Localization of Three Distinct Heparin-binding Domains of Laminin by Monoclonal Antibodies*

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Monoclonal antibodies were utilized to localize novel heparin-binding domains of laminin. A solid-phase radioligand binding assay was designed such that [3H]heparin bound to laminin in a time- and concentration-dependent manner. Tritiated heparin binding to laminin was saturable and specific as determined by competition with unlabeled heparin, dextran sulfate, and dermatan sulfate. By Scatchard analysis, two distinct dissociation constants were calculated (Kd = 50 and 130 nM), suggesting the presence of at least two binding sites for heparin on laminin. Tritiated heparin bound to thrombin-resistant (600 kDa) and chymotrypsin-resistant (440 kDa) laminin fragments, both known to lack the terminal globular domain of the long arm. Sodium dodecyl sulfate-polyacrylamide gels of chymotrypsin- and thermolysin-digested laminin chromatographed on a heparin-Sepharose column showed multiple proteolytic fragments binding to the column. Monoclonal antibodies generated against laminin were tested for their ability to inhibit [3H]heparin binding to laminin. Four monoclonal antibodies significantly inhibited the binding of [3H]heparin to laminin in the range of 15–21% inhibition. Laminin–monoclonal antibody interactions examined by electron microscopy showed that one antibody reacted at the terminal globular domain of the long arm, domain Hep-1, while epitopes for two of these monoclonal antibodies were located on the lateral arms of laminin, domain Hep-2, and the fourth monoclonal antibody bound below the cross-region of laminin, domain Hep-3. When two monoclonal antibodies recognizing distinctly different regions of laminin were added concomitantly, the inhibition of [3H]heparin binding to laminin increased almost 2-fold. These results suggest that at least two novel heparin-binding domains of laminin may be located in domains distinct from the terminal globular domain of the long arm.

Interaction of the extracellular matrix protein laminin with glycosaminoglycans and proteoglycans is important in the formation of basement membranes, the adhesion of cells, and possibly the outgrowth of neurites (1–3). Laminin, an 850-kDa glycoprotein, is produced by a variety of cells including embryonic, epithelial, and tumor cells (4, 5). Early studies showed that the extracellular matrix components collagen, glycosaminoglycans, and fibronectin readily interact with each other (6–9). Laminin reacts with glycosaminoglycans, in particular heparin, as shown by its ability to bind to heparin-affinity columns (10–14) as well as cell surfaces (15–19). Furthermore, laminin reacts with glycosaminoglycans, in particular heparin, as shown by its ability to bind to heparin-affinity columns (20, 21). More recent studies have used proteolytic fragments of laminin in conjunction with heparin-affinity chromatography to localize a heparin-binding domain (designated Hep-1) on the globule at the end of the long arm of laminin (22). Since previous studies were performed on enzymatically derived laminin fragments, it is possible that other heparin-binding domains on laminin were not detected, due to their susceptibility to the enzymatic treatment.

In this study, we searched for additional heparin-binding sites on laminin by use of heparin-affinity chromatography and a solid-phase radioligand binding assay (RLBA). We used laminin fragments produced after short-term limited proteolytic treatment with various enzymes, in addition to monoclonal antibodies generated against laminin. These antibodies were shown to inhibit heparin-laminin interactions and were used in assays where the laminin molecule is present in its intact form. Our results indicate that there are at least two more heparin-binding domains present on laminin. One of these heparin-binding domains, Hep-2, is present on the middle of a lateral short arm of a B chain, while the other novel heparin-binding domain, Hep-3, is present below the cross-region where the A, B1, and B2 chains intersect.

MATERIALS AND METHODS

Protein Purification and Fragment Generation—Laminin was isolated from the Engelbreth-Holm-Swarm tumor as described previously (23) and a weight coefficient of A480nm = 8.3 was used to calculate laminin concentrations (24). A purified 600-kDa fragment of laminin which was isolated from a 7-h thrombin digest was kindly provided by Dr. S. L. Palm (University of Minnesota, Minneapolis, MN). This fragment’s isolation, purity, and structure have been previously described (25). Purified laminin was also digested with chymotrypsin or thermolysin for 24 or 7 h, respectively, as previously described.

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1The abbreviations used are: RLBA, radioligand binding assay; PBS, Dulbecco’s phosphate-buffered saline without CaCl2 or MgCl2, pH 7.4; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin.
described (25). A 440-kDa fragment was isolated from the chymotrypsin digest by gel filtration chromatography on Sephacryl S-300 (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) (2.6 × 100 cm column) in Dulbecco’s phosphate-buffered saline without CaCl₂ or MgCl₂, pH 7.4 (PBS), containing 0.5 M NaCl, where it eluted just after the void volume. Molecular weight determinations and verification of purity of isolated proteolytic fragments were done by sodium dodecyl sulfate (SDS)-polyacylamide gel electrophoresis as described below.

Monoclonal Antibody Preparation—Five monoclonal antibodies (AL-1 to AL-5) were generated against laminin by immunization of male LOU/MCr rats (Frederick Cancer Research Facility, National Cancer Institute, Bethesda, MD). The preparation, purification, and characterization of three of the antibodies (AL-1 to AL-3) has been described previously (26). All of the monoclonal antibodies were of the IgG class except AL-5 which was an IgM. Further purification of the monoclonal antibodies for this study was done to remove any potential contaminants which might bind to heparin and interfere in the inhibition assays described below. This was achieved by incubating 3 mg of the antibodies per ml of heparin-Sepharose beads (Pharmacia) in 6 mM phosphate, 0.1 M NaCl, 65 μM CaCl₂, pH 6.8, for 1.5 h with gentle agitation at room temperature. The beads were allowed to settle and the supernatant was collected. The beads were then rinsed with 1 ml of the same buffer until no further protein rinsed off as determined by absorbance at 280 nm. The rinses were pooled, concentrated, and dialyzed against the same buffer.

Polyclonal Antibodies—Commercially available normal rat immunoglobulin was obtained from Cooper Biomedical, Inc., Malvern, PA. Hyperimmune rat sera were obtained from LOU/MCr rats which were repeatedly immunized with the purified 440-kDa fragment of laminin (25).

Solid-phase Radioimmunoassays—The binding of [³H]heparin (0.3 nCi/trog) Du Pont-New England Nuclear Research Products, Wilmington, DE) to laminin, laminin fragments, and bovine serum albumin (BSA) (fatty acid free, fraction V, ICN Immunobiologicals) was quantitated by a solid-phase RLBA in 96-well polystyrene Immulon 1 plates (Dynatech Laboratories, Inc., Alexandria, VA). Fifty microliters of 1.76 pmol of laminin. After a 2-h incubation, the wells were washed three times, and the [³H]heparin bound was quantitated in a scintillation counter. All determinations were repeated a minimum of three times in triplicate.

Various sulfated polysaccharides were tested for their ability to compete with [³H]heparin for binding to laminin. In these studies, 50 μl of various concentrations of heparin (porcine intestinal mucosa, grade I; 15 kDa), dextran sulfate (8 kDa), or dermatan sulfate (porcine skin, type B; 20 kDa) (all from Sigma) in RLBA buffer were added concomitantly in a total volume of 50 μl with each antibody at 0.88 pmol; 1.76 pmol of laminin. After a 2-h incubation, the wells were washed three times, and the [³H]heparin bound was determined as described above.

Chromatography—Thrombin, chymotrypsin, and thermolysin digests of laminin (10 mg at 1 mg/ml) were chromatographed on a 1.5 × 5-cm column of heparin-Sepharose (Pharmacia) equilibrated with PBS at room temperature. The column was rinsed with PBS and 3.0-mM NaCl in PBS. Aliquots of the fractions were suspended in nonreducing electrophoresis sample buffer (25) and separated on 2–15% (w/v) gradient SDS-polyacrylamide gels (25). The gels were stained with Coomassie Blue R-250 to detect protein fragments.

Electron Microscopy—Laminin-antibody complexes were visualized by electron microscopy after rotary shadowing as previously described (26).

Results

Time Course of [³H]Heparin Binding to Various Concentrations of Laminin—The binding of [³H]heparin to laminin was measured by a direct solid-phase RLBA as described under “Materials and Methods” (Fig. 1). Binding of [³H]heparin to laminin increased as a function of time, and was detected as early as 5 min when high concentrations of laminin were used. Furthermore, significant binding of [³H]heparin was achieved when wells contained greater than 0.44 pmol of laminin, and the binding of [³H]heparin increased as the amount of laminin added was increased. Equilibrium binding of heparin to laminin was reached by 2 h, irrespective of the amount of laminin used in the wells.

Heparin Binding to Laminin as a Function of [³H]Heparin Concentration—In order to test whether the binding of heparin concomitantly in a total volume of 50 μl, with each antibody at 50 μg/ml. The antibodies were incubated in the wells for 90 min at 37 °C. Fifty microliters of [³H]heparin (15,000 dpm) in RLBA buffer were then added directly to the wells and incubated for 15 min at 37 °C. The unbound [³H]heparin and antibodies were aspirated and the wells were rinsed three times with wash buffer. Bound [³H]heparin was solubilized with 200 μl of 0.5 N NaOH and 1% SDS, and quantitated in a scintillation counter. All determinations were repeated a minimum of three times in triplicate.

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Heparin Binding to Laminin as a Function of [³H]Heparin Concentration—In order to test whether the binding of heparin

![Fig. 1. Time course of [³H]heparin binding to various concentrations of laminin. [³H]Heparin (15,000 dpm) was added to wells coated with laminin: 0, 0.44 pmol; △, 0.88 pmol; ●, 1.76 pmol; ○, 3.53 pmol. The nonspecific binding of [³H]heparin in the presence of a 100-fold excess of unlabelled heparin has been subtracted, and ranged from 30 to 80 dpm depending upon the length of incubation. Each value represents the mean of three separate determinations, and the S.E. was less than 5% in each case. Four separate experiments gave similar results.](image-url)
Polysaccharides, heparin (15 kDa), dextran sulfate (8 kDa), required for 50% displacement of [3H]heparin. These results bound to laminin for 2 h, and a 100-fold excess of unlabeled

tural features of heparin.

Assay, [3H]heparin and various concentrations of the sulfated

teration assay was conducted to determine

testablished in the presence of additional sulfated polysaccharides to de-
terminate the specificity of heparin binding to laminin. In this assay, [3H]heparin was

and various amounts of [3H]heparin were added in the absence or presence of a 100-fold excess of unlabeled heparin to wells coated with 1.76 pmol of laminin and incubated for 3 h. The specific binding of [3H]heparin to laminin (\( K_a \)) was determined by subtracting the amount of [3H]heparin bound to laminin in the presence of a 100-fold excess of unlabeled heparin (\( K_d \)) from the amount bound in the absence of unlabeled heparin (\( K_d \)). Each value represents the mean of three

determinations, and the S.E. was less than 5% in each case. Three separate experiments gave similar results. Inset, Scatchard plot of the specific binding data. Linear regression of the data predicts two lines as shown:

\[ K_a = 50 \text{ nM}, \quad r = 0.9876, \quad p < 0.01 (\odot); \quad K_d = 130 \text{ nM}, \quad r = 0.9908, \quad p < 0.01 (\bullet). \]

**Reversibility of [3H]Heparin Binding to Laminin**—A competition assay was conducted to determine if heparin binding to laminin was reversible. In this assay, [3H]heparin was bound to laminin for 2 h, and a 100-fold excess of unlabeled heparin was then added (Fig. 3). Within 12 min, 50% of the laminin-bound [3H]heparin was removed by the unlabeled heparin, and less than 10% of the [3H]heparin remained bound to the laminin by 2 h. When only RLBA buffer was added to the [3H]heparin, no more than 15% of the [3H]heparin was displaced, even after 4 h.

**Competition of [3H]Heparin Binding to Laminin by Sulfated Polysaccharides**—A second competition assay was conducted in the presence of additional sulfated polysaccharides to determine the specificity of heparin binding to laminin. In this assay, [3H]heparin and various concentrations of the sulfated polysaccharides, heparin (15 kDa), dextran sulfate (8 kDa), or dermatan sulfate (20 kDa), were added concomitantly to wells coated with 1.76 pmol of laminin (Fig. 4). Fifty percent of the [3H]heparin bound to laminin was displaced by 5 \( \times \) 10\(^{-8}\) M heparin, while 20 times more dextran sulfate (1 \( \times \) 10\(^{-6}\) M) or 60 times more dermatan sulfate (3 \( \times \) 10\(^{-6}\) M) was required for 50% displacement of [3H]heparin. These results suggest significant specificity in the heparin-laminin interaction, and indicate that this binding is due to specific structural features of heparin.

**Identification of Heparin-binding Fragments of Laminin by Heparin-Sepharose Chromatography**—Previous studies with laminin fragments have suggested that although the globule at the end of the long arm of laminin contains a major binding site for heparin, other binding sites may be present and also that the duration of enzymatic digestion may alter or destroy the heparin-binding ability of these sites (22). Therefore, we used various methods (heparin-affinity chromatography, solid-phase RLBA, and monoclonal antibodies) to localize these other heparin-binding sites to specific laminin domains. Laminin was digested with thrombin for 7 h and the major 600-kDa protease-resistant fragment was shown to bind to a heparin-Sepharose column (data not shown).
than blue staining fragments were generated (Fig. 6, present in these smaller fragments of laminin located near approximately 70% of the thermolysin digest passed through the heparin-Sepharose column (Fig. 6, lane B), whereas only minor cold inhibition of the heparin-Sepharose column (Fig. 6, lane B) bound to the heparin-Sepharose column and were eluted with 0.5 M NaCl in PBS. This finding indicates that heparin-binding domain(s) are present in these smaller fragments of laminin located near the cross-region yet away from the globule at the distal end of the long arm, Hep-1.

Heparin Binding to Purified Proteolytic Fragments of Laminin—Tritiated heparin was next tested in the solid-phase RLBA to determine whether it would bind in a specific manner to the purified proteolytic fragments of laminin as it had to intact laminin. In this assay, equimolar amounts of intact laminin, the purified 600-kDa thrombin-resistant fragment of laminin, and the purified 440-kDa chymotrypsin-resistant fragment of laminin, and BSA were dried onto wells. 

Heparin was added to the wells and incubated for 2 h. [3H]Heparin bound to laminin and fragments of laminin in a concentration-dependent manner (Fig. 7), whereas only minimal background binding of [3H]heparin was observed to BSA. As laminin was digested to smaller proteolytic fragments (from 850 to 600 to 440 kDa), less [3H]heparin was observed to bind to equimolar amounts of the laminin fragments. However, all of these fragments bound heparin at levels which were significantly above background (i.e. binding of [3H]heparin to laminin in the presence of a 100-fold excess of unlabeled heparin). These purified fragments did not bind [3H]heparin as well as intact laminin, indicating that heparin-binding domain(s) exist in regions of laminin removed and/or destroyed by the proteolytic enzymes used in this study.

Epitope Localization of Monoclonal Antibodies on Laminin by Electron Microscopy—The domains of laminin to which the monoclonal antibodies bound were localized by electron microscopy after rotary shadowing (26). At least 61 clear-cut images of the binding of each monoclonal antibody to laminin were examined. Each of the five monoclonal antibodies bound

![Figure 4](image1.png)

**FIG. 4.** Competition of [3H]heparin binding to laminin by sulfated polysaccharides. Wells coated with 1.76 pmol of laminin were incubated for 2 h with [3H]heparin (15,000 dpm) and various concentrations of unlabeled heparin (O), dextran sulfate (C), or dermatan sulfate (L). The nonspecific binding of [3H]heparin to laminin in the presence of a 100-fold excess of unlabeled heparin has been subtracted, and was never more than 80 dpm. Each value represents the mean of three separate determinations, and the S.E. was less than 5% in each case. Three separate experiments gave similar results.

![Figure 5](image2.png)

**FIG. 5.** Model of laminin and proteolytic fragments of laminin. Model of laminin based on previous studies (25, 50, 51): (a) 850-kDa intact, nonreduced laminin with A, B₁, and B₂ chains (held together by disulfide bonds; the coiled coil α-helical regions are not shown for simplification); (b) the purified 600-kDa thrombin-resistant laminin fragment of laminin; and (c) the purified 440-kDa chymotrypsin-resistant fragment of laminin.

fragment is comprised of the two lateral short arms and part of the long arm of laminin, yet it lacks the globule at the end of the long arm (Fig. 5b) (25). Digestion of laminin with chymotrypsin for 24 h yielded many fragments including a major 440-kDa chymotrypsin-resistant fragment which also bound to a heparin-Sepharose column (Fig. 5c) (25). This 440-kDa fragment is comprised of parts of the two lateral short arms lacking the globules and part of the long arm of laminin; it, too, lacks the globule at the end of the long arm. When this 440-kDa chymotrypsin-resistant fragment was further digested for 7 h with thermolysin, ~10 major Coomassie blue staining fragments were generated (Fig. 6, lane A). Approximately 70% of the thermolysin digest passed through the heparin-Sepharose column (Fig. 6, lane B), whereas ~30% of the digest bound to heparin. Five major fragments (120, 110, 76, 65, and 35 kDa) (Fig. 6, lane C) bound to the heparin-Sepharose column and were eluted with 0.5 M NaCl in PBS. This finding indicates that heparin-binding domain(s) are present in these smaller fragments of laminin located near the cross-region yet away from the globule at the distal end of the long arm, Hep-1.

![Figure 6](image3.png)

**FIG. 6.** Thermolysin-digested fragments of laminin binding to a heparin-Sepharose column. Laminin digested for 7 h with thermolysin was chromatographed on a heparin-Sepharose column and the unbound and bound fragments were obtained. Fifty micrograms of the laminin fragments were suspended in nonreducing electrophoresis sample buffer, separated on a 2–15% SDS-polyacrylamide gel, and stained with Coomassie Blue R-250. Thermolysin-digested laminin: Lane A, before chromatography on a heparin-Sepharose column; Lane B, unbound fragments; and Lane C, bound and eluted fragments. Molecular mass standards were: fibronectin, 220 kDa; phosphorylase a, 94 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; and soybean trypsin inhibitor, 21 kDa.

Electron microscopy images of the binding of each monoclonal antibody to laminin are shown for simplification; (b) the purified 600-kDa thrombin-resistant laminin fragment of laminin; and (c) the purified 440-kDa chymotrypsin-resistant fragment of laminin.
Novel Heparin-binding Domains of Laminin

**FIG. 7. Binding of [3H]heparin to purified proteolytic fragments of laminin.** [3H]Heparin (15,000 dpm) was incubated for 2 h in wells containing laminin (LMN), the purified 600-kDa thrombin-resistant fragment of laminin (thrombin-LMN), the purified 440-kDa chymotrypsin-resistant fragment of laminin (chymo-LMN), or BSA. The following picomoles of each protein were dried down to the wells: (a) 0.44, (b) 0.88, (c) 1.76, and (d) 3.53. Each value represents the mean of four separate determinations, and the S.E. was less than 5% in each case. Three separate experiments gave similar results.

to a different region of the laminin molecule. Between 67 and 89% of the total antibody binding events for each of the antibodies were readily categorized to one distinct region of laminin or another (Fig. 8A); the remaining antibody binding events were randomly distributed over the entire laminin molecule. Antibody AL-1 bound on or near the cross-region, AL-2 bound on the long arm near the cross-region, AL-3 bound on the lateral short arms in an area ranging from near the cross-region to the middle of the short arms, AL-4 bound on the globule at the end of the long arm, and AL-5 bound on the lateral short arms in an area ranging from near the middle to the globules at the edges. Representative examples of electron micrographs of monoclonal antibodies AL-1, AL-2, and AL-3 binding to laminin have been recently published (26); while examples of monoclonal antibodies AL-4 and AL-5 binding to laminin are shown in Fig. 8B.

**Inhibition of [3H]Heparin Binding by Monoclonal Antibodies—** Wells precoated with laminin were incubated with each of the purified monoclonal antibodies. [3H]Heparin was then added to the wells and the amount of [3H]heparin bound was determined. At a concentration of 50 μg/ml, monoclonal antibody AL-5 was capable of inhibiting [3H]heparin binding to laminin by 21%; AL-2, AL-3, and AL-4 caused 15–16% inhibition of [3H]heparin binding; while AL-1 caused no significant inhibition (Fig. 9). Polyclonal immunoglobulin from rabbits hyperimmunized with laminin inhibited [3H]heparin binding to laminin by 31%. No inhibition of [3H]heparin binding was observed when wells were incubated with RLBA buffer containing no immunoglobulin, and only 4% inhibition was observed with normal rabbit or rat immunoglobulin. These results suggest that there are at least three potential heparin-binding domains of laminin (Fig. 10); Hep-1 localized to the globule at the distal end of the long arm (shown by AL-4 inhibition), Hep-2 present on the lateral short arms of laminin (shown by AL-3 and AL-5 inhibition), and Hep-3

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3 A. P. N. Skubitz, unpublished data.
those antibodies which caused a significant inhibition of [3H]heparin binding was quantitated as 100 minus ((amount of [3H]heparin specifically bound when added to the test immunoglobulin divided by the amount of [3H]heparin specifically bound when added to normal rat immunoglobulin) times 100). Each value represents the mean of three separate determinations, and the S.E. was less than 5% in each case. Duplicate experiments gave similar results.

![Graph](image)

**Table I**

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DISCUSSION

Laminin has previously been shown to interact with heparin (1, 20–22). In this study, the binding of heparin to laminin was quantitatively analyzed. We have identified multiple heparin-binding sites on laminin and have mapped these sites to three specific domains of laminin. In a solid-phase radioligand binding assay, [3H]heparin was shown to bind to laminin in a time- and concentration-dependent manner. The [3H]heparin binding to laminin was saturable and could be reversed by unlabeled heparin. Specificity of [3H]heparin binding to
laminin was indicated by competition assays using dextran sulfate or dermatan sulfate.

The data suggest the presence of at least two other heparin-binding domains on laminin, in addition to the previously described (22) domain on the globule at the distal end of the long arm (Hep-1). This conclusion was based on the following findings: (a) by Scatchard analysis, at least two distinct dissociation reactions were measured; (b) enzymatically derived fragments of laminin that lack the globule of the long arm were retained on heparin-Sepharose affinity columns; (c) [3H]heparin bound directly in a solid-phase RLBA to the 600-kDa thrombin-resistant fragment and the 440-kDa chymotrypsin-resistant fragment both known to lack the Hep-1 domain; and (d) inhibition studies using a series of well-characterized monoclonal antibodies in various combinations strongly suggested that at least two other heparin-binding sites exist.

One of the novel heparin-binding domains, Hep-2, was localized to the lateral short arms of laminin as determined by inhibition studies with antibodies AL-3 and AL-5 which bind to this region of laminin by rotary shadowing and recognize the B chains of reduced laminin as determined by immunoblotting techniques. The other novel heparin-binding site, Hep-3, located below the intersection of the cross on the long arm of laminin, has not yet been localized to the A, B1, or B3 chain of laminin, or a combination of the chains. This is because monoclonal antibody AL-2, which was used to localize this domain, recognizes both A and B chains, as determined by electron microscopy and immumoblot analysis of thoroughly reduced laminin (26). All of these monoclonal antibodies recognize the polypeptide portion of laminin, suggesting that the polypeptides are involved in heparin-binding, not the carbohydrate moieties on laminin.

Previous studies utilizing proteolytic digests of laminin may not have identified these two novel heparin-binding domains perhaps due to their sensitivity to enzymatic treatment. For example, Ott et al. (22) carried out most of their proteolytic digestions of laminin for 24 h, which may have partially altered the heparin-binding capability of some of their fragments. They found that a 2-h limited digestion of laminin with staphylococcal V8 protease resulted in one-third of the protein binding to heparin-Sepharose; the majority of which was a large fragment S1-4, corresponding to the cross-region and the globules of the short arms (22). These results suggest that short-term, limited proteolytic digestion of laminin do not destroy the heparin-binding domains located near the cross-region; whereas long-term digestion may alter the capability of some fragments to bind to heparin. This hypothesis is supported by our findings that generation of fragments by proteolytic treatment of laminin with thrombin or chymotrypsin decreases the binding of [3H]heparin (Fig. 7), and indicates that prolonged digestion of laminin with certain enzymes results in a decrease in heparin-binding capabilities.

The finding that multiple heparin-binding domains exist in laminin is interesting but not surprising. Large extracellular matrix proteins are known to have various domains involved in similar or identical functions. For example, in fibronectin, multiple heparin-binding domains have been identified (27-30). Laminin is known to have more than one domain involved in the binding to type IV collagen (13, 14, 17, 31) and to various cell surfaces, including hepatocytes (32), tumor cells (17, 26, 32, 33), and neurites (3, 34).

The complex process of cell adhesion to extracellular matrices may depend upon cell surface glycosaminoglycans or proteoglycans. Previous studies have shown that extracellular matrix proteins in addition to laminin (20, 21), such as serum spreading factor (35), thrombospondin (36), and fibronectin (30, 37) all bind to glycosaminoglycans. Furthermore, it has been suggested that cell surface proteoglycans play an important role in the adhesion of cells to extracellular matrices based upon the findings that heparin-binding domains from laminin (34), fibronectin (38-40), thrombospondin (41), and platelet factor 4 (42) all support cell adhesion and spreading in vitro. In addition, focal adhesions isolated from adherent cells were found to be enriched in heparan sulfate and chondroitin sulfate proteoglycans (43-46). Taken together, these studies suggest that cell surface proteoglycans may act as receptors or binding constituents for extracellular matrix proteins. For example, the domain of laminin responsible for neurite outgrowth is located in close proximity to the heparin-binding domain Hep-1 (3, 34). It is possible that tumor cells which bind to a region of laminin near the intersection of the cross (26, 35) are influenced in a similar manner by the other two heparin-binding domains of laminin located near the cross-region, Hep-2 and Hep-3. This type of heparin-laminin interaction may be an alternative way for cells to bind to the extracellular matrix rather than just by way of the conventional 67-kDa laminin receptor (47, 48). This interaction may be used by metastatic cells in conjunction with or as an alternative to the 67-kDa receptor to invade the basement membrane. Recently we have shown that when highly metastatic murine fibrosarcoma cells are pretreated ex vivo with purified heparin-binding fragments of fibronectin or laminin, a decrease in pulmonary metastatic nodules are observed following tail vein injection of the cells (49). This study, therefore, provides further evidence for the role of cell surface proteoglycans in the adhesion and metastasis of certain malignant neoplasms.

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Novel Heparin-binding Domains of Laminin