the cell surface binding site have been identified [2,12–14] (Fig. 1). The type IV collagen binding sites are present on the short chains, whereas the heparin binding site is located at the globular end of the long chain. A cell surface binding site has been localized to the region where the two or three short chains interact [12,13] and others probably exist, depending on the type of cell and the presence of type IV collagen [14]. Due to these multiple interactions, it is likely that laminin has a major role in maintaining the stability of the matrix as well as in anchoring cells to the basement membrane.

Cell Adhesion

Laminin at levels of 5 μg/ml promotes the attachment of various epithelial cells to plastic or to type IV collagen-coated substrates. Unlike fibronectin, laminin is able to bind either to the cell surface or to collagen, although laminin appears to be most effective as an attachment protein for epithelial cells when bound to type IV collagen. Cells attach rapidly under these conditions and spread or elongate (Fig. 2). Using affinity chromatography or Western blotting types of studies, it has been possible to demonstrate specific, high affinity receptors (M_r = 67,000) for laminin on epithelial, muscle, and tumor cells [13,15–17]. The exact cellular distribution of the laminin receptor is not yet known. Many epithelial cells (Table II) recognize laminin and use it for attachment. Certain fibroblasts do not recognize laminin and will not survive well in culture if excess laminin is present [26]. Embryonic fibroblasts, however, behave differently, and a subpopulation of these cells can bind to laminin and can grow in its presence [24,48]. Some cells, such as hepatocytes [19,20,24], endothelial cells [8,49], and Schwannoma cells [35], can recognize both laminin and fibronectin. These cells presumably have receptors for both proteins.

Cell Growth

Laminin has been found to promote the growth of various cell types (Table III). Of the cells tested, most respond with a two- to fourfold increase in cell growth compared to that seen on tissue culture plastic (Table IV). The response can be observed when laminin is present on the substrate at the time of plating or if it is added one day after the cells have attached. Thus, it is not just increasing cell growth by promoting the initial adhesion of the cells. The amount required to increase the growth rate of cells is much higher than that required for cell adhesion. The reason for the large amounts required is unclear. Soluble laminin may be inactivated, as it is not very stable ($t_{1/2}$ 49°C, 30 min) [50]. Thus, higher levels of the soluble form may be required. Alternatively, laminin may act at a different site such as, for example, in binding heparan sulfate proteoglycan. Also, because laminin has been shown to have a role in matrix assembly (see below) and matrices promote cell growth [51,52], it

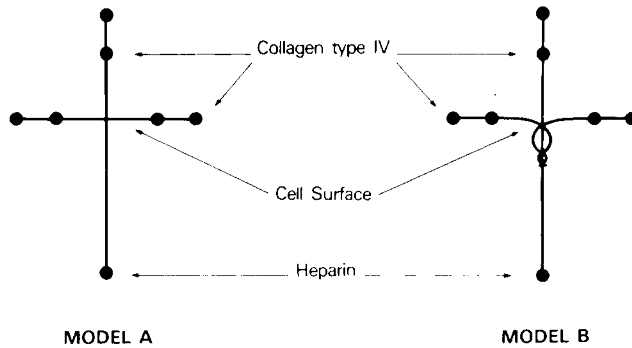

Fig. 1. Structural organization and localization of domains of laminin [12–14]. Two possible models are presented. Model A consists of three short chains held to the long chain by disulfide bonds. Model B shows two short chains which have one end each coiling around the long chain near the center.

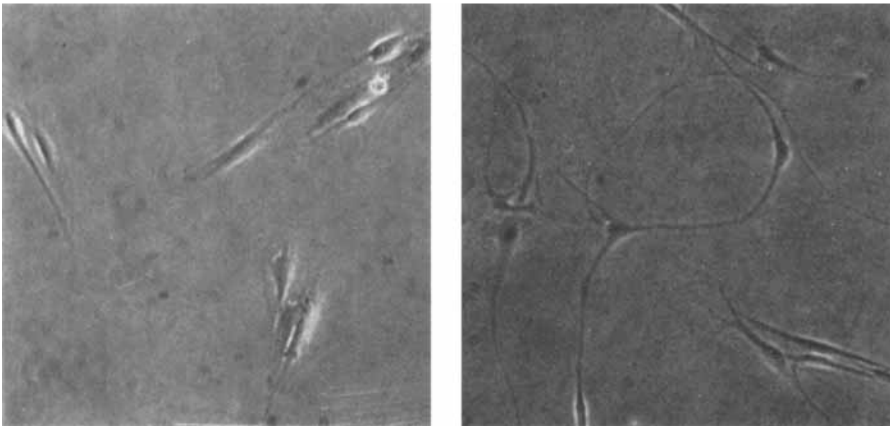

Fig. 2. Effect of laminin on Schwann cell morphology. Embryonic rat Schwann cells were cultured in the presence (right) and absence (left) of 100 μg/ml of laminin for 24 hr. Laminin caused the cells to assume a more elongated appearance. This phenotype is more typical of the in vivo morphology where the cells stretch out and wrap around the neurons.

TABLE II. Cells Binding to Laminin

Cell type	Reference
PAM 212, endothelial cells, parietal yolk sac cells, choroid epithelial cells, pigmented eye epithelial cells, carcinoma and sarcoma cells	8,18
Hepatocytes	19,20
Metastatic melanoma cells	21
Adenovirus transformed cells	22
Neuroblastoma	23
Fibroblasts (embryonic)	24,49
Schwann cells	25
HT 1080 fibrosarcoma	49

TABLE III. Cells Whose Growth is Stimulated by Laminin

Cell type	Reference
Schwann cells	25
PAM 212	26
Neurite	27, 28, 31
Hepatocytes	L. Reid, unpublished
B16 melanoma	33, see Table IV
Renal tubular cells	29
Embryonal carcinoma cells	30
M-50-76 reticulosarcoma	V.P. Terranova, unpublished

TABLE IV. Effect of Laminin on B16 Melanoma Cell Growth and Pigmentation

Laminin added μg	Cell number $\times 10^3$	Pigment absorbance 400 nm μ
0	4.0	0.03
5	5.2	0.04
10	8.3	0.07
20	6.3	0.05
50	10.6	0.09
100	10.0	0.10
200	12.3	0.17

Cells (2×10^5 per 16mm/dish) were grown for 5 days on substrates containing the indicated amounts of laminin per dish. The surfaces were coated with laminin one hour before the cells were added. Laminin did not affect significantly the attachment of the cells. Therefore, the increase in cell number is due to increased growth. Cell number was determined by a Coulter counter and the amount of pigment synthesized was determined from the cell layer after freeze-thawing the cells to release the pigment. No pigment was seen in the absence of laminin. Although the increased pigment was not normalized for cell number, visible pigment was seen in the cell layer and the media after exposure to laminin. Thus, laminin increased both cell number and the pigment production by the cells.

may act by providing a favorable substrate for the cells. Additionally, changes in cell shape have been correlated with changes in cell growth [51,53]; therefore, laminin, which alters the morphology of cells (see below), may exert its growth promoting effects by indirectly altering the shape of the cells, thus making receptors for growth factors more available.

Laminin also has been shown to promote neurite outgrowth [27,28,31]. This has been observed both with human spinal ganglion explants [27] and with cultured central and peripheral neurons [28]. In addition, a specific site near the heparin binding on the long chain of laminin has been found to contain the activity [31]. Thus,

laminin may be important during nerve development and may be useful therapeutically to promote nerve repair.

Cell Morphology

Sugrue and Hay [32] were the first to observe that laminin altered cellular morphology and that epithelial cells devoid of basement membrane produced blebs from their basal surface suggesting that the basal plasmalemma was unstable. Addition of laminin stabilized the membrane and stopped the blebbing. Since then, various cells have been observed to undergo shape changes in response to laminin (Table V). Unlike fibronectin, which primarily causes cell spreading, laminin causes distinct morphological changes that are dependent on the cell type. For example, Sertoli cells become more columnar on a laminin substrate [34], whereas Schwann cells become more elongated [25,35]. B16 melanoma cells become more aggregated [33], whereas oligodendrocytes send out long processes [Dubois-Dalcq, unpublished]. In the presence of laminin, parotid gland cells no longer grow as suspended aggregates but flatten on the culture dish in groups of cells to form a lumen [54]. Laminin thus promotes a morphology for these cells, which is more characteristic of the in vivo appearance of a particular cell population.

Cell Differentiation

Various observations suggest that laminin has a key role in development. The small chains of laminin appear in the developing embryo at the four- to eight-cell stage [36,37], and both chains have been demonstrated in 16-cell stage embryos [37,38]. The synthesis of laminin during kidney differentiation coincides with the appearance of type IV collagen and heparan sulfate proteoglycan [39]. Direct evidence for the role of laminin in differentiation has come from cell culture studies. Laminin has a key and perhaps essential role in epithelium formation and in the differentiation of F9 cells [40]. When added to sparse F9 cells, low concentrations of laminin stimulate embryoid body formation and α-fetoprotein synthesis, whereas antibody to laminin blocks differentiation of these cells. Darmon [41] showed that, depending on the cell density, laminin causes teratocarcinoma cells to differentiate into either neurites or multinucleated striated muscle cells. The differentiation of B16 melanoma cells is stimulated by laminin (Table IV). Melanin synthesis is increased several-fold when laminin is present on the substrate, but not as well when it is added to the culture medium. To date, only a few cell types have been tested, but it is likely that the biological activities of a wide variety of cultured cells will be affected by the presence of laminin.

TABLE V. Cells Whose Morphology is Altered by Laminin

Cell type	Morphology change	Reference
Corneal epithelium	Blebbing stops	32
Sertoli	Become columnar	34
Schwann	Elongate 3-fold	25, 35
B16 melanoma	Aggregate	33
Oligodendrocytes	Assume stellate shape	Dubois-Dalcq, unpublished
Parotid, lacrimal, and pancreatic acinar	Form glandlike structures with lumen	54
HT-1080	Become spread	49

Matrix Assembly

Laminin, as the major component of basement membranes, has a key role in the organization of this matrix. It binds to various basement membrane components including type IV collagen, heparan sulfate proteoglycan, entactin, and nidogen [8–11,47]. It was first shown to bind to type IV collagen in cell attachment assays [8]. Later, using direct measures of binding by ELISA and affinity chromatography, it was demonstrated that laminin recognizes native type IV collagen and not the denatured protein [9]. Recent studies which report that laminin does not bind to type IV collagen may have employed partially denatured type IV collagen [55]. Laminin also was found to bind to heparin [10] and later to heparan sulfate proteoglycan [9,11]. Because it has these multiple interactions, it is not surprising that a role in the assembly of basement membranes has been documented both in culture and with various matrix components. For example, when added to cultured thyroid cells that produce only small segments of basement membrane, laminin causes the rapid deposition of a continuous basement membrane [56].

In vitro studies with pure and partially purified components have yielded significant information about the interactions of laminin. When incubated separately, laminin, type IV collagen, and proteoglycan are soluble; when mixed together, they form a large floccular precipitate containing laminin, type IV collagen, and heparan sulfate proteoglycan in molar ratios of 1:1:0.1 [57]. These are comparable to the proportions of these components present in basement membranes [58]. Recently, unfractionated extracts of the EHS tumor have been found to reconstitute into a gel-like structure under physiological conditions [33]. Both type IV collagen and heparan sulfate proteoglycan increase the total amount of material that incorporates into the gel, but the proportions of each remain constant (Fig. 3). The gel is enriched in laminin (60%), with lesser amounts of entactin, nidogen, type IV collagen, and heparan sulfate proteoglycan (Fig. 3). At the ultrastructural level, the gel supplemented with type IV collagen and heparan sulfate proteoglycan is seen to be composed of a network with strands similar in thickness and in appearance to the lamina densa of authentic basement membranes [33]. These observations are in agreement with the concept that laminin is an integral basement membrane component [7]. Morphological examination suggests that laminin coats type IV collagen filaments to form 3–8 nm wide cords that make up the lamina densa [59]. Therefore, laminin could play an organizational role in matrix assembly via its multiple interactions with other basement membrane components.

OTHER ACTIVITIES OF LAMININ

Haptotaxis

Laminin has been shown to promote cell migration. In vitro, Schwannoma cells show increased directed movement to laminin [60], and both fibronectin and laminin promote the haptotactic migration of B16 mouse melanoma cells in vitro [61]. Rabbit neutrophils migrate in response to concentrations of laminin similar to that of f-Met-Leu-Phe, a very potent chemoattractant [62]. Such cell movements are probably important during embryogenesis, wound repair, and tumor cell metastasis, and it is expected that laminin plays a role in these events.

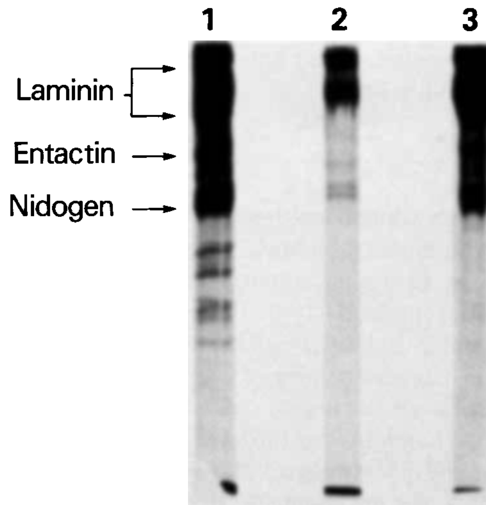

Fig. 3. SDS gel electrophoresis of the components present in the 2M urea EHS tumor extract and in the gel formed at 37°C. The EHS tumor was washed with 3.4M NaCl, 0.05M Tris-HCl, pH 7.4, containing protease inhibitors. Then the tissue was extracted with 2.0M urea, 0.05M Tris-HCl, pH 7.6, and dialyzed into 0.15M NaCl, 0.05M Tris-HCl, pH 7.4. When warmed, the solution formed a gel. Shown are the total protein in the extract (lane 1) and the material present in the gel (lane 2). When the extract is supplemented with type IV collagen and heparan sulfate proteoglycan, more protein incorporates into the gel (lane 3).

Agglutination

Like fibronectin, laminin agglutinates fixed erythrocytes [63]. However, sugars do not block this effect, and it is not clear that laminin is a lectin with simple sugar specificity. Certain gangliosides ($GD_{1a} > GT_{1b}$) and charged phospholipids have been reported to inhibit this agglutination, suggesting that these components also interact with laminin. Whether these components have a role in the biological activity of laminin has not yet been demonstrated.

Tumor Cell Metastases

Invasive tumor cells must traverse basement membranes to spread to secondary sites. Recent studies suggest that this process is likely enhanced by the strong interactions of these cells with laminin. Subpopulations of tumor cells with high metastatic propensity can be selected from the mass of tumor cells based on their rapid adhesion to laminin in vitro [21]. This was expected, as certain metastatic tumor cells adhere preferentially to laminin better than do their nonmetastatic counterparts [21] and have greater numbers of laminin receptors on the cell surface [64]. However, when tumor cells, such as B16F1 melanoma cells, are cultured for one week in laminin, they become more metastatic [64]. Thus, laminin may be allowing for the selection of more metastatic tumor cells or causing cells that are not metastatic to become metastatic. Alternatively, the shape of cultured tumor cells has been shown to be correlated with the metastatic potential [65]. Because laminin can affect cell shape, it may be exerting its effect in this manner. In addition, when laminin is added

to certain tumor cells just before injection into the animal, the metastatic activity is increased [66]. Taken together, these data demonstrate that laminin increases the metastatic phenotype of tumor cells.

CONCLUSIONS

Basement membranes contain laminin as a major component. Due to its multiple interactions with other basement membrane components and its potent effects on cell behavior in vitro, it is likely an important, integral component of the extracellular matrix. Because it can bind to type IV collagen, heparan sulfate proteoglycan, nidogen, and entactin and form large insoluble complexes, laminin probably interacts in the tissue in a similar manner and plays a role in matrix organization. Laminin also affects epithelial cell behavior, including cell adhesion, growth, morphology, differentiation, and migration. Laminin thus links resident cells to the basement membrane and modulates in part the phenotype of the cells. Laminin probably exerts these activities in vivo during tissue development, in pathological conditions, including the spread of tumor cells, and in wound repair.

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