Role of Laminin Receptor in Tumor Cell Migration

Ulla M. Wewer, Giulia Taraboletti, Mark E. Sobel, Reidar Albrechtsen, and Lance A. Liona

ABSTRACT

Polyclonal antisera were made against biochemically purified laminin receptor protein as well as against synthetic peptides deduced from a complementary DNA clone corresponding to the COOH-terminal end of the laminin receptor (U. M. Wewer et al., Proc. Natl. Acad. Sci. USA, 83: 7137-7141, 1986). These antisera were used to study the potential role of laminin receptor in laminin-mediated attachment and haptotactic migration of human A2058 melanoma cells. The anti-laminin receptor antisera reacted with the surface of suspended, nonpermeabilized melanoma and carcinoma cells. The anti-laminin receptor antisera blocked the surface interaction of A2058 cells with endogenous laminin, resulting in the inhibition of laminin-mediated cell attachment. The A2058 melanoma cells migrated toward a gradient of solid phase laminin or fibronectin (haptotaxis). Anti-laminin antisera abolished haptotaxis on laminin but not on fibronectin. Synthetic peptide GRGDS corresponding to the fibronectin cell-binding domain inhibited haptotaxis on fibronectin but not on laminin. Both types of anti-laminin receptor antisera inhibited haptotaxis on laminin but not on fibronectin. Using immunohistochemistry, invading human carcinoma cells in vivo exhibited a marked cytoplasmic immunoreactivity for the receptor antigen. Together these findings indicate a specific role for the laminin receptor in laminin-mediated migration and that the ligand binding of the laminin receptor is encompassed in the COOH-terminal end of the protein.

INTRODUCTION

Basement membranes are specialized forms of the extracellular matrix which separate various epithelial and endothelial cells from the underlying interstitium and individually surround muscle, nerve, fat, and decidual cells (1). The major noncollagenous glycoprotein of the basement membranes is laminin (2-6). Laminin is important for cell attachment, cell spreading, neurite outgrowth, cell migration, and binding to other matrix components (7). Antibodies to laminin have been used to study the organization of basement membranes in normal versus malignant tissue (8-14). Invasive carcinomas consistently exhibited defective basement membranes adjacent to the invading tumor cells in the stroma. These and other studies showed that the development of a malignant tumor is accompanied by profound alterations in the composition and distribution pattern of the adjacent extracellular matrix. The question therefore arose as to whether changes in the manner in which a tumor cell interacts with the extracellular matrix may influence the malignant phenotype. The recent identification of a number of extracellular matrix cell surface receptors offers a new approach to study how regulatory signals transmitted from components of the extracellular environment to the cell may influence cell behavior, including cell attachment and migration, during tumor progression.

A cell surface laminin receptor (Mr ~68,000) has been identified and characterized by several laboratories (15-23). We have recently purified and molecularly cloned a human laminin receptor cDNA (24) to investigate the structure, function(s), and regulation (22) of the protein. The level of laminin receptor mRNA (determined by Northern analysis) correlated with the number of laminin receptors on the cell surfaces of a variety of human carcinoma-derived cell lines (determined by Scatchard analysis of laminin receptor binding assays). The data further suggested that the ability of a tumor cell to bind laminin may be controlled by the amount of laminin receptor mRNA available for translation. Furthermore, it has been suggested that the laminin receptor may play a role in the attachment and migration of tumor cells in vitro to laminin-coated surfaces (18, 23, 24) and in vivo during lung colonization by i.v. injected tumor cells (25). In the present study we investigated the potential role of the laminin receptor in aspects of the metastatic process involving laminin-mediated tumor cell attachment and migration. This was evaluated using antisera directed against specific synthetic peptides derived from the external (cell surface) domain of the laminin receptor. These antisera were studied for their effect on laminin- and fibronectin-mediated tumor cell migration. The antisera were also used to study the expression of the laminin receptor antigen in vivo using immunohistochemistry on tissue sections. An augmented content of laminin receptor antigen was noted in tumor cells and invading trophoblasts in a state of apparent active invasion and migration.

MATERIALS AND METHODS

Purification of the Human Laminin Receptor. Laminin receptor was isolated from liver metastases of human breast carcinomas (22). The purification procedure involved preparation and extraction of microsomal membranes and application of these extracts to an affinity matrix of laminin-Sepharose 4B. The affinity matrix was extensively washed including a step with 400 mM NaCl. The laminin receptor was eluted from the laminin affinity matrix by 1 M NaCl. Laminin receptor was also eluted with denaturing agents such as sodium dodecyl sulfate or urea. Material eluted directly and material cut out of the polyacrylamide gels were used for production of polyclonal antisera. Rabbits were given s.c. injections with approximately 50 μg of protein mixed with complete Freund's adjuvant. Four weeks later and at subsequent 3-week intervals, the rabbits were boosted with material in incomplete Freund's adjuvant and were bled 10 days after each injection.

cDNA-derived Peptides and Computer Analysis of Secondary Structure. Peptides were synthesized according to predicted amino acid sequences based on the nucleotide sequence of a cDNA-encoding human laminin receptor (22). The purification procedure involved preparation and extraction of microsomal membranes and application of these extracts to an affinity matrix of laminin-Sepharose 4B. The affinity matrix was extensively washed including a step with 400 mM NaCl. The laminin receptor was eluted from the laminin affinity matrix by 1 M NaCl. Laminin receptor was also eluted with denaturing agents such as sodium dodecyl sulfate or urea. Material eluted directly and material cut out of the polyacrylamide gels were used for production of polyclonal antisera. Rabbits were given s.c. injections with approximately 50 μg of protein mixed with complete Freund's adjuvant. Four weeks later and at subsequent 3-week intervals, the rabbits were boosted with material in incomplete Freund's adjuvant and were bled 10 days after each injection.

Received 4/22/87; revised 7/29/87; accepted 8/5/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This study was supported in part by the Danish Cancer Society.

2 To whom requests for reprints should be addressed, at University Institute of Pathological Anatomy, Frederik V's Vej 11, 2100 Copenhagen, Denmark.

3 The abbreviations used are: cDNA, complementary DNA; DMEM, Dulbec-co's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline (137 mM NaCl, 1.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.2-7.4).
Fig. 1. Amino acid sequence of the COOH-terminal end of the human laminin receptor as predicted by a cDNA clone (22). The two areas underlined have a high probability of a turn formation (p > 0.75 × 10⁻⁵), as calculated by the method of Chou and Fasman (26). Synthetic 20-mer peptides corresponding to these areas were used for production of polyclonal antisera.

**LCNTDSPLRYVDIAIPCNKNGKASHVGLMMVLAREV**

**LRMGQTISREHPWVMPDLYFYRDPEEIEKEQAAAE**

**KAVTKEFFQGGEWTAPAFTATQOPEAVDSEQVQV**

**SVPIQOFPTEDWSAQAPETDWSAAPTAQETWGA**

**1.46 × 10⁶**

**1.54 × 10⁵**

**HUMAN LAMININ RECEPTOR AND TUMOR CELL HAPTOTAXIS**

**Immunofluorescence Staining of the Laminin Receptor on the Cell Surface of Cells in Suspension.** Cultured cells were harvested by treatment with 0.1% trypsin/0.5% EDTA. Cell surface proteins were allowed to regenerate during a 30-60 min incubation period in a test tube at 37°C in growth medium containing 10% fetal bovine serum. During this period the cells were kept in suspension by occasional shaking. The cells were centrifuged, washed, and resuspended to a concentration of 10⁶ cells/ml of DMEM with 1% fetal bovine serum. Incubation of the cells (500,000 cells in 0.5 ml) with specific antiserum or preimmune serum (diluted 1:50-200) was carried out in microfuge tubes on ice for 2 h. The cells were washed twice in DMEM with 1% fetal bovine serum and were reacted with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (diluted 1:100; Miles Laboratories) for 30 min at room temperature. After a wash, 50 μl of 50% glycerol in PBS were added to the cell pellet, and portions of the cell suspension were applied to clean glass microscope slides, mounted with a coverslip, and examined in a Leitz fluorescence microscope.

**Cell Attachment and Haptotaxis Assays.** Suspensions of cultured A2058 cells were harvested as for the immunofluorescence staining experiments. The cells were preincubated on ice for 30 min with specific antiserum or preimmune serum in indicated dilutions (1:25-1000). After this period, cells were washed once in DMEM containing 1% fetal bovine serum added to bacteriological quality multiwell plates (20,000 cells/well) (Immunolon 2; Dynatech Laboratories, Inc., Alexandria, VA) and incubated for indicated periods of time at 37°C in 5% CO₂/95% air. Subsequently, cells which had attached and spread were counted under a phase microscope. The results were obtained when PBS with 1% bovine serum albumin was used as the incubation medium.

**Haptotaxis was assayed in modified miniature multwell Boyden chambers (Neuprobe, Inc.) as described previously (24, 34-36).** Nucleopore 8-μm-pore filters (Nucleopore Corporation, Pleasanton, CA) were coated with laminin or fibronectin on one side of the filter which faced the lower chamber. Laminin was purified from the EHS (Engelbreth-Holm-Swarm) tumor essentially as described (3). The chosen concentration of laminin was based on laminin dose-response studies correlating density units with cell number. The test antisera were added in indicated dilutions to the lower chamber. Laminin was purified from the EHS (Engelbreth-Holm-Swarm) tumor essentially as described (3). The chosen concentration of laminin was based on laminin dose-response studies (data not shown) to determine the lowest concentration of laminin which promotes the maximum haptotactic response. Fibronectin was purchased from Collaborative Research (Bedford, MA). Pentapeptides GRGD and GRGES were kindly provided by Dr. Kenneth Yamada, NIH. The tumor cells (400,000 in 200 μl DMEM containing 0.1% bovine serum albumin) were introduced into the upper chamber and the test antisera were added in indicated dilutions to the lower chamber. The chambers were then incubated for 4 h at 37°C. The number of cells migrating through the filter in response to laminin was time dependent, with a chosen assay time being 4 h. After the incubation, the filters were removed from the chambers and stained with hematoxylin. The number of cells which had migrated through the filter to the lower side of the filter were quantified using a LKB 2202 Ultrascan Laser Densitometer (LKB, Bromma, Sweden). The number of migrating cells was calculated from a standard curve (r = 0.99 linear correlation coefficient) correlating density units with cell number.

**Immunolocalization of the Laminin Receptor in Ethanol/ACetic Acid Fixed Paraffin-embedded Tissue Specimens.** Forty-eight carcinomas and nitrocellulose papers for 4 h at room temperature with shaking. After three 10-min washings in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.05% Tween 20, the nitrocellulose papers were incubated for 1 h at room temperature with secondary antibody, which was affinity purified goat anti-rabbit Fc alkaline phosphatase (Promega Biotech, Madison, WI) diluted 1:9,000 (0.9 μg/ml) in the nonfat dry milk-containing solution. The filters were washed again and stained with nitrotritrazolium (N-6876; Sigma) and 5-bromo-4-chloro-3-indolyl phosphate (B-8503; Sigma). The staining solution was prepared by mixing 200 μl of 50 mg/ml nitrotritrazolium dissolved in 70% N,N-dimethylformamide and 100 μl of 50 mg/ml 5-bromo-4-chloro-3-indolyl phosphate dissolved with 100 μl of 100 mM Tris-HCl, pH 9.4, and 100 mM NaCl. The staining reaction was stopped by extensive washing in distilled water. For control of effective transfer of proteins, nitrocellulose papers were stained with 0.04% amido black in 7% acetic acid.
normal tissue adjacent to the tumors (8, 13) were examined immunohistochemically for the presence of laminin receptor antigenicity. Tissue specimens were fixed in cold ethanol/acetic acid overnight as described (8, 12, 13) and embedded in paraffin using standard procedures. Freshly cut sections were prepared, deparaffinized, hydrated, and treated with 10% H2O2 in methanol for 10 min to abolish endogenous peroxidase.

The unlabeled peroxidase anti-peroxidase technique was used as described (37). Briefly, the sections were incubated with the specific antiserum or preimmune serum (diluted 1:100–1:500 in 50 mM Tris-HCl, pH 7.2) overnight at 4°C in a moist chamber. Swine anti-rabbit and peroxidase-antiperoxidase (Dakopatt a/s Accurate Chemicals, Westbury, NY) were diluted to 1:50 and were incubated for 30 min with the sections. Washing between the individual steps was carried out in 50 mM Tris-HCl containing 250 mM NaCl. The immune complexes were finally visualized by staining with 3,3'-diaminobenzidine tetrahydrochloride.

Internalization Studies. Labeling of laminin with colloidal gold was performed as described by Geoghenan and Ackerman (38). Briefly, colloidal gold was made by reducing chlorauric (HAuCl4 3H2O; Polysciences, Inc., Warrington, PA) with trisodium citrate. Gold particles were prepared with an average diameter of 5 nm. The colloidal gold solution was adjusted to pH 5.0, the approximate isoelectric point of laminin. Laminin purified from the L2 rat yolk sac carcinoma (39) was dialyzed against three changes of distilled deionized water and centrifuged at 10,000 × g for 20 min to remove denatured protein. Based on titration experiments, 160 μg of laminin were added to 10 ml of the gold solution together with 50 μl of H2O2 and 1 ml of 10% NaCl. After 3 h the solution was centrifuged at 10,000 × g for 30 min, and concentrated 10 × with silica gel. Excess free gold particles were removed by gel chromatography on Sepharose CL-6B (21 x 1.7 cm) in 0.02 M Tris-HCl, pH 8.2, with 0.1% bovine serum albumin. The gold-labeled laminin appeared in the void volume. The labeling was confirmed by electron microscopy after rotary shadowing. The number of gold particles conjugated to each laminin molecule averaged 10–15, and no free gold particles were observed.

Suspensions of cultured cells were harvested as for the immunofluorescence staining experiments. After a 30-min regeneration period, the cells were washed and resuspended in DMEM with 1% fetal bovine serum and gold-labeled laminin at 4°C. The mixture was incubated for 1 h at 4°C with occasional shaking. Apliquots were then fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The remaining cells were then fixed for 1 h and fixed (40, 41). Cells were embedded in 1% agar using standard techniques. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Jeol 500 electron microscope.

RESULTS

ELISA Demonstration of Reactivity and Specificity of Polyclonal Antisera. Two different 20-mer peptides predicted from the COOH-terminal end of the cDNA clone encoding laminin receptor (Fig. 1) were used to raise rabbit antisera. The reactivity of the peptide antisera with the respective peptide was demonstrated by ELISA (Fig. 2). When peptides were coated directly onto the microtiter wells (5 μg/well) and reacted with serial dilutions of specific antisera for 1 h, a positive signal was detected at a dilution of up to 1:25,000 (Fig. 2A). Reactivity was specific for each peptide with no cross-reactivity of antisera. The Asn-20-Arg antiserum to Pro-20-Ala peptide and vice versa (Fig. 2 A and B). Furthermore, the Asn-20-Arg peptide was conjugated to bovine serum albumin and keyhole limpet hemocyanin. In this assay, much less peptide was required (0.4 μg/well) to elicit the same or higher absorbance value for a given antiserum dilution (Fig. 2B). This result indicates that linking of peptide to a carrier significantly increases the availability of the peptide for reaction with the antiserum in ELISA. Both peptide antisera and polyclonal antiserum to the natural receptor reacted (Fig. 2C) with the biochemically purified human laminin receptor (Fig. 2D).

COOH Terminal of Laminin Receptor on Outside of the Cell Membrane. The presence of laminin receptor protein was demonstrated by immunoblotting technique. Polyclonal antisera against the synthetic peptides specifically corresponding to the COOH-terminal domain as well as antiserum to the natural receptor, recognized a M, ~68,000 band from Nonidet P-40 extracts of cultured MCF-7 breast carcinoma cells (Fig. 3, top).
Similar results were obtained from extracts of A2058 melanoma, A431 vulva squamous carcinoma, and HU lung carcinoma cell lines. Immunofluorescence staining with both the antiserum to the natural receptor and antisera to the peptides showed positive immunoreaction of the cell surfaces of living, nonpermeabilized tumor cells (Fig. 3, center). Using preimmune serum, no significant cell surface staining was observed (Fig. 3, bottom). This result suggests that the COOH-terminal domain of the laminin receptor is localized outside the cell membrane of these tumor cell lines.

Effect of Anti-Laminin Receptor Antisera on Laminin-mediated Cell Attachment. It has been observed previously that tumor cells expressing a metastatic phenotype are rich in laminin-like substances on their cell surfaces (42, 43). The A2058 human melanoma cells produced significant amounts of laminin as demonstrated by ELISA (0.5 μg/10⁶ cells/24 h) and immunoprecipitation studies (data not shown). By immunofluorescence staining, laminin immunoreactivity could be detected on the cell surfaces (data not shown). We also noted that the A2058 melanoma cells attached via this endogenously produced laminin to a bacteriological (non-surface-treated) plastic substratum (Fig. 4A). This cell attachment was significantly inhibited when the cells were preincubated with anti-laminin antisera (Fig. 4A). Preincubation of A2058 melanoma cells with polyclonal antiserum to the natural laminin receptor resulted in a marked dose-dependent inhibition of cell attachment (Fig. 4B). The effect of the two peptide antisera was also examined. The antiserum raised to the synthetic peptide closest to the COOH
terminus (Pro–20–Ala) appeared to have a significant inhibitory effect (Fig. 4B), whereas the other antisera (against Asn–20–Arg) had only a small effect. In all cases, preimmune serum had no significant effect on the cell attachment. This suggests that the laminin receptor of the A2058 cells may play a significant role in the laminin-mediated attachment of these cells and furthermore that areas located in the COOH-terminal portion of the laminin receptor might represent the actual binding domain. This is consistent with our previous observation that an anti-laminin receptor monoclonal antibody which blocks laminin binding to cells (21) specifically recognizes fusion proteins which contain only COOH-terminal domains of the laminin receptor (22).

Effect of Anti-Laminin Receptor Antisera on Laminin-mediated Haptotaxis. A2058 human melanoma cells migrate in response to a gradient of laminin (Fig. 5; Table 1) as assayed in a modified Boyden chamber with laminin-coated nucleopore filters. In this system, the tumor cells are placed on one side of a Nucleopore (8 μm pore size) filter and the opposite face of the filter is coated with laminin (10 μg/ml). The tumor cells were allowed to attach to the filter in the top part of the chamber and the antisera were then added in the lower part of the chamber. In this manner, the laminin receptor function associated with migration on laminin substrata could be separated from its effect on primary attachment. The polyclonal antisera against the natural laminin receptor and the polyclonal antisera against the synthetic peptides derived from the receptor cDNA clone all inhibited the laminin haptotaxis of A2058 cells in a dose-dependent manner compared to preimmune controls (Table 1). The A2058 cells also exhibited a significant haptotactic response to a gradient of fibronectin (Fig. 5). Anti-laminin antisera abrogated haptotactic response to laminin but not to fibronectin. In contrast, 50 μg/ml of the synthetic peptide GRGDS significantly inhibited haptotaxis on fibronectin. The control peptide GRGES, which is not an active binding sequence of fibronectin, did not have an inhibitory effect on fibronectin haptotaxis. There was no inhibition of laminin haptotaxis with these fibronectin peptides. Furthermore, the anti-laminin receptor antisera did not significantly inhibit fibronectin haptotaxis compared to controls. These results support the concept that different receptors are involved in laminin versus fibronectin haptotaxis.

In Vivo Expression of Laminin Receptor in Human Tumors Correlated with Their Invasive and Migratory Capacities. Bearing in mind the in vitro results, we next investigated the possibility that the laminin receptor might also be preferentially associated with cells involved in in vivo invasion and migration. Invading trophoblasts of decidua basalis in early human pregnancy which are characterized by invasive and migratory properties (44) exhibited a prominent cytoplasmic laminin receptor immunoreactivity (Fig. 6). In the malignant human tumors investigated, the tumor cells aggressively invading the extracellular matrix also exhibited intense cytoplasmic staining with the polyclonal anti-laminin receptor antisera (Fig. 7). The identical topographic distribution pattern was found using antisem to the natural laminin receptor or synthetic peptide antisera. Surrounding normal tissues had only little immunoreactivity, except for some proliferating blood vessels. Metastatic carcinoma cells in liver were strongly immunoreactive, whereas the surrounding parenchyma was essentially nonreactive with these antisera (not shown). There appeared to be a correlation between the degree of differentiation and the level of laminin receptor-positive cytoplasmic immunostaining (Table 2). In 33 of 48 cases studied, the moderately or poorly differentiated carcinomas had more abundant and intense laminin immunoreactivity compared to the well-differentiated carcinomas (15 cases of 48). The observation that the laminin receptor expression in vivo might be augmented in highly malignant tumor cells which are involved in invasion and migration is also supported by our finding that metastatic tumor tissue is a successful source for biochemical purification of the laminin receptor (22).

Table 1 Inhibition of laminin haptotaxis of A2058 cells by polyclonal antiserum to the natural laminin receptor (Anti-LR), antisera to the laminin receptor-synthetic peptides (Anti-LRP) Asn–20–Arg or Pro–20–Ala, compared to preimmune serum (Preimm).

<table>
<thead>
<tr>
<th>Antiserum (dilution)</th>
<th>Laminin haptotaxis (% of inhibition of migration compared to untreated cells; mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-LR 1:100</td>
<td>87.0 ± 13.0</td>
</tr>
<tr>
<td>Anti-LR 1:1000</td>
<td>55.0 ± 4.5</td>
</tr>
<tr>
<td>Anti-LRP (Asn–20–Arg) 1:100</td>
<td>85.8 ± 14.0</td>
</tr>
<tr>
<td>Anti-LRP (Asn–20–Arg) 1:1000</td>
<td>73.3 ± 8.0</td>
</tr>
<tr>
<td>Anti-LRP (Pro–20–Ala) 1:100</td>
<td>30.0 ± 4.1</td>
</tr>
<tr>
<td>Preimm 1:100</td>
<td>9 ± 0.0</td>
</tr>
<tr>
<td>Preimm 1:1000</td>
<td>0 ± 0.0</td>
</tr>
</tbody>
</table>

* Low level inhibition by the preimmune serum was sporadically observed in some experiments. This effect was abolished by heat inactivating (56°C) the preimmune serum. Heat inactivation had no effect on the ability of the antilaminin (Anti-LR) receptor serum to block laminin haptotaxis.

Some Cellular Laminin Receptor May Result from Receptor Protein Internalized Together with the Laminin Ligand. Cytoplasmic laminin receptor could reflect an increased pool of newly synthesized protein. Alternatively, in some biological processes, e.g., invasion, the transit time of the laminin receptor...
HUMAN LAMININ RECEPTOR AND TUMOR CELL HAPTOTAXIS

Fig. 6. Invasive trophoblasts (44) in decidua basalis of early pregnancy exhibit intensely positive cytoplasmic immunostaining with polyclonal antiserum to the natural laminin receptor (arrowheads). Faint positive immunoreactivity is seen along the basement membranes of the chorion villi (C). Large mature decidual cells (D) and endothelial cells of the blood vessels (V) appear unreactive with this antiserum. Antiserum dilution, 1:500. Counterstained with hematoxylin. x 350.

Fig. 7. Colon carcinoma with single laminin receptor-positive tumor cells invading the muscular layers of the bowel wall. Arrowheads, presence of laminin receptor-positive tumor cells inside a small vessel. Surrounding stroma appear unreactive with this antiserum. Immunoperoxidase staining was with polyclonal antiserum to the Pro-20-Ala laminin receptor peptide. Antiserum dilution, 1:100. Counterstained with hematoxylin. x 500.

Table 2 Immunoperoxidase staining of laminin receptor in 48 human carcinomas

<table>
<thead>
<tr>
<th>Tumor (cases)*</th>
<th>Highly differentiated</th>
<th>Moderately differentiated</th>
<th>Poorly differentiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinomas (16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma</td>
<td>+ (8)</td>
<td>+ (2)</td>
<td>+++ (5)</td>
</tr>
<tr>
<td>Papillomatous carcinoma</td>
<td>+ (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon/rectal carcinomas (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung carcinomas (10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>+++ (5)</td>
<td>+++ (2)</td>
<td></td>
</tr>
<tr>
<td>Adenomatous carcinoma</td>
<td>+ (3)</td>
<td>+ (2)</td>
<td>+++ (3)</td>
</tr>
<tr>
<td>Gastric carcinomas (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal carcinomas (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>+ (2)</td>
<td></td>
<td>+++ (2)</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>+ (2)</td>
<td></td>
<td>+++ (2)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, cases with the indicated staining reactivity. 
++++, ++, and +, strength of the immunoreactivity in order of decreasing intensity.

DISCUSSION

Tumor cell attachment and migration is thought to play a crucial role in the complex multistep process of tumor invasion and metastasis. In order to dissect the various biochemical and molecular mechanisms involved, we studied the role of laminin/laminin receptor in these processes using in vitro attachment and haptotaxis assays. We demonstrated that laminin-mediated attachment and haptotaxis of the A2058 melanoma cells are perturbed by antisera to the laminin receptor. The antibodies included polyclonal antisera to the natural receptor as well as antisera to two synthetic peptides. These two 20-mer peptides from the COOH-terminal part of the laminin receptor were selected because their amino acid sequence predicted a high probability for β-turn, a secondary structure which is often found on the external surface of proteins and which is associated with receptor-ligand binding domains (45). Immunocytochemical studies of various human carcinoma-derived cell lines further suggested that these COOH-terminal areas of the laminin receptor are located at the exterior part of the cell membrane. Thus, it appears that the binding domain of the laminin receptor to laminin may reside in the COOH-terminal portion of the receptor exposed to the extracellular space.

on the cell surface might be very short. Laminin receptor could conceivably be internalized together with its ligand, laminin. We approached this question by studying the fate of gold-labeled laminin following cell surface binding (Fig. 8A). Gold-labeled laminin subsequently became endocytosed (Fig. 8B) and was seen in lysosome-like structures in the cytoplasm (Fig. 8C).
Haptotaxis is defined as directed migration of cells along a gradient of a substratum-bound insolubilized factor (46). It can be distinguished from chemotaxis, in which the cell is responding to a soluble gradient of attractant. Laminin has been demonstrated previously to be involved in both chemotaxis and haptotaxis (23, 24, 34, 43). Furthermore, the addition of exogenous laminin to laminin-deficient tumor cell lines significantly increased their motility in vitro (43). Since laminin usually exists in a solid form in the basement membrane, laminin haptotaxis may be more physiologically relevant than laminin chemotaxis (24). A step gradient of laminin in the Boyden chamber assay is analogous to the in vivo situation in which the tumor cells encounter preformed host basement membranes as they invade blood vessels, nerves, or muscle. McCarthy et al. (34, 35) have shown previously that certain tumor cells can exhibit a haptotactic response to laminin and to fibronectin, although the cell surface receptors involved were not defined. In the present studies, A2058 melanoma cells were shown to require different receptor mechanisms to migrate in response to laminin versus fibronectin (Fig. 5). The $M_r$ ~68,000 laminin receptor appears to play an important role in laminin but not in fibronectin haptotaxis of A2058 cells. The GRGDS peptide which is the active domain on fibronectin (47) inhibited haptotactic response to fibronectin but not to laminin. In vivo, tumor cells migrate through different types of extracellular matrix, some containing laminin, and many others containing fibronectin or related adhesion proteins. The data presented in this study suggest that the same cell can express receptors for different matrix molecules and may use the appropriate receptor for migration on the appropriate substrate.

The human A2058 melanoma cell line produces significant amounts of laminin. Some of the secreted laminin becomes bound to the cell surface. We show in the present report that the tumor cells utilize the endogenous cell surface laminin to adhere and spread to bacteriological grade plastic substratum. Cell attachment was inhibited when the cells were preincubated with anti-laminin antiserum as compared to a preimmune control serum. The anti-receptor antisera significantly inhibited the attachment in a dose- and time-dependent manner compared to preimmune sera from the same rabbits. These data support the concept that the laminin receptor plays a significant role in conveying the endogenous laminin-mediated attachment of A2058 cells to a substratum. In this context, it is interesting to note that previous independent studies have reported that increased content of tumor cell surface laminin augments the ability of these cells to produce metastases following i.v. injection (25, 42). In contrast, fragments of the receptor-binding region of laminin which lack the globular end regions of the short and long arm significantly inhibit metastases (25). These data may be interpreted as follows. Tumor cells with exposed laminin receptors may use these receptors to bind in vivo to laminin in the basement membrane. If tumor cells synthesize laminin, the endogenous laminin may bind to the cell surface via the $M_r$ ~68,000 laminin receptor. Thus, the cell surface laminin may serve an adhesion function which requires the globular end regions of the laminin molecule arms. The exposed cell surface laminin globular end regions may promote metastases by interacting with cellular or matrix elements within the circulation, the endothelium, or the basement membrane. Endogenously produced cell surface laminin, then, may also play a significant role in the migration of tumor cells through tissues outside the circulation.

These in vitro studies suggested a role for the laminin receptor in migratory tumor cells. Therefore, we also examined cells in vivo in a state of apparent active migration and invasion, using immunohistochemistry. This class of cells appeared to express augmented amounts of cytoplasmic laminin receptor antigen. This was particularly the case for highly malignant, poorly differentiated carcinomas (Table 2). Perhaps tumor cells process the laminin receptor antigen in a manner intrinsically different from nonneoplastic cells. On the other hand, the cytoplasmic distribution may reflect an internalization of the receptor in actively invading and migrating cells not associated with a formed basement membrane. In these cells the laminin receptors may have a short cell surface transit time. As shown in Fig. 8, the laminin bound to tumor cell surface receptors apparently can be endocytosed in vitro. Internalization of laminin may be involved in the attachment-detachment cycle which is required during migration. We can speculate that an actively invading carcinoma cell may coat its own surface with endogenous laminin and then internalize the receptor and the ligand.

ACKNOWLEDGMENTS

The excellent technical assistance of Brit Valentin, Bodil Lindholm, and Hanne Kobbernagel is greatly appreciated. We thank Sue Hostler for secretarial expertise.

REFERENCES


Role of Laminin Receptor in Tumor Cell Migration

Ulla M. Wewer, Giulia Taraboletti, Mark E. Sobel, et al.