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Laminin receptor on human breast carcinoma cells

(type IV collagen/basement membrane/metastasis)

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ABSTRACT **Human MCF-7 breast carcinoma cells possess a receptor-like moiety on their surface that has a high binding affinity (K_d = 2 nM) for laminin, a glycoprotein localized in basement membranes. Laminin preferentially stimulates (8-fold) MCF-7 cells to attach to type IV (basement membrane) collagen, whereas fibronectin stimulates attachment only 2-fold for these cells on type I collagen. The attachment properties of two other human breast carcinoma cell lines to type IV collagen were also studied. The attachment of ZR-75-1 cells was stimulated 4-fold by laminin and 5-fold by fibronectin, whereas T47-D cell attachment was stimulated 2-fold by laminin and 7-fold by fibronectin. By employing protease-derived fragments of laminin, the major domains of the laminin molecule that participate in MCF-7 cell attachment to type IV collagen were identified. The whole laminin molecule has the configuration of a four-armed cross with three short arms and one long arm. A major cell-binding domain was found to reside near the intersection point of the short arms, and the type IV collagen-binding domain was associated with the globular end regions of the short arms. The receptor for laminin on the surface of these tumor cells may be involved in the initial interaction of tumor cells via laminin with the vascular basement membrane to facilitate invasion and subsequent promotion of metastasis.**

Interaction of cells with the extracellular matrix plays an important role in both normal and neoplastic processes (1, 2). Recent studies indicate that normal cells require an extracellular matrix for survival, proliferation, and differentiation (1, 3). Attachment of cells to these extracellular matrices are mediated by specific glycoproteins that bind the cells to discrete collagen types. For example, many cells of mesenchymal origin, such as fibroblasts, myoblasts, and smooth muscle cells, utilize fibronectin to bind to interstitial type I and type III collagens (1, 4). Chondrocytes, which synthesize type II collagen, cartilage collagen, use another glycoprotein, chondronectin, to adhere to this collagen (5). Laminin, the major glycoprotein of the basement membranes, mediates the attachment of epithelial and neoplastic cells to type IV collagen (2, 6, 7).

Laminin (M_r 10^6) upon reduction migrates on polyacrylamide gels as two subunits with apparent molecular weights of 200,000 (α3 subunits) and 400,000 (β subunit). Recent electron microscopic studies by Engel *et al.* (8) using rotary shadowing showed that the configuration of the laminin molecule is an asymmetric cross with one long arm (77 nm) and three identical short arms (37 nm). We have recently shown that the long arm of the cross is the β subunit and the three short arms make up the α3 subunit, which consists of three similar M_r 200,000 chains (9).

Protease-derived fragments of fibronectin have been utilized to identify specific molecular domains that participate in cell and collagen binding (10, 11). In the present study, we have used a similar approach to identify the domains of the laminin molecule that participate in the attachment of human breast carcinoma cells to type IV collagen.

The basement membrane is a specialized extracellular matrix composed of type IV collagen, glycoproteins such as laminin, and proteoglycans. During the many stages of the metastatic process (12), tumor cells encounter and traverse basement membranes. These tumor cells attach to the vascular basement membrane in capillaries prior to both intravasation and extravasation (13, 14). Thus, tumor cells that have the ability to preferentially attach to areas of exposed basement membrane may have a selective advantage during hematogenous metastases formation.

In previous work it was shown that metastatic cells bind preferentially to type IV collagen (2, 15). For highly metastatic murine tumor cells, laminin was identified as the specific attachment factor that mediates adherence to type IV collagen (2). The present studies extend these observations to human tumor cells. A high-affinity receptor for laminin was identified on the surface of human breast carcinoma cells.

MATERIALS AND METHODS

Cell Lines. The MCF-7 human breast cancer cell line was provided by the Michigan Cancer Foundation (16). Characterization of the cells as to human and mammary origin has been summarized (17). The cells were shown to be invasive and to metastasize in *nude* mice (18). They were also invasive *in vitro* when human amnion connective tissue was used as a barrier (15).

The ZR-75-1 and T47-D human breast carcinoma lines were provided by L. Engel (Laboratory of Pathology, National Cancer Institute). These cells were verified to be carcinomas by electron microscopy criteria, to be human in origin by karyotypic analysis, and to contain mammary gland specific secretory milk proteins. Both of these lines grew poorly in *nude* mice with no gross metastases, even when subcutaneous xenotransplantation was performed in newborn *nude* mice. Normal human skin fibroblasts (CRL 1507 and CRL 1477) were obtained from the American Type Culture Collection.

Preparation of Substrate, Antibody, Attachment Factors, and Laminin Fragments. Type I collagen was prepared from lathyritic rat skin (19), and type IV collagen (20) and laminin (21) were prepared from the Engelbreth–Holm–Swarm tumor. Purified antibodies to laminin were prepared as described (22). Laminin was iodinated by the lactoperoxidase method (23).

Purified human α thrombin was kindly supplied by John W. Fenton (New York State Department of Health, Albany, NY). Digestion with α thrombin was performed at pH 7.6 and 25°C, using an enzyme-to-substrate weight ratio of 1:100 as described (9, 24, 25). Laminin digestion with pepsin and cathepsin G was performed as described (8, 9, 24). Protease fragments were isolated by high-performance liquid chromatography (Beck-

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Abbreviation: P_i/NaCl, phosphate-buffered saline.

man) and studied by gel electrophoresis (9, 24). Polyacrylamide slab gels (5%) were prepared and electrophoresis was performed with the Laemmli discontinuous buffer system (26).

Attachment Assay. The attachment assay was adapted from that described by Klebe (27) and modified by Terranova *et al.* (7).

Cell Binding. Binding of laminin to MCF-7 and T47-D breast carcinoma cells and adult human fibroblasts CRL 1477 and CRL 1507 was performed with monolayer cell cultures. All cell lines were replica plated in multiwell culture dishes (FB-6-TC, Limbro) in complete growth media. When the cells were 50–70% confluent the medium was changed to Dulbecco's modified Eagle's medium with 0.1% bovine serum albumin for a 2-hr wash. The binding medium consisted of Dulbecco's modified Eagle's medium, 0.1% bovine serum albumin, and 20 mM Hepes buffer (pH 7.4). ^{125}I-Labeled laminin (^{125}I-laminin) with either excess unlabeled laminin or excess unlabeled laminin fragments was added in 250 μl of phosphate-buffered saline (P_i/NaCl) to initiate the binding assay. After various times of incubation at 20°C, 25°C, and 37°C, the binding medium was rapidly aspirated, and monolayers were washed three times with ice-cold P_i/NaCl containing 0.2% bovine serum albumin to remove unbound material. The cells were then removed by using 0.02% EDTA in P_i/NaCl and cell-bound radioactivity was determined in a Searle (Skokie, IL) Autogamma counter. Specific binding was defined as the total radioactivity bound minus the amount bound in the presence of a 100-fold excess of unlabeled material.

Collagen Binding. Procollagen type IV was dissolved in 0.5 M acetic acid and neutralized with 0.05 M Tris·HCl/0.9 M NaCl, pH 7.4. Five microliters of the solution (25 μg) was placed on a 13-mm SCWP nitrocellulose filter with 8-μm pore diameter (Millipore). The filters were then immersed in P_i/NaCl with 3% bovine serum albumin at 4°C overnight. After washing, ^{125}I-labeled laminin (1 mg/ml) or purified ^{125}I-labeled laminin fragments were applied to the filters (10 μl) and incubated in a 100% relative humidity chamber for 20 min. After intensive washing in P_i/NaCl the bound radioactivity was quantitated with a gamma counter.

Rotary Shadowing and Electron Microscopy. These techniques were performed according to the method of Engel *et al.* (8), modified as described (9, 24).

RESULTS

Cell Attachment to Collagen. When freshly trypsinized MCF-7, ZR-75-1, and T47-D cells were added to various substrates, the MCF-7 cells preferred type IV collagen over type I collagen or plastic (data for types I and IV collagen shown in Table 1). In all experiments the MCF-7 cells attached more rapidly and to a greater extent to type IV collagen substrate compared to the ZR-75-1 and T47-D cell types. To determine whether the adherence of the cells to specific substrates was mediated by the presence of specific attachment factors, we tested the effect of exogenous laminin and fibronectin on cell attachment to type I and type IV collagen, respectively. To eliminate the effect that endogenous synthesis of these factors might have on adherence, cells were treated with cycloheximide in the incubation media to inhibit protein synthesis. In the presence of cycloheximide, laminin stimulated the attachment of all three cell lines to type IV collagen. The MCF-7 cells showed an 8-fold stimulation by laminin compared to a 4-fold stimulation for the ZR-75-1 cells and 2-fold stimulation for the T47-D cells (Fig. 1). Fibronectin, when added to type I collagen in the presence of cycloheximide, caused a 7-fold increase in the attachment of the T47-D cells, a 5-fold increase in attachment of the ZR-75-1 cells, and only a 2-fold increase in the attachment of the MCF-7 cells (Fig. 1). These data indicate that the MCF-7 cells prefer type IV collagen as a substrate and use laminin as an attachment factor. Laminin in the same dose range failed to stimulate attachment of these cells to type I collagen (data not shown). In contrast, the T47-D cells utilized fibronectin to bind preferentially to type I collagen, whereas ZR-75-1 cells exhibited no preference for either laminin or fibronectin for attachment to type IV or type I collagen substrate, respectively.

Table 1. Attachment of human breast carcinoma cells

Cells	% attachment	
	Type I	Type IV
MCF-7	22	68
ZR-75-1	54	44
T47-D	62	33

Attachment of cells to types I and IV collagen-coated dishes was measured after 3-hr incubation in serum-free Dulbecco's modified Eagle's medium. Attachment assay is detailed in the legend to Fig. 1. The results are the means of quadruplicates, which did not differ by more than 5%.

The attachment of the MCF-7 cells in the presence of affinity-purified antibody to laminin was studied to verify that attachment to type IV collagen in the absence of cycloheximide involved laminin. Anti-laminin antibody inhibited the attachment of the MCF-7 cells to type IV collagen. In the presence of anti-laminin antibody the thrombin-derived α3 fragment of laminin antagonized the inhibitory effect of the anti-laminin on cell attachment to type IV collagen (Fig. 2).

Binding Properties of Laminin Fragments. In order to investigate which regions of the laminin molecule mediate the

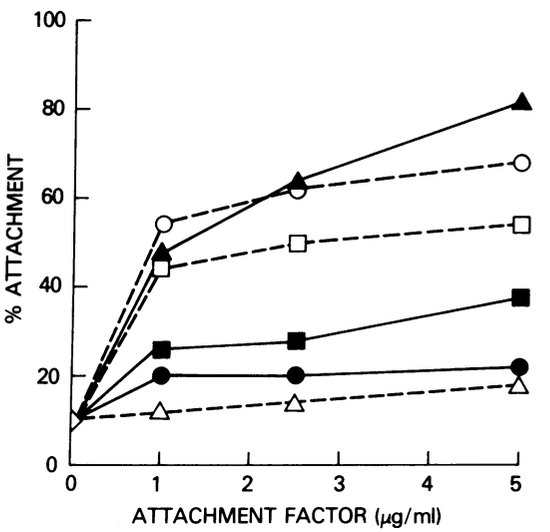

FIG. 1. Dose–response curve for laminin-mediated attachment of MCF-7 (▲), ZR-75-1 (■), and T47-D (●) cells to type IV collagen-coated dishes and fibronectin mediated attachment of MCF-7 (△), ZR-75-1 (□), and T47-D (○) cells to type I collagen-coated dishes. Cells were pretreated for 4 hr in RPMI 1640 medium with 10% fetal bovine serum containing cycloheximide at 25 μg/ml. After incubation the cells were trypsinized, with 0.01% trypsin/0.1% EDTA, centrifuged (1,000 × *g*) for 5 min, and resuspended in serum-free Dulbecco's modified Eagle's medium containing cycloheximide at 25 μg/ml. The cells were then incubated for 3 hr in the presence of the indicated concentrations of either fibronectin or laminin. The attached cells were removed by trypsinization and counted electronically. Data points represent the mean of triplicates not differing by more than 10%. Fibronectin did not mediate the attachment of MCF-7 cells to type IV collagen. Laminin did not mediate attachment of T47-D cells to type I collagen.

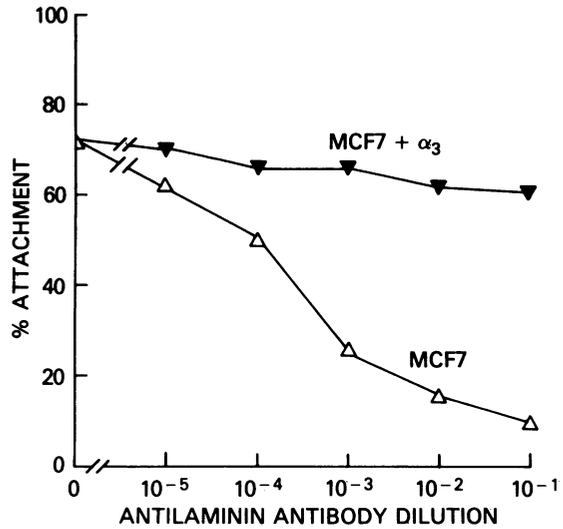

FIG. 2. Effect of affinity-purified anti-laminin antibody (100 μg of protein per ml initial concentration) at various dilutions on the attachment of MCF-7 cells to type IV collagen. The antibody was added at the onset of the attachment assay. In a separate experiment, the M_r 600,000 α3 fragment of laminin (1 μg/ml) was added with the affinity-purified anti-laminin. Cells were incubated for 3 hr and harvested. Each value represents the mean of triplicate assays not differing by more than 5%.

attachment of cells to type IV collagen, protease-derived fragments of laminin were tested for their attachment properties. The thrombin-derived α3 fragment of laminin stimulated the attachment of MCF-7 cells to type IV collagen to the same extent as native laminin did (Fig. 3). The α3 fragment is as active on a microgram basis, but it is less active than native laminin on a molar basis. The β component of laminin showed no attachment activity. The data in Figs. 2 and 3 support the conclusion that the α3 component of laminin contains the biologically active sites for both cell and collagen binding. The effect of the α3 laminin component and a pepsin-derived "P1" M_r 280,000 laminin fragment on the ability of the MCF-7 and T47-D cells to attach to type I and type IV collagen was measured in the presence of the native laminin molecule. The α3 component stimulated adherence to type IV collagen, whereas the P1 fragment markedly inhibited attachment (Table 2). Neither fragment had an effect on attachment to type I collagen (data not shown). However, the T47-D cells attached to type IV collagen by utilizing laminin or laminin fragments to a much lesser degree (Table 2). A dose–response experiment using the P1 fragment is shown in Fig. 4. Attachment of both the MCF-7 cells and the T47-D cells to type IV collagen was completely inhibited by the P1 fragment at 1.0 μg/ml. A further laminin fragment (M_r 350,000) produced by cathepsin G digestion (named "C1") completely inhibited the attachment of the MCF-

Table 2. Ability of laminin and laminin fragments to bind to type IV collagen and to mediate human breast carcinoma cell adhesion to type IV collagen

Laminin or fragment	% binding to collagen	% attachment	
		MCF-7	T47-D
Laminin	80	84	28
α3	85	81	29
P1	15	13	11
C1	10	11	10
None	—	48	22

Binding of whole laminin or purified protease-derived fragments of laminin to procollagen type IV immobilized on nitrocellulose was measured. Ten micrograms of the ligand was applied to 25 μg of type IV collagen. Percent attachment of MCF-7 and T47-D cell to type IV collagen substrate in the presence of various laminin fragments is indicated. Freshly trypsinized cells were incubated for 3 hr in serum-free Dulbecco's modified Eagle's medium with laminin added at 1 μg/ml, M_r 600,000 α3 laminin fragment at 1 μg/ml, P1 laminin fragment at 100 μg/ml, or C1 laminin fragment at 100 μg/ml. Unattached cells were removed and counted, the dishes were washed with P_i/NaCl, and the attached cells were removed with 0.01% trypsin/0.1% EDTA and counted electronically. The data are the mean of quadruplicates with a range less than 10% of the mean; sensitivity was as low as 500 ng/ml.

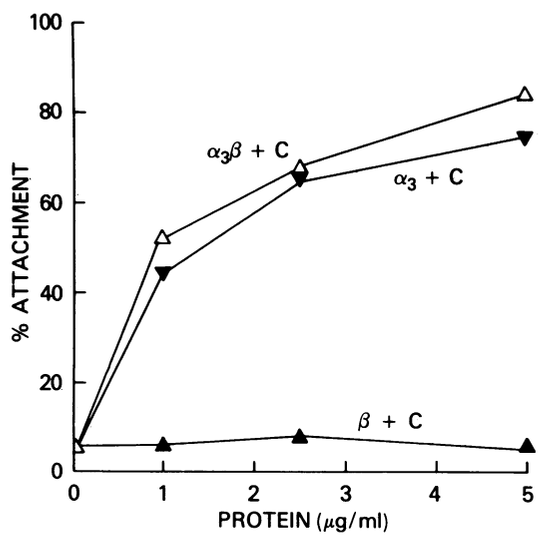

FIG. 3. Dose–response curve for laminin-mediated attachment and laminin-fragment-mediated attachment of MCF-7 cells to type IV collagen-coated dishes in the presence of cycloheximide (+ C). Thrombin-derived α3 (M_r 600,000) laminin fragment, and β (M_r 400,000) laminin fragment are compared to the native molecule. Data points represent the mean of quadruplicates not differing by more than 10%. The entire experiment was repeated three times with mean values differing by not more than 5%.

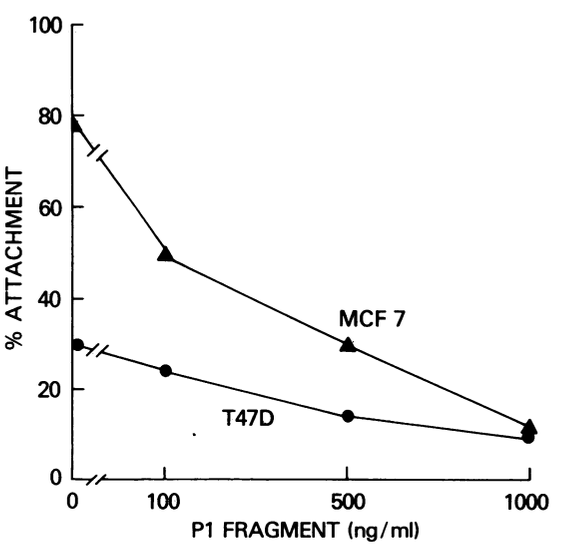

FIG. 4. Competition of P1 fragment and native laminin in mediating attachment: Dose–response curve for P1 (M_r 280,000) fragment-mediated inhibition of laminin-mediated attachment of MCF-7 and T47-D cells to type IV collagen-coated dishes. Cells were incubated for 3 hr with native laminin at 0.5 μg/ml at the start of the experiment and harvested as described. Data points represent mean of triplicate assays not differing by more than 3%.

7 cells to type IV collagen when used at concentrations of 1 μg/ml.

Laminin Binding to Cells. If laminin mediates attachment of epithelial cells to type IV collagen, then these cells may possess specific surface receptors that are involved in recognizing laminin. Moreover, cells such as fibroblasts, which utilize fibronectin rather than laminin as an attachment factor, should lack these laminin-binding sites. Experiments were conducted to determine whether ^{125}I-laminin binds to cells with high affinity and specificity. Binding of laminin to the MCF-7, T47-D, and the CRL 1477 and CRL 1507 fibroblast cell lines was time dependent. Equilibrium binding was reached after 90 min for all cell lines tested (Figs. 5 and 6). The human fibroblast cell lines, CRL 1477 and 1507, showed no evidence of a specific laminin receptor (Fig. 6) whereas the epithelial T47-D cells exhibited a low level of laminin binding when compared to the MCF-7 cells. Scatchard binding analysis (using MCF-7 cells) gave a roughly linear curve, with an estimated K_d of 50–2.2 nM. Calculations suggest 10,000–50,000 binding sites per cell. The receptor for laminin could be extracted from the cell membrane by 0.1% Triton X-100 and had a molecular weight of 60,000–75,000 after isolation by laminin affinity chromatography. Laminin fragments α3 and P1 both competed for ^{125}I-laminin binding at a level similar to whole laminin (Fig. 5). Laminin bound to both attached and suspended MCF-7 cells. For the latter, binding was identified 2 hr after trypsinization followed by incubation in Dulbecco's modified Eagle's medium containing 0.5% bovine serum albumin. Heat-denatured laminin and fibronectin were 1/50th to 1/500th as effective as native laminin in competing for ^{125}I-laminin binding. Both ^{125}I-laminin and ^{125}I-labeled α3 laminin fragment maintained biological activity when used in an attachment assay. Therefore, a major domain of the laminin molecule that binds to the MCF-7 and T47-D cells is retained on both the α3 laminin component and the C1 or P1 laminin fragment (Fig. 5, Table 2).

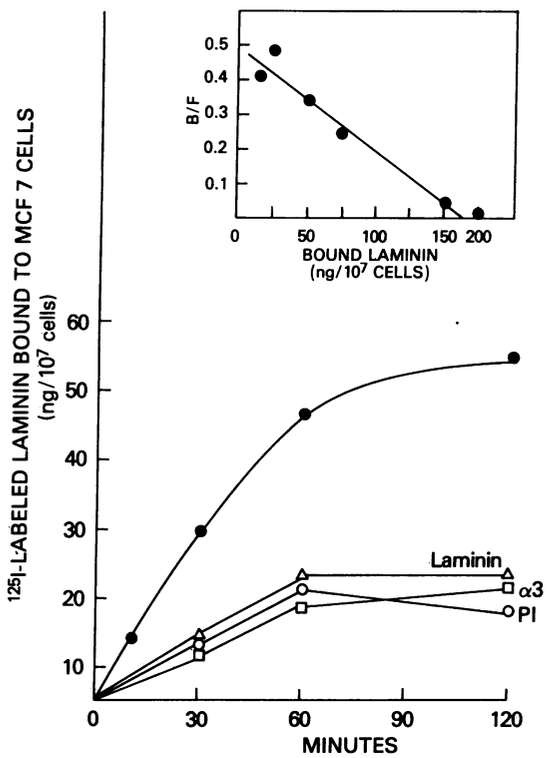

FIG. 5. Binding of ^{125}I-laminin to MCF-7 human breast carcinoma cells. A time course of binding is shown with or without 100× competition by unlabeled laminin or purified unlabeled protease-derived fragments of laminin. ●, Total laminin binding with no competitor; △, unlabeled laminin; □, α3, M_r 600,000 α thrombin digest fragment of laminin; ○, P1 pepsin fragment of laminin. (*Inset*) Scatchard plot of the ^{125}I-laminin binding to the MCF-7 cells. A least-squares analysis for the data shown yielded an r value of 0.98 for a linear fit. The MCF-7 cells were incubated with a series of concentrations of ^{125}I-laminin for 90 min. The amount of laminin bound to the MCF-7 cell surface is shown on the abcissia. The ratio of bound to free laminin is shown on the ordinate. Binding affinity of the isolated laminin receptor (M_r 60,000–75,000) was similar to that shown here for whole cells.

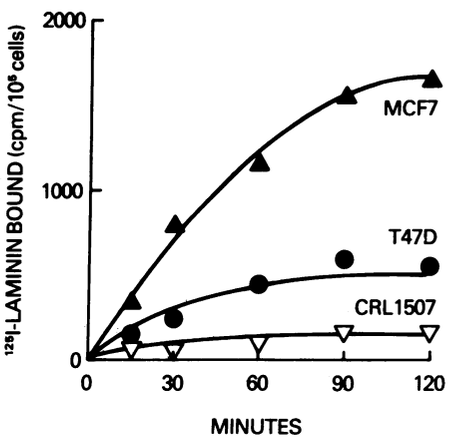

FIG. 6. Comparison of the time course of total ^{125}I-laminin binding to MCF-7 breast carcinoma (▲), T47-D breast carcinoma (●), and human fibroblast CRL 1507 and 1477 (▽) (Note: data are shown for CRL 1507 cells; data for CRL 1477 cells were identical.)

Laminin Binding to Collagen. In contrast, the same laminin fragments (α3 and P1) showed a marked difference in their ability to bind to type IV collagen immobilized on nitrocellulose. Native laminin and the α3 fragment bound equally well to type IV collagen (Table 2). The P1 or C1 fragments exhibited no capacity to bind to type IV collagen (Table 2). The structural and binding properties of the various laminin fragments are summarized in Fig. 7.

DISCUSSION

The present study demonstrates that three different lines of human breast carcinoma cells differ markedly in their utilization of laminin and fibronectin for attachment to type IV collagen. Furthermore, a high-affinity laminin receptor has been dem-

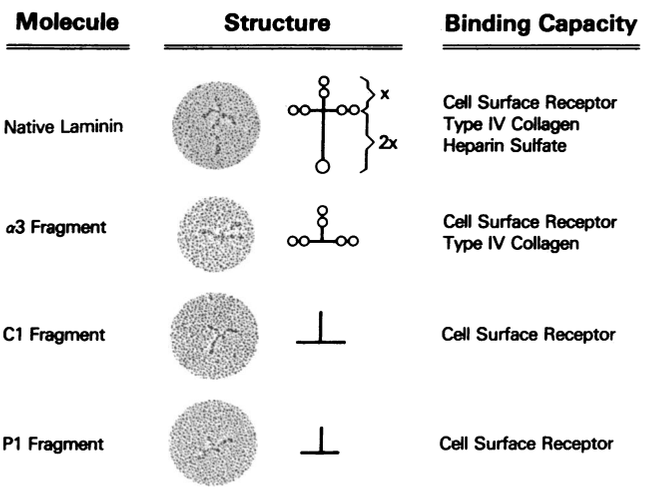

FIG. 7. Structural and functional properties of laminin and its fragments. Circles represent globular end regions. Representative (9, 24) electron micrographs of laminin or purified fragments are shown.

onstrated on the surface of MCF-7 cells. Exogenous laminin stimulates up to 80% of the MCF-7 cells to bind to type IV collagen. In contrast, fibronectin stimulates only 20% of the MCF-7 cells to bind to the same collagen substrate. The ZR-75-1 and T47-D breast carcinoma lines can use either fibronectin or laminin to attach to type IV collagen. These latter two cell lines show a decreased laminin-binding capacity.

Various hormones and growth factors are thought to be involved in the regulation of breast tumor growth or metabolism. These include estrogens, androgens, progestins, glucocorticoids, insulin, iodothyronines (for review see ref. 28), and epidermal growth factor (29). Cell surface receptors for all of these agents have been identified. The present study demonstrates a specific high-affinity receptor for laminin. The receptor was present on attached as well as suspended MCF-7 cells. Because the MCF-7 cells are stimulated to attach to type IV collagen by the addition of laminin at low concentrations, it is not surprising that these cells possess high-affinity cell binding sites for this attachment factor. The K_d of the MCF-7 laminin receptor is similar to the value found for growth factors such as epidermal growth factor (30, 31). Indeed, laminin may act like a growth factor that promotes cell attachment and spreading and subsequently stimulates cell division.

Tumor cells are thought to retain less fibronectin or laminin on their surface compared to their normal counterparts (32). Recently it has been shown that laminin promotes the adhesion and metastasis of highly metastatic murine tumor cells (2). The existence of an abundance of free laminin binding sites on the tumor cell surface may facilitate interaction of these cells by laminin with the vascular basement membrane. Because tumor cells survive for only a short time in the circulation, it is likely that any biochemical event that stimulates interaction of the circulating tumor cell with the exposed vascular basement membrane will enhance its ability to extravasate and initiate a metastatic focus.

A model for the domains of laminin that participate in cell attachment to type IV collagen can be derived from these results. Experiments employing protease-derived fragments of laminin in the cell attachment and collagen binding assays indicate the domains of the laminin molecule that bind to the cell surface and which domains bind to the type IV collagen substrate. The native laminin molecule binds to type IV collagen, binds to the cell surface, mediates cell attachment, and also binds to heparin (33) (Fig. 7). Protease-derived fragments of laminin each retain some, but not all, of these binding properties (Fig. 7). With rotary shadowing electron microscopy, the whole laminin molecule appears as a cross-shaped structure with three short arms and one long arm (8, 9, 24). Digestion of laminin with α thrombin does not affect the cell binding or type IV collagen attachment properties (Fig. 7). The $\alpha 3$ fragment (M_r 600,000) derived from thrombin digestion is missing the long arm (M_r 400,000 fragment) of the laminin molecule and retains the three similar short arms with their globular end regions (9). Digestion of laminin with pepsin or cathepsin G, yielding P1 and C1 fragments, respectively, removes the long arm of the molecule and also alters the globular end regions of the short arms. The P1 and C1 fragments bind to the cell surface (9, 24), block cell attachment to type IV collagen (Fig. 4), and also fail to directly bind to type IV collagen immobilized on nitrocellulose (Table 2). On the basis of the properties of these fragments, we conclude that a major cell receptor binding site on the laminin molecule resides on the midregion or intersection point of the three short arms of the laminin molecule. The type IV collagen-binding domain apparently resides on or near the globular regions of the short arms because their removal by pepsin or cathepsin G abolishes attachment to type IV collagen. The finding that the P1 or C1 fragment inhibits cell attachment can be explained by the ability of these fragments to saturate the cell surface receptor for laminin. However, because these fragments lack the type IV collagen-binding domains they fail to mediate attachment of the cells to this substrate.

Characterization of the specific domain of laminin that modifies tumor cell attachment may lead to the synthesis of a cell-binding analogue or type IV collagen-binding site analogue that could be used therapeutically to reduce metastasis from the hematogenous route.

1. Kleinman, H. K., Klebe, R. J. & Martin, G. R. (1981) *J. Cell Biol.* **88**, 473–485.
2. Terranova, V. P., Liotta, L. A., Russo, R. G. & Martin, G. R. (1982) *Cancer Res.* **47**, 2265–2269.
3. Hay, E. D. (1981) in *Cell Biology of Extracellular Matrix*, ed. Hay, E. (Plenum, London), pp. 1–39.
4. Grinnell, F. & Field, M. K. (1979) *Cell* **18**, 117–128.
5. Hewitt, A. T., Kleinman, H. K., Pennypacker, J. P. & Martin, G. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 385–388.
6. Chung, A. E., Jaffe, R., Freeman, I., Vergnes, J., Braguiski, J. & Carlin, B. (1979) *Cell* **16**, 277–281.
7. Terranova, V. P., Rohrbach, D. H. & Martin, G. R. (1980) *Cell* **22**, 719–728.
8. Engel, J., Odermatt, E., Engel, A., Madri, J. A., Furthmayr, H., Rohde, H. & Timpl, R. (1981) *J. Mol. Biol.* **150**, 97–120.
9. Rao, C. N., Margulies, I. M. K., Tralka, T. S., Terranova, V. P., Madri, J. A. & Liotta, L. A. (1982) *J. Biol. Chem.* **257**, 9740–9744.
10. Balian, G., Click, E. M., Crouch, E., Davidson, J. M. & Bornstein, P. (1979) *J. Biol. Chem.* **254**, 1429–1432.
11. McDonald, J. A. & Kelley, D. G. (1980) *J. Biol. Chem.* **255**, 8848–8858.
12. Liotta, L. A., Tryggvason, K., Garbisa, S., Hart, I., Foltz, C. M. & Shafie, S. (1980) *Nature (London)* **284**, 67–68.
13. Liotta, L. A. & Hart, I. R., eds. (1982) *Tumor Invasion and Metastasis* (Nijhoff, The Hague, Netherlands).
14. Kramer, R. H., Gonzales, R. & Nicolson, G. L. (1982) *Int. J. Cancer* **26**, 639–645.
15. Murray, J. C., Liotta, L. A., Rennard, S. I. & Martin, G. R. (1980) *Cancer Res.* **40**, 347–351.
16. Barnes, D. & Sato, G. (1979) *Nature (London)* **281**, 388.
17. Engel, L. W. & Young, N. A. (1978) *Cancer Res.* **38**, 4327–4339.
18. Shafie, S. & Liotta, L. A. (1980) *Cancer Lett.* **11**, 81–87.
19. Bornstein, P. & Piez, K. A. (1966) *Biochemistry* **5**, 3460–3473.
20. Garbisa, S., Liotta, L. A., Tryggvason, K. & Siegal, G. P. (1981) *FEBS Lett.* **127**, 257–262.
21. Timpl, R., Rohde, H., Gehron, R. P., Rennard, S. I., Foidart, J.-M. & Martin, G. R. (1979) *J. Biol. Chem.* **254**, 9933–9937.
22. Foidart, J. M., Bere, E. W., Yaar, M., Rennard, S. I., Gullino, M., Martin, G. R. & Katz, S. I. (1980) *Lab. Invest.* **42**, 336–342.
23. Thorell, J. I. & Johansson, B. G. (1969) *Biochim. Biophys. Acta* **251**, 363–369.
24. Rao, C. N., Margulies, I. M. K., Goldfarb, R. H., Madri, J. A., Woodley, D. T. & Liotta, L. A. (1982) *Arch. Biochem. Biophys.* **219**, 65–70.
25. Liotta, L. A., Goldfarb, R. H. & Terranova, V. P. (1981) *Thromb. Res.* **21**, 663–673.
26. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
27. Klebe, R. J. (1974) *Nature (London)* **250**, 248–251.
28. Osborne, C. K. & Lippmann, M. E. (1978) in *Breast Cancer: Advances in Research and Treatment*, ed. McGuire, W. L. (Plenum, New York), Vol. 2, pp. 103–148.
29. Osborne, C. K., Hamilton, B., Titus, G. & Livingston, R. B. (1980) *Cancer Res.* **40**, 2361–2366.
30. Salomon, D. S. (1980) *Exp. Cell Res.* **128**, 311–327.
31. Salomon, D. S., Liotta, L. A. & Kidwell, W. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 382–386.
32. Hayman, E. G., Engvall, E. & Ruoslahti, E. (1981) *J. Cell Biol.* **88**, 352–357.
33. Sakashita, S. & Ruoslahti, E. (1980) *FEBS Lett.* **116**, 243–246.