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Role of Galaptin in Ovarian Carcinoma Adhesion to Extracellular Matrix In Vitro

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Immunohistochemical studies indicated that galaptin is a major protein of ovarian carcinoma cells present in patient effusions and it is distributed throughout the cytoplasm. Enzyme-linked immunoadsorbent assay (ELISA) and immunoprecipitation experiments demonstrated that galaptin is also a major protein of the A121 ovarian carcinoma cell line, constituting ≤1% of extractable protein bound by DEAE Sephacel. Western blot analyses revealed that the galaptin present in ovarian carcinoma consists of a 14.5 KD subunit. Ovarian carcinoma and mesothelial cells isolated from patient effusions display surface receptors for galaptin with an apparently greater density of receptors present on the carcinoma cells. A121 cells also display surface receptors for galaptin: binding sites/cell = 3×10^8 and Ka = 1.2×10^9 M^{-1}. The presence of galaptin in bovine corneal endothelial cells (BCEC) and BCEC-derived extracellular matrix (ECM) was demonstrated by ELISA. Of the total ECM-bound galaptin, about 75% appears to be insoluble in phosphate-buffered saline (PBS) lactose. ECM was also found to contain abundant receptors for galaptin. Treatment of ECM with lactose increased the apparent galaptin receptor density:binding sites/cm^2 = 7×10^{13} and Ka = 2.6×10^9 M^{-1}. Pretreatment of A121 cells with galaptin inhibited adhesion to ECM. The addition of exogenous galaptin to ECM had variable effect on cell adhesion. The data presented here suggest that early adhesion events may be carbohydrate-specific involving interaction between ECM-bound galaptin and cell surface galaptin receptors.

Key words: ovarian carcinoma, endogenous lectin, extracellular matrix, receptors, cell adhesion, beta-galactoside

Galaptin is an evolutionarily conserved β-galactoside-binding lectin present in a wide variety of tissues and cells of vertebrate origin [1]. The function of galaptin is unknown; however, data has been presented by our laboratory and by others that suggest that this lectin may play a role in cellular adhesion phenomena [2]. Immunohistochemical studies have shown that galaptin appears to be densely located in the extracellular matrix (ECM) for a variety of tissues [1,3]. The density of galaptin in the ECM appears

Abbreviations used: ELISA, enzyme-linked immunoadsorbent assay; ECM, extracellular matrix; BCEC, bovine corneal endothelial cells; PBS, Dulbecco's phosphate-buffered saline; BSA, bovine serum albumin

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to be depressed in human malignancy (unpublished observations). The expression and density of cell surface galaptin in murine and rat systems are modulated by viral transformation. Highly metastatic rat and murine cell lines have been shown to display higher cell surface galaptin density than corresponding unselected parent cell lines. Anti-galaptin monoclonal antibody has been shown to inhibit colony formation in soft agar and to inhibit lung colonization by B16 melanoma cells [2,4].

Human ovarian carcinoma exhibits unusual growth properties in vivo that involves metastatic growth on the peritoneum, shedding of cells into the peritoneal cavity, growth of multicellular clusters in effusions, and readherence of cells to the peritoneum [5]. The studies reported here were initiated to assess the role of galaptin in adhesion of ovarian carcinoma cells to ECM. ECM used in these studies was that synthesized in vitro by BCEC. This ECM was shown to act as a model substratum for the preferential attachment of human ovarian tumor cells in vitro [6].

MATERIALS AND METHODS

For the immunolocalization of galaptin, ovarian carcinoma cells were isolated from patient effusions as described [5]. Cell pellets were fixed in Lillie's solution [7] and embedded in paraffin. Sections, 5 μM, were cut and processed for immunoperoxidase staining essentially as described in the Vectastain ABC kit (Vector Laboratories, Burlingame, CA). The primary anti-galaptin serum was that previously described [8]. Blocking solution and primary antiserum diluent was 20% normal goat serum. Buffers contained Nonidet P-40 (50 μl of 1% NP-40: 500 ml). Control sections were treated with preimmune serum or with anti-galaptin serum blocked with purified galaptin. Anti-galaptin dilution utilized was 1:600. Sections were counterstained with hematoxylin.

The antiserum utilized in the immunoprecipitation and ELISA assays was raised against the 14.5 KD galaptin subunit prepared from human spleen. In brief, galaptin was isolated by affinity chromatography on asialofetuin-Sepharose [9] followed by DEAE Sephacel chromatography in 0.025 M NaCl-0.01 M Tris-0.01 M mercaptoethanol, pH 7.4. Galaptin was step eluted with 0.2 M NaCl and subjected to preparative slab gel SDS-PAGE as described for analytical gels [9]. The region of gel containing the 14.5 KD subunit was extracted for injection into rabbits. Antiserum obtained on day 126 (booster injection #5) was utilized for the studies reported here.

The specificity of this antiserum was assessed by Western blot analysis [10]. Tissues were homogenized in 1.2 M Tris-0.037 M EDTA-2% SDS-10 M urea-0.001 M dithiothreitol-0.25 mM phenylmethyl sulfonylfluoride, pH 8.6. Solid dithiothreitol was added to the soluble extract to give 30 mM followed by incubation at room temperature under N_2 for 2 h. Solid iodoacetamide was then added to give 100 mM followed by incubation at room temperature under N_2 for 30 min. The extracts were equilibrated with sample buffer by passing through desalting gel filtration columns prior to SDS-PAGE on 12% acrylamide slab gels [11]. The gel was blotted onto nitrocellulose and the blot was blocked with 5% nonfat dry milk. Immunodetection was carried out with anti-galaptin serum (1:1,000) followed by goat anti-rabbit IgG-peroxidase conjugate (Kinkegaard and Perry Laboratories, Gaithersburg, MD) with 4-chloronaphthol as peroxidase substrate.

For immunoprecipitation of galaptin, A121 cells were radiolabelled for 4 h with [^{35}S]methionine in methionine-free RPMI 1640 medium-10% dialyzed fetal calf serum. Immunoprecipitation, SDS-PAGE, and fluorography were carried out as described [12] except that all solutions contained 50 mM lactose.

For ELISA, galaptin (10 ng for human, 20 ng for calf) was adsorbed to the wells of Immulon I ELISA plates (Dynatech, Chantilly, VA). Anti-galaptin serum, 1:10,000 in 10% normal goat serum, was mixed with an equal volume of various dilutions of samples containing an unknown amount of galaptin. After 1 h at 37°C, the solutions were applied to the coated wells. After 1 h at 37°C, the plates were washed and incubated with goat anti-rabbit IgG-peroxidase conjugate (1:500). After 1 h at 37°C, the plates were washed and assayed for peroxidase activity [13]. Standard solutions of human or calf galaptin were assayed in parallel. All wash buffers contained 0.5% Triton X-100 and all buffers contained 0.1 M lactose. The dilution of unknown, which gave 50% inhibition of antibody binding, was used to calculate galaptin concentrations. Additional details are given in Results.

Immunoreactive galaptin remaining in ECM following lactose treatment was also determined by ELISA. Primary anti-galaptin antiserum (1:200 and 1:1,000 dilutions) was added to triplicate wells. After incubation and washing of the wells, bound antibody was detected as above. Control wells containing lactose-insoluble ECM were treated with preimmune serum. Calibration curves were constructed by coating blank wells with 5–200 ng galaptin. Additional details are given in Results.

Carcinoma cell and ECM receptor assays were carried out with ^{14}C-labeled human splenic galaptin. For radiolabeling, 1 mg galaptin was adsorbed to 0.5 ml DEAE Sephacel in a small column wrapped in foil at room temperature. [^{14}C]Iodacetamide (50 μCi) was dissolved in 300 μl of 0.02 M NaCl-0.01 M Tris-0.1 M lactose, pH 7.4, and applied to the column. After 60 min, 500 μl of 0.1 M iodoacetamide in the preceding buffer was applied to the column. After 30 min, the column was washed. The radiolabeled galaptin was eluted with 0.2 M NaCl-0.01 M Tris, pH 7.4, and was repurified by affinity chromatography. The specific activity of galaptin varied from 1,500 to 2,400 cpm/μg.

For cell binding assays, near confluent cultures were harvested by EDTA-mediated detachment and scraping. The cells were washed and suspended in PBS-1% BSA. Additional details are given in Results. Binding assays were also carried out with ovarian carcinoma cell clusters and mesothelial cells isolated from patient effusions [5] as described in Results.

For ECM binding assays, BCEC were grown to confluency in 24 well plates. The plates were treated briefly with 0.02 M NH_4OH to remove the cells and then washed with PBS. The wells were then treated for 18–72 h, 4°C, with PBS-1 mM Ca^{++}-1% BSA with and without 0.1 M lactose. The plates were washed with PBS prior to binding assay. Additional details are given in Results.

Saturation binding curves were subjected to Scatchard analysis [14]. Cell adhesion assays were carried out with A121 cells and cell-free ECM. Details are given in Results.

A121 cells, BCEC cultures, and BCEC-derived ECM have been previously described [6,15,16]. Sepharose and DEAE Sephacel were obtained from Pharmacia (Piscataway, NJ). [^{35}S]Methionine, 1,125 Ci/mM, was obtained from Dupont NEN (Wilmington, DE). [^{14}C]Iodoacetamide, 17.9 mCi/mM, and [^{3}H]thymidine, 80 Ci/mM, was obtained from ICN (Irvine, CA). Liquid scintillation counting was carried out with Scintiverse cocktail (Fisher Scientific, Rochester, NY).

RESULTS

The specificity of antiserum raised against affinity purified human lung galaptin was previously demonstrated by immunodiffusion assay [8]. The monospecificity of the

antiserum raised against human splenic galaptin was demonstrated by Western blot analysis (Fig. 1). A single 14.5 KD polypeptide was detected in the tissues assayed. The detection of only the 14.5 KD polypeptide was critically dependent on alkylation of samples prior to SDS-PAGE.

Ovarian carcinoma cells prepared from patient effusions stained intensely for galaptin (Fig. 2). The galaptin was distributed throughout the cytoplasm and frequently appeared to be in the nucleus, but was absent from vacuoles. Absorption of antiserum with purified galaptin blocked immunostaining (not shown).

Cultured ovarian carcinoma cells (A121) were assayed for the presence of galaptin. Anti-galaptin serum precipitated a 14.5 KD polypeptide from extracts of ^{35}S-methionine-labeled cells (Fig. 3). The presence of galaptin in A121 cells was confirmed by ELISA. The lectin represents a major protein of the cells, constituting $\leq 1\%$ of extractable protein bound by DEAE-Sephacel (Table I).

Immunohistochemical studies had shown that galaptin was present in abundant amounts in the ECM of normal tissues [1,3] (unpublished observations). It was of interest, therefore, to determine if galaptin receptors were present at the cell surface that might interact with ECM-bound galaptin. The results of galaptin binding assays are shown in Figure 4A and B. Data for human Widr colon and murine Lewis lung carcinoma cells are shown for comparison. The bound galaptin increased as a function of galaptin concentration. Binding was completely inhibited by 50 mM lactose.

Ovarian serous adenocarcinoma cell clusters isolated from effusions were also shown to bear receptors for galaptin as do mesothelial cells (Fig. 4B). However, the mesothelial cell receptors appeared to be of lower density and/or lower binding affinity.

The binding of galaptin by A121 cells was shown to be a saturable process (Fig. 5). Scatchard analysis of the binding data revealed a high density of high affinity binding sites; binding sites/cells $= 3 \times 10^8$ and Ka $= 1.2 \times 10^9$ M^{-1}.

It was of interest to determine if BCEC-derived ECM contained galaptin that might interact with cell surface receptors. The results of ELISA for BCEC and ECM extracts are given in Table I. Galaptin is a major protein of BCEC representing 0.4% of the DEAE Sephacel-bound protein. Galaptin was also present in the soluble fraction of ECM, constituting 0.02% of the DEAE Sephacel-bound protein.

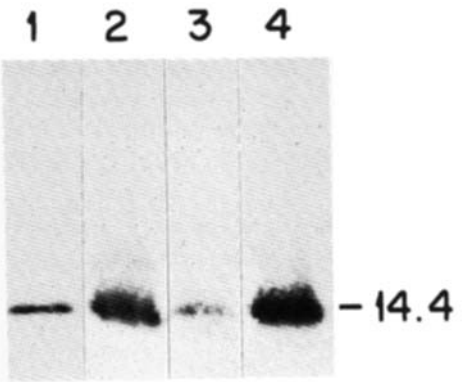


Fig. 1. Western blot analysis of tissue extracts with anti-spleen galaptin. **Lane 1:** Normal cutaneous tissue. **Lane 2:** Ovarian serous adenocarcinoma. **Lane 3:** Normal plasma. **Lane 4:** Affinity purified galaptin (20 ng). The migration position of a 14.4 KD molecular mass marker is indicated.

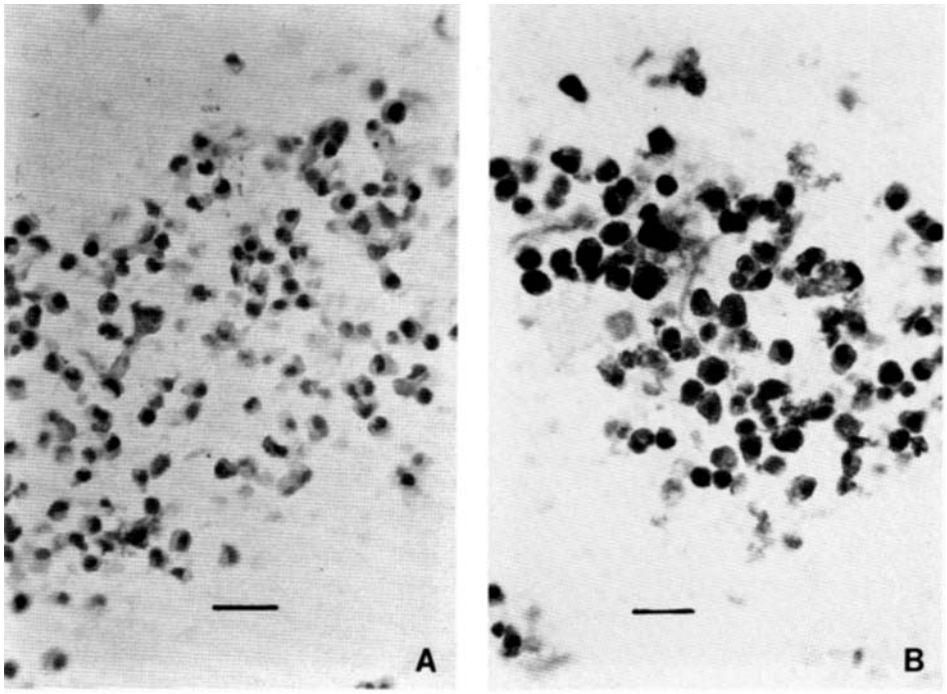


Fig. 2. Immunoperoxidase localization of galaptin in ovarian carcinoma cells isolated from a patient effusion. Cells shown were isolated from a patient with poorly differentiated serous adenocarcinoma. **A:** Preimmune serum control. **B:** Section immunostained with anti-galaptin serum. The immunoreactive galaptin is distributed throughout the cytoplasm. Dense immunostaining of nuclei is demonstrated in this view. Bar = 40 μ.

Assays were carried out to determine if BCEC-derived ECM contained immunoreactive galaptin not solubilized in the presence of lactose. For these assays, BCEC were grown in 24 well plates. At confluency, the cells were removed with 1 mM EDTA and the ECM analyzed by ELISA. The results are shown in Figure 6. Of the total immunoreactive galaptin, 27% was released from the matrix by 0.1 M lactose. The total immunoreactive galaptin content of BCEC-free ECM was 30 ng/cm^2. The ECM content of soluble galaptin was higher in these experiments than for those reported in Table I. This may be due to the inefficient removal of ECM from the plates as described in Table I.

BCEC-derived ECM was also shown to have galaptin receptors (Fig. 7). The apparent density of galaptin receptors could be increased by pretreating the ECM with 0.1 M lactose. Binding of radiolabeled galaptin to ECM was inhibited by 50 mM lactose. The binding of galaptin by ECM pretreated with lactose was saturable (Fig. 8). Scatchard analysis of the data indicated the presence of 7×10^{13} binding sites/cm^2 with Ka = 2.6×10^9 M^{-1}.

The presence of galaptin and galaptin receptors in carcinoma cells and ECM suggested that an interplay between these components may have a role in ovarian carcinoma cell adhesion to ECM. Experiments were carried out to determine if exogenously applied galaptin might influence cell adhesion in vitro. The results are summa-

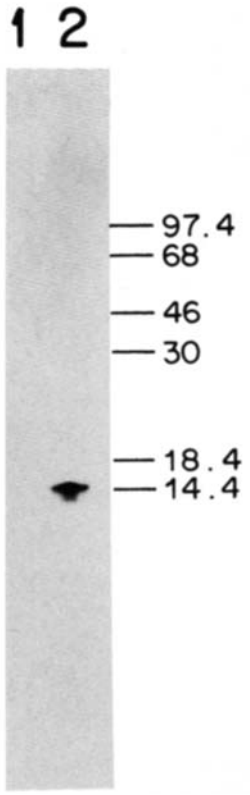


Fig. 3. Homogeneity of galaptin species synthesized by human A121 ovarian carcinoma cells. Immunoprecipitates of ^{35}S-methionine-labeled cells were dissolved in 2% SDS-50 mM dithiothreitol followed by alkylation with 0.1 M iodoacetamide. The samples were electrophoresed on 10–20% polyacrylamide slab gels followed by fluorography. **Lane 1:** Preimmune serum. **Lane 2:** Antigalaptin serum. The migration positions of molecular mass markers are indicated.

TABLE I. Galaptin in Cell and ECM Extracts[a]

Sample	Protein (μg)[c]	Galaptin (μg)	ng Galaptin/cm^2 ECM
BCEC[b]	877	3.7	
ECM[b]	859	0.17	2.9
A121[d]	1524	15.9	

[a]Galaptin was quantitated by ELISA using rabbit anti-human galaptin serum. Human and calf galaptin were used as standards.

[b]Bovine corneal endothelial cells were grown to confluency in 50 mm culture dishes. The cells were removed by EDTA treatment and extracted with 0.5% Triton X-100-0.1 M lactose. The ECM was scraped off the culture dish in the presence of Triton X-100-lactose and sonicated. Three cultures were pooled for each assay. The average of 3 assays is shown.

[c]Prior to assay, galaptin was partially purified by adsorption to and elution from DEAE Sephacel. Protein values are for the galaptin-containing 0.2 M NaCl eluates from DEAE.

[d]A121 ovarian carcinoma cells were grown in T75 flasks to confluency. The cells were detached by EDTA treatment and assayed as for BCEC. The average of 6 samples is shown.

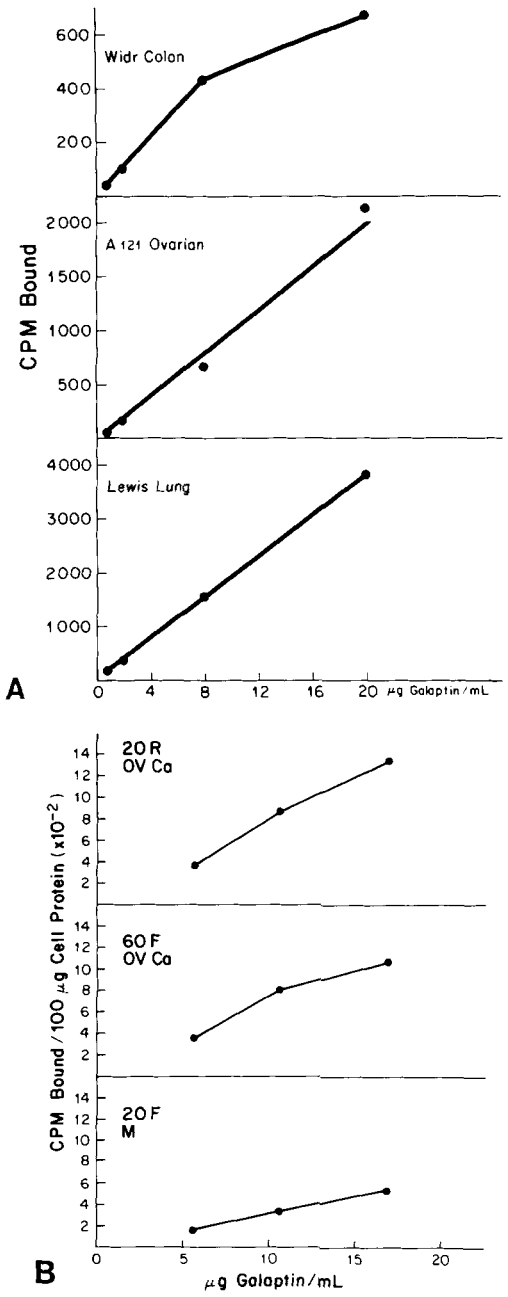


Fig. 4. Binding of human ^{14}C-galaptin by carcinoma cells. Binding assays were carried out in triplicate in 0.5 ml PBS-1% BSA at 4°C for 90 min. Cells were harvested on glass fiber filters, presoaked in 1% BSA, for liquid scintillation counting. Data are corrected for nonspecific binding that was less than 5% in the presence of 50 mM lactose. **A:** A121 cells were at 1×10^6/ml; Widr human colonic and Lewis murine lung carcinoma cells were not accurately counted because of aggregates. **B:** Cells isolated from an effusion. 20R are cell clusters retained on a nylon net with pores 20 μ in diameter; 60F are cell clusters that passed through a nylon net with pores 60 μ in diameter but were retained by a nylon net with 20 μ diameter pores; 20F are mesothelial cells that passed through a nylon net with 20 μ diameter pores. Data are corrected for nonspecific binding that was less than 5%.

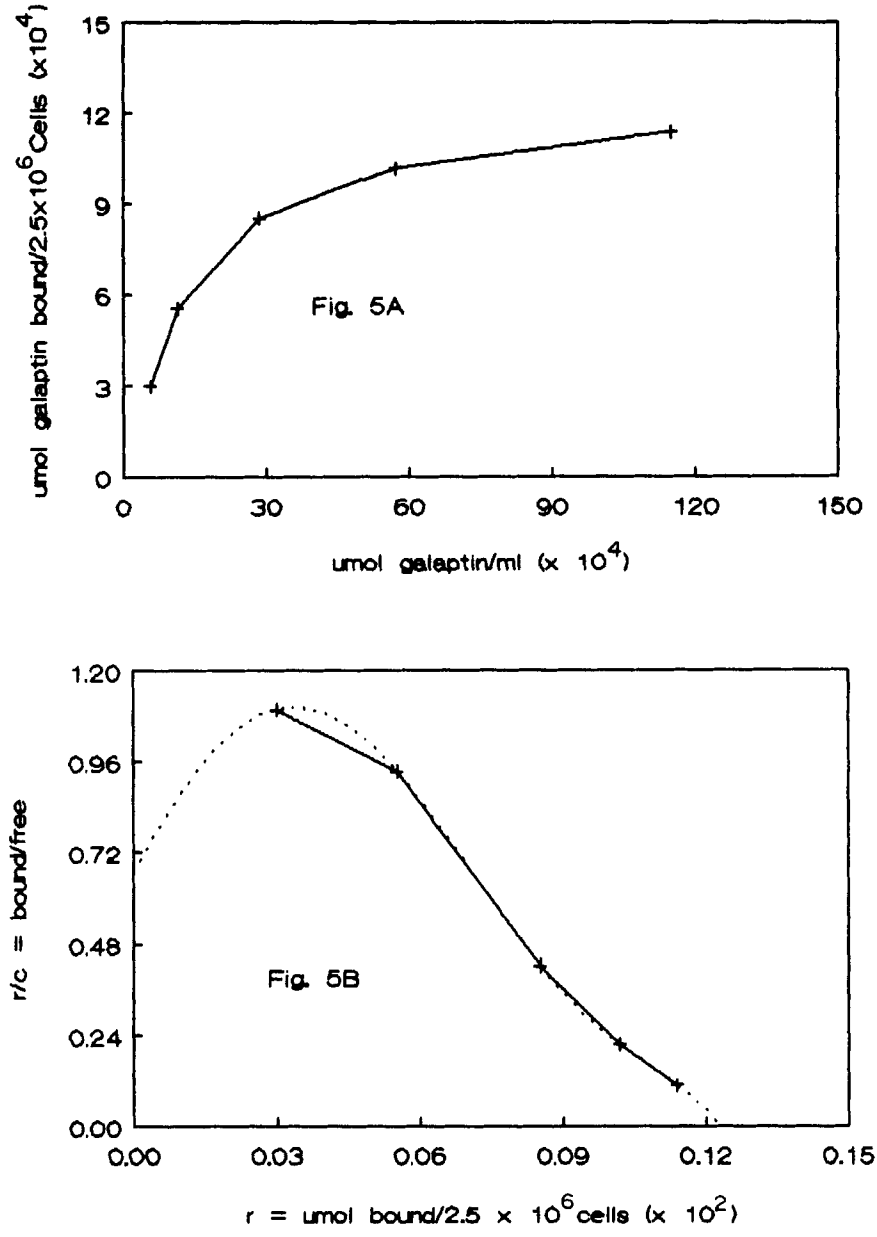


Fig. 5. Saturation binding curve for galaptin receptors on A121 ovarian carcinoma cells. A121 cells were harvested by EDTA treatment and were suspended in PBS. Binding assays were carried out in 0.5 ml PBS-0.5% BSA with 1.25×10^6 cells and variable amount of [^{14}C]galaptin. After 90 min at 4°C, the cells were washed on glass fiber filters, presoaked in 1% BSA, followed by liquid scintillation counting. Control tubes included 50 mM lactose. **A:** Binding curve. **B:** Scatchard plot. The plot is approximated with a sixth-order polynomial to estimate Ka ~ 1.2×10^9 M^{-1} and binding sites/cell ~3×10^8.

rized in Table II. When A121 cells were pretreated with galaptin, cell adhesion could be inhibited by as much as 75%. Pretreatment of ECM with galaptin had variable, but slight effect on A121 cell adhesion. The presence of 10 mM thiodigalactoside completely or partially inhibited the affect of galaptin on cell adhesion, depending on galaptin concentration: compare 112% vs. 59% and 58% vs. 25% of control (Table II).

DISCUSSION

Owing to the unusual growth properties of ovarian carcinoma in vivo [5], an elucidation of the mechanisms of cell-cell and cell-substration adhesion for this tumor system could lead to profound clinical benefits for the tumor host. BCEC-derived ECM has been shown to promote the efficient adhesion of ovarian carcinoma cells in vitro [6]. The final state of stable adhesion is likely to result from a sequence of highly specific interactions between cell and ECM and the molecular responses to these interactions. The vast data on altered cell surface glycoconjugates of transformed cells [17,18] and on endogenous lectins [4,8,19] drives the hypothesis that these glycoconjugates and lectins may play a role in metastasis via modulation of cell adhesion. Therefore, we have commenced an analysis of this carcinoma-ECM system as a model to probe the role of galaptin and galaptin receptors in cell-substratum adhesion.

Immunoperoxidase analysis revealed that ovarian carcinoma cells present in effusions contain an abundant amount of galaptin distributed throughout the cytoplasm (Fig. 2). What amount, if any, may be present at the cell surface could not be determined by the methods used here. Immunofluorescence analysis of viable cells will be of value in assaying surface-localized galaptin. Intracellular vacuoles and extracellular lumina formed by multicellular clusters were always devoid of immunoreactive galaptin. Nuclei stained variably, from no staining to intense staining, for galaptin. To what extent this is attributable to variations in cell cycle and/or sectioning artifact is not known. Similar nuclear immunostaining has been observed for EBV-induced B lymphoblastoid cells (unpublished). Western blot analysis of tumor extracts indicate that the galaptin species present consists of a 14.5 KD subunit (Fig. 1). This probably corresponds to the native 30 KD dimer found in many tissues [1,8]. The results of ELISA showed that abundant galaptin was also present in A121 ovarian carcinoma cells (Table I). Preliminary immunoperoxidase assays of A121 cell smears suggest that low, but variable, density of galaptin may be present at the cell surface. Fluorographic analysis of immunoprecipitates of A121 cell extracts showed that these cells synthesize the expected 14.5 KD galaptin subunit and no other immunoreactive polypeptides (Fig. 3).

It is noted here that if samples for SDS-PAGE are not adequately alkylated, or if thiol reducing agents are not present in the stacking and separating gels, galaptin-related polypeptides differing in molecular mass are detectable. This is the case for Western blot analysis, fluorographic analysis, and silver and Coomassie blue staining of affinity purified galaptin. We hypothesize that the SH groups present in galaptin apparently easily form disulfide bonds and generate homo- and heteroaggregates. This phenomenon may be responsible for detection of some of the higher molecular mass species of galaptin that are occasionally reported in the literature.

The presence of cell surface galaptin receptors was demonstrated for carcinoma cells and mesothelial cells (Fig. 4). Binding of galaptin was completely blocked by 50 mM lactose demonstrating carbohydrate-specific interaction. The ovarian carcinoma cells (20R and 60F) isolated from an effusion were present as multicellular clusters of

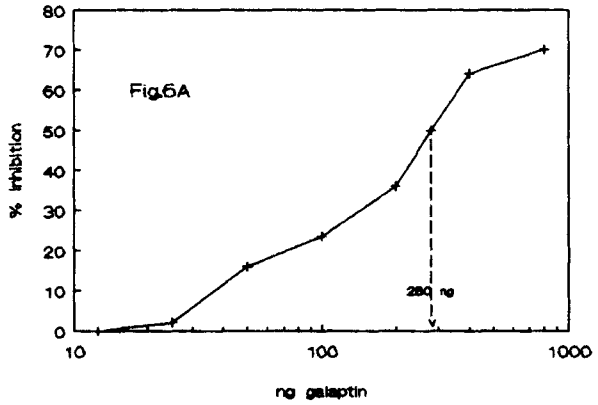


Fig.6A
% inhibition
ng galaptin
280 ng

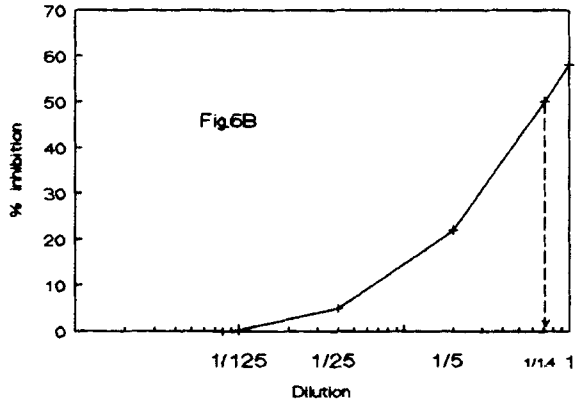


Fig.6B
% inhibition
Dilution
1/125
1/25
1/5
1/1.4
1

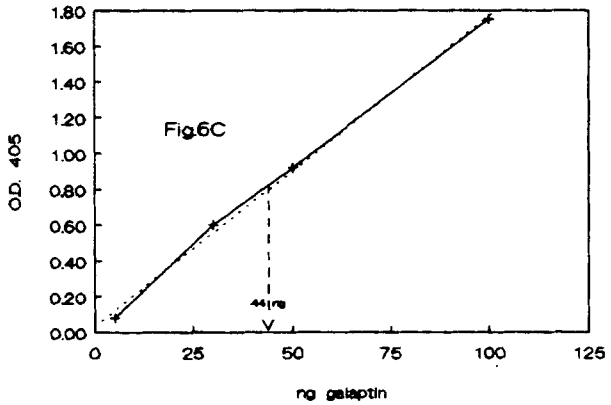


Fig.6C
O.D. 405
ng galaptin
44 ng

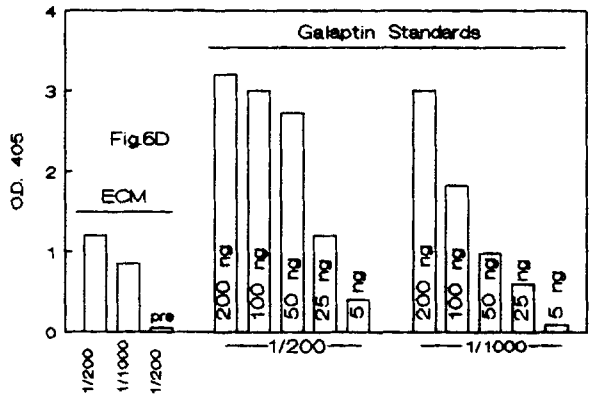


Fig.6D
O.D. 405
ECM
Galaptin Standards
200 ng
100 ng
50 ng
25 ng
5 ng
pre
1/200
1/1000
1/200
1/200
1/1000

Figure 6

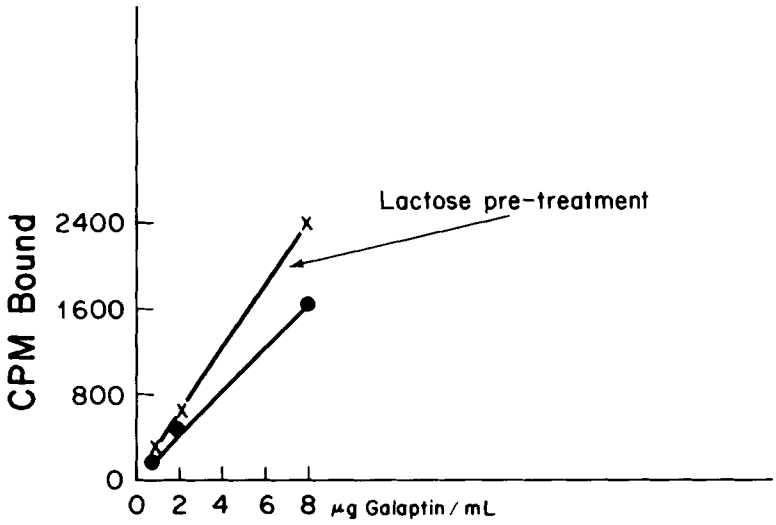
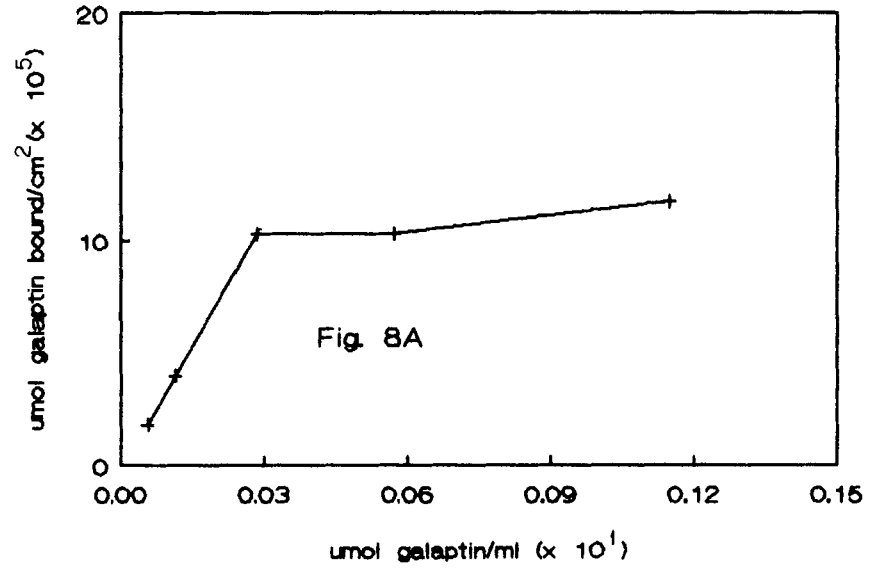


Fig. 7. Binding of human galaptin by ECM. Cell-free ECM was obtained from confluent BCEC cultures in 24 well plates. The plates were blocked with 1% BSA and incubated with and without 0.1 M lactose. After washing the plates, [^{14}C]galaptin in PBS-1 mM Ca^{++}-1% BSA was added in triplicate wells. Incubation was carried out at 4°C for 90 min. After washing the plates, bound galaptin was solubilized with 2% SDS and aliquots taken for liquid scintillation counting. Control wells contained 0.1 M lactose. Data are corrected for nonspecific binding that was <5%.

slightly dissimilar average size and they bound similar amounts of galaptin on a cellular protein basis (Fig. 4B). Although the mesothelial cells were present as single cells in suspension, galaptin binding was quantitated relative to cellular protein for comparison with the carcinoma cell clusters. Although the cell surface area available for galaptin binding was much greater for the mesothelial cells relative to the ovarian carcinoma cell clusters, the mesothelial cells bound less galaptin. The data indicate that the ovarian

Fig. 6. Quantitation of galaptin in bovine corneal endothelial cell-derived extracellular matrix synthesized in vitro. BCEC were grown to confluency in 24 well plates. The cells were removed by treatment with 1 mM EDTA. One milliliter of 0.1 M lactose – 1% BSA in PBS – 1 mM Ca++ – 1 mM Mg^{++} was added to each of 24 wells. The plate was incubated at 4°C for 72 h. The soluble contents of the wells were pooled, dialyzed against deionized water, and lyophilized. The residue was dissolved in buffer containing lactose and assayed for galaptin using an inhibition of antibody binding ELISA. Briefly, dilutions of the soluble ECM fraction were mixed with equal volumes of a 1:10,000 dilution of anti-galaptin antiserum. After incubation, the mixture was placed in wells of an ELISA plate containing adsorbed calf spleen galaptin. After incubation, the plates were washed and bound antibody was detected with goat anti-rabbit IgG-peroxidase conjugate. A calibration curve was constructed with affinity purified soluble calf spleen galaptin. The concentration of galaptin in the soluble ECM fraction was calculated relative to 50% inhibition values for antibody binding.

The ECM fraction remaining after lactose treatment was washed with PBS- Ca^{++}—Mg^{++}. Primary anti-galaptin antiserum (1:200 and 1:100 dilutions) was added to triplicate wells. After incubation and washing of the wells, bound antibody was detected with goat anti-rabbit IgG-peroxidase conjugate. Control wells were treated with preimmune serum or peroxidase conjugate. Calibration curves were constructed by coating blank wells with 5-200 ng galaptin. **A:** Calibration curve for soluble galaptin. **B:** Antibody inhibition curve for the lactose-soluble fraction of ECM. **C:** Calibration curve for insoluble galaptin; anti-galaptin dilution = 1:1,000. **D:** Binding of anti-galaptin to lactose-insoluble ECM and to plastic-adsorbed standard galaptin.



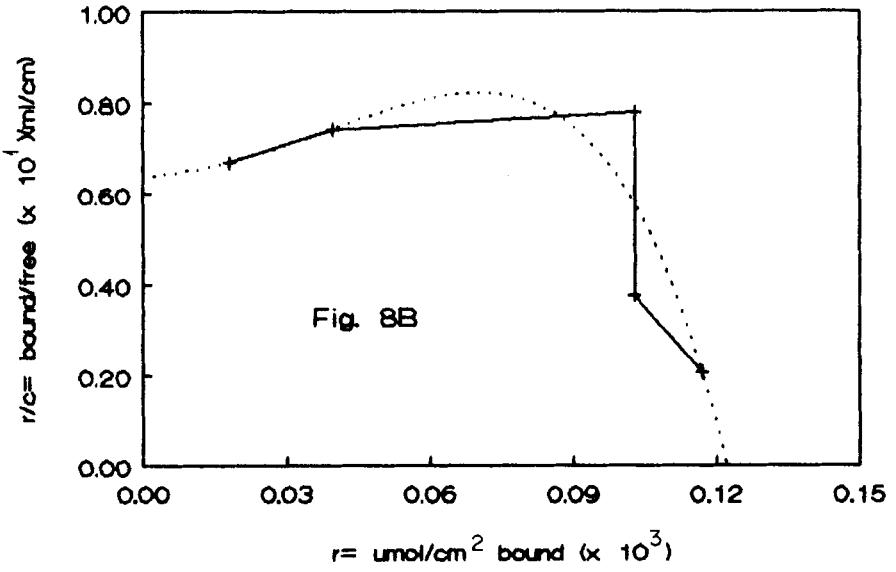


Fig. 8. Saturation binding curve for galaptin receptors in ECM. Cell-free ECM was obtained from confluent BCEC cultures in 24 well plates. The wells were incubated in PBS-1 mM Ca^{++}-1 mM Mg^{++}-0.3% Tween 20-0.1 M lactose at 4°C, 72 h. After washing the wells in PBS, varying amounts of [^{14}C]galaptin in 0.5 ml PBS-1% BSA were added to the wells. After 90 min at 4°C, the wells were washed with PBS-1% BSA. The bound [^{14}C]galaptin was solublized with 2% SDS for liquid scintillation counting. Control wells included 0.1 M lactose. The assays were done in triplicate. **A:** Binding curve. **B:** Scatchard plot. The Scatchard plot in **B** is approximated by a sixth-order polynomial to estimate Ka ~ 2.6×10^9 M^{-1} and binding sites/cm^2 ~ 7×10^{13}.

TABLE II. Influence of Galaptin on Carcinoma Cell Adhesion to ECM[a]

Pretreated	[Galaptin]	[TDG][b]	% of Control[c]
Cells	0	0	100[d]
Cells	1 ug/ml	0	59
Cells	10	0	56
Cells	100	0	25
ECM	1	0	122
ECM	10	0	98
ECM	100	0	80
Cells	1	10 mM	112
Cells	100	10	58

[a]BCEC were grown to confluency in 24 well plates. The cells were removed by mild treatment with 0.02 M NH_4OH. The plates were washed with PBS, treated with 1% BSA at 37°C for 1 h and then washed with PBS. A121 ovarian carcinoma cells labeled with [^{3}H]thymidine were harvested at confluency by EDTA treatment. The cells were washed with PBS and suspended in PBS-1 mM Ca^{++}-1 mM Mg^{++}- 1% BSA with galaptin and/or TDG. The cells were incubated for 1 h at 4°C followed by washing with PBS. The cells were suspended in PBS-1 mM Ca^{++} = 1 mM Mg^{++}- 1% BSA and aliquots placed in the ECM-containing wells. After 2 h at 37°C, the plates were washed. Adherent cells were solublized with 2% SDS and aliquots taken for scintillation counting. Alternatively, ECM was treated with galaptin similarly to the cells. The experiments were done in triplicate.

[b]Thiodigalactoside: a galaptin inhibitor.

[c]Control adhesion was 59% of the radioactivity (cells) added to the wells. This was normalized to 100% to facilitate comparisons.

[d]Control.

carcinoma clusters have galaptin receptors of greater surface density and/or greater binding affinity relative to mesothelial cells. Saturation binding experiments revealed a high density of high affinity galaptin receptors for A121 cells: binding sites/cell = 3×10^8 and Ka = 1.2×10^9 M^{-1} (Fig. 5). For comparison, 6C3HED murine ascites cells possess 3.6×10^6 lentil lectin binding sites with Ka = 4.2×10^6 M^{-1} [20].

ECM was also found to contain both galaptin and galaptin receptors. Immunoreactive galaptin was solublized from mechanically dislodged ECM by sonication in the presence of Triton X-100-lactose or more efficiently by prolonged incubation of plastic-adherant ECM in PBS-lactose at 4°C. This soluble fraction corresponded to 8 ng galaptin/cm^2 of ECM. Subsequent solid phase immunoassays on lactose-insoluble ECM demonstrated that 73% of the total ECM galaptin is present in the insoluble fraction, corresponding to 22 ng galaptin/cm^2 (Fig. 6). The nature of the linkages involved in anchoring the insoluble galaptin is unknown. The presence of immunoreactive galaptin in this ECM is consistent with numerous immunohistochemical observations on various tissues [1,3] (unpublished observations). It is possible that BCEC-derived ECM contains more than one type of immunoreactive polypeptide. Sufficient material has not yet been available to assess this possibility. However, an argument against this is the observation that the antiserum used here reacted with only the 14.5 KD galaptin subunit on Western blots of several tissues including human plasma and matrix-rich human cutaneous tissue (Fig. 1) and bovine spleen (not shown). Similar studies are in progress for BCEC and BCEC-derived ECM to determine the potential heterogeneity of immunoreactive galaptin in ECM.

The ECM-soluble galaptin may be bound to saccharides via its carbohydrate binding site. This possibility is derived from the observation that lactose pretreatment of

ECM increased the density of ECM-bound receptors and the binding of galaptin to ECM is completely inhibited by 0.1 M lactose. The specificity of the release of endogenous galaptin from ECM has not been determined. It is possible that other saccharides might promote the same result by some unknown mechanism. The binding of galaptin by ECM is a saturable process. The form of the binding curve and the Scatchard plot is unusual, however, and may be influenced by the physical state of the receptors as well as receptor heterogeneity (Fig. 8). Nevertheless, the data indicate the presence of a high density of high affinity receptors: binding sites/cm^2 of ECM $= 7 \times 10^{13}$ and Ka $= 2.6 \times 10^9$ M^{-1}.

Cell adhesion to substrata is a complex phenomenon involving a sequence of events. The data shown here (Table II) suggest that one of the early events could be a specific interaction between ECM-bound galaptin and cell surface receptors. Pretreatment of A121 cells with galaptin inhibited adhesion and inhibition was relieved by thiodigalactoside. This inhibition could have resulted from blocking the cell surface receptors from interacting with galaptin in ECM. Alternatively, the inhibition at high galaptin concentration could be due to cytoagglutination. Pretreatment of ECM with galaptin had little affect on cell adhesion. This implies that sufficient galaptin was already present in ECM to mediate optimal adhesion.

The perturbation of galaptin-galaptin receptor interaction in vivo may facilitate weakening of cell-substratum adhesion and lead to shedding of cells into the peritoneal cavity as is frequently observed with human ovarian carcinoma. Such a process is plausible in view of the fact that carcinoma cells isolated from effusions release to culture medium lactosaminoglycan-bearing glycoconjugates, which could function as galaptin inhibitors [21,22]. Additional studies with cell lines and cells isolated from effusions are required to adequately evaluate the role of galaptin and galaptin receptors in cell adhesion.

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