Isolation and Characterization of Epinectin, a Novel Adhesion Protein for Epithelial Cells

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ABSTRACT A 70,000-mol-wt protein was isolated from A431 carcinoma cell extracellular matrix that promotes cell substratum adhesion of these epidermoid tumor cells. Extracellular matrix was isolated by a modification of a procedure described by Hedman et al. (Hedman, K., M. Kurkinen, K. Alitalo, A. Vaheri, S. Johansson, and M. Höök, 1979 *J. Cell Biol.*, 81:83–91) and Yamada and Weston (Yamada, K., and J. A. Weston, 1974, *Proc. Natl. Acad. Sci. USA*, 71:3492–3496). Cells were solubilized with 0.5% deoxycholate, 10 mM Tris, 0.9% NaCl, and 1 mM phenylmethylsulfonyl fluoride, pH 8.0. The residual matrix was then removed from the plates with 6 M urea and 1 mM phenylmethylsulfonyl fluoride and phosphate-buffered saline. SDS PAGE gels of the 6 M urea extract showed one major band at 70,000-mol-wt by Coomassie Blue staining. A 70,000-mol-wt isotopically-labeled band could also be extracted from the matrix of cells incubated with [^{35}S]methionine. Because of the presence of this protein on squamous-derived epithelial cells we have called the 70,000-mol-wt molecule epinectin. Indirect immunofluorescence with polyclonal rabbit antibodies against epinectin stained A431 cells pericellularly in dense punctate accumulations and along the plasma membrane. Enzyme-linked immunoassays and gel-transfer immunolocalization studies showed that the extract did not cross-react with antibodies to fibronectin, laminin, serum-spreading factor, epibolin, or keratin. Additionally, antibodies to epinectin did not cross-react with these proteins. Further studies showed that epinectin does not bind to gelatin. Cell-adhesion assay, using radiolabeled A431 carcinoma cells on various adhesion-promoting substrates, showed that epinectin has similar adhesion-promoting capacity as serum-spreading factor, was somewhat less active than fibronectin, but more effective than laminin or epibolin. Epinectin appears to be a unique protein isolated from epidermoid tumor cells that is distinct from other known adhesion proteins.

Cell adhesion is a complicated phenomenon with profound biological significance. Cell-substratum attachment is involved in development, wound healing, cell migration, inflammation, and metastasis. Various molecules, such as glycoproteins, proteoglycans, and glycosyl transferases, may be involved in the attachment of cells to matrices. Much effort has focused on glycoproteins present either in serum, basement membranes, or the cell matrix produced by cells in culture. At present, a limited number of glycoproteins, including fibronectin (1), laminin (2), epibolin (3), serum-spreading factor (SSF)[1] (4), and a 140,000-mol-wt glycoprotein (5) are known to mediate attachment. These adhesion proteins are often of high molecular weight, have disulfide bonding, and sometimes exist in multimeric forms. Fibronectin is a disulfide-bonded, 440,000-mol-wt protein present in plasma, cell matrices, and basal lamina (for reviews see references 1, 6). Fibronectin promotes the adhesion and migration of a variety of cell types. Studies have shown that specific regions of the fibronectin molecule bind to collagen, heparan sulfate, and fibrin, or have other unique functions (1, 6). A small peptide fragment of fibronectin has been observed to promote the attachment of cells (7). Laminin is a disulfide-

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DME, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; SSF, serum-spreading factor.

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bonded, 850,000-mol-wt protein composed of subunits of 400,000 and 200,000 mol wt. Laminin is located in basement membranes throughout the body, including vascular, glomerular, and pulmonary basement membranes, etc. (6, 8, 9). Laminin promotes the attachment of epithelial cells to type IV collagen (2) and promotes the adhesion of highly metastatic tumor cells to substrata (10). Original studies proposed that laminin could only promote cell attachment of epithelial cells. However, in more recent studies, laminin promoted attachment of cells of nonepithelial origin (11), and, in fact, is produced by fibrosarcoma cells and perhaps fibroblasts (12).

Two other adhesion-promoting molecules are epibolin and SSF. Epibolin is a 65,000-mol-wt protein, originally isolated from human plasma (3). The location of epibolin in tissues has not been determined. Epibolin promotes epithelial, specifically epidermal cell movement and spreading (3). SSF consists of 65,000- and 75,000-mol-wt proteins. It can be isolated from serum, platelets, and placenta. SSF promotes adhesion and spreading of a wide variety of cells of diverse origins (4).

It is possible that some attachment molecules are tissue or function specific, or that several molecules may act in concert to promote adhesion of any one cell. Our laboratory and others have tried to isolate novel molecules from the extracellular milieu that may be involved in the cell-attachment process. This report describes the isolation and characterization of a new cell attachment protein derived from the A431 epidermoid carcinoma line (13). This cell line was derived from a vulvar squamous carcinoma and has been studied extensively by laboratories working on epidermal growth factor receptors (14). The protein produced by these cells promotes significant attachment of the A431 cells and is distinct by functional and immunoassays from laminin, fibronectin, epibolin, and SSF. Indirect immunofluorescent staining of cells in vitro with antibodies produced to this protein show the protein to be distributed along the cell membrane and also in dense pericellular accumulations.

MATERIALS AND METHODS

Cells: A431 carcinoma cells were purchased from Meloy Laboratories (Springfield, VA). This cell line was originally isolated from a vulvar carcinoma (13). Cells were grown in 75-cm^2 Falcon flasks (Falcon Labware, Oxnard, CA) in Dulbecco's modified Eagle's medium (DME) (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) with 6% fetal calf serum (Flow Laboratories, Rockville, MD) at 37°C, 5% CO_2, and passaged at confluence at a 1:10 ratio every 4 d.

Isolation of Matrix: The cell matrix was extracted from confluent layers of A431 carcinoma cells using a modification of the urea extract procedure described (15, 16). Cells were grown for 4 d (until confluence) in DME with 6% fetal calf serum, rinsed three times with serum-free DME, fed with serum-free medium and grown for an additional 2 d. To extract matrix, we first rinsed cells three times with Dulbecco's phosphate-buffered saline (PBS) at room temperature, then treated with 0.5% deoxycholate-sodium salt (Sigma Chemical Co., St. Louis, MO), 0.9% NaCl, 1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co.), 10 mM Tris buffered at pH 8.0. Flasks were rotated vigorously on a Tektator V (American Scientific Products, McGaw Park, IL) rotating platform at room temperature for 10 min, the supernatant discarded, and the treatment repeated two additional times. Studies have shown that what remains after this or somewhat similar treatment is an extracellular matrix (15, 16). This residual matrix was rinsed with PBS, pH 7.4, and then extracted with 6 M urea in PBS with 1 mM phenylmethylsulfonyl fluoride for 2–3 h at 37°C. The urea soluble extract was dialyzed extensively against PBS, pH 7.4, and concentrated by evaporation. Extracts were analyzed by 7.5% SDS PAGE with a 5% stacking gel, using formulations of Blattler et al. (17), and the Laemmli buffer system (18).

Metabolic Labeling: A431 cells were rinsed in serum-free medium after 4 d in culture and labeled for 2 d with 25 μCi/ml [^{35}S]methionine (New England Nuclear, Boston, MA; specific activity 1,220 Ci/mMol). Radiolabeled cells were treated as described above to isolate cell matrices and radiolabeled proteins were similarly extracted with urea. Conditioned medium and cell extracts were analyzed on SDS PAGE with a 5% stacking gel and a 7.5% running gel. Autoradiography was performed by treating gels with En^{3}Hance (New England Nuclear), followed by drying, and exposure of Kodak XAR5 autoradiography film.

Antibody Production: The urea extract of the cell matrix was electrophoresed on 7.5% SDS polyacrylamide gels and a predominant 70,000-mol-wt protein was observed. The 70,000-mol-wt band was cut from the gel and protein was electrophoretically eluted from the polyacrylamide into small dialysis bags, concentrated by evaporation and injected subcutaneously every 2 wk into New Zealand white rabbits. The initial injection was in complete Freund's adjuvant (Cappel Laboratories, West Chester, PA) and all subsequent injections were in incomplete Freund's adjuvant (Gibco Laboratories, Grand Island, NY). Antibody titers were assayed by enzyme-linked immunoassay (ELISA). Studies showed that rabbits developed antibodies to the 70,000-mol-wt protein and also to albumin, which presumably was present in trace quantities (from serum in growth medium) in the original immunizing preparation. Therefore, antisera was purified on an affinity column of bovine serum albumin (BSA)–Affigel 10 (Bio-Rad Laboratories, Richmond, CA) prepared as recommended by the manufacturer; this removed contaminating antialbumin antibodies and the residual antibody not binding albumin was used in all further studies.

Additional Antibodies: Affinity-purified antibodies to fibronectin and laminin were prepared in this laboratory and previously characterized by Palm and Furcht (19). These antibodies were used to rule out the possibility that the 70,000-mol-wt protein was a proteolytic fragment of either higher molecular weight cell-adhesion molecule. Antihuman keratin was purchased from Accurate Scientific and Chemical Co. (Westbury, NY), anti-BSA antibodies were purchased from Cappel Laboratories (West Chester, PA). Because the 70,000-mol-wt protein derived from A431 cells has attachment promoting activities, as described below, studies were performed to ascertain any similarity of the protein with SSF and epibolin, proteins with similar activities and approximately similar molecular weights. The antigens and antibodies to SSF were kindly provided by Dr. David Barnes (University of Pittsburgh) and to epibolin by Dr. Kurt Stenn, (Yale University).

ELISA were performed as described (20), using primary antisera to the 70,000-mol-wt protein, fibronectin, laminin, SSF, epibolin, keratin, and albumin, starting at 1:100 dilution; this was then followed by a 1:500 dilution of peroxidase-conjugated goat anti-rabbit immunoglobulin G (Cappel Laboratories). Additional studies characterized the cell matrix preparation by gel-transfer immunolocalization (Western blots) as previously described (21). An "immunodot" analysis was also performed, which is very similar to gel-transfer immunolocalization, except that solutions of purified proteins are applied directly to nitrocellulose rather than being transferred from gels. Localization with antibodies is performed identically in each procedure (19).

Immunofluorescence: Cells were grown on glass coverslips for 2 d past the point of confluence and then fixed with 3% formaldehyde in PBS. Cells were rinsed five times in PBS with Ca^{++} (0.68 mM $CaCl_2$); then reacted with a 1:20 dilution of antibodies for ½ h in a humidified atmosphere. Cells were rinsed five times in PBS-Ca^{++} and reacted with a 1:40 dilution of rhodamine conjugated goat anti-rabbit immunoglobulin G (Cappel Laboratories) followed by five rinses in PBS-Ca^{++}. Fluorescence was observed on a Zeiss Universal microscope with an epifluorescence attachment and photographed with 3M ISO 1000 color slide film (3M, St. Paul, MN).

Cell Adhesion: A431 cells were radiolabeled for 1–2 d with 25 μCi/ml of ^{3}H-amino acids (ICN Radiochemical, Irvine, CA) in serum-free medium and then harvested at 80–100% confluence. Cells were washed free of radiolabeled free amino acids, released with trypsin, and trypsin was inhibited with 1 mg/ml soybean trypsin inhibitor (Sigma Chemical Co.). Cells were diluted to 5–8 × 10^4 cells/ml in DME with HEPES buffer and then 1 ml of a cell suspension was placed in each well. Before this, wells of 24 well dishes (Costar, Cambridge, MA) were coated for 2 h at 37°C with test proteins diluted in 0.05 M sodium carbonate buffer, pH 9.6. Wells were then coated for 3 h with 5 mg/ml BSA (Miles Lab, Elkhart, IN) to minimize any nonspecific adhesion. Triplicate samples of cells were incubated for 45 min at 37°C. Wells were then rinsed three times with PBS-Ca^{++} to remove unattached cells. Radiolabeled attached cells were solubilized in 1% SDS, 0.5 N NaOH, dissolved in Aquasol 2 (New England Nuclear), and then radioactivity was quantitated with a Beckman LS 230 Scintillation System. For antibody-blocking experiments, immunoglobin fractions of anti-70,000-mol-wt serum and preimmune serum were purified by DEAE (Whatman, Kent, England) batch processing. Antiepinectin and preimmune serum were both concentrated six times over the original sera and used at equivalent concentrations in antibody-blocking experiments. Antifibronectin and antilaminin were used in a range that completely blocks cell adhesion on fibronectin and laminin, respectively.

Assessment of Possible Gelatin Binding of 70,000-mol-wt Preparation: The ability of the 70,000-mol-wt protein isolated from A431 cells to bind to gelatin was assessed on a gelatin-agarose column made as described (22). A preparation of the 70,000-mol-wt protein was applied to the column (2 × 1 cm) in PBS with approximately equal amounts of fibronectin, which is known to bind gelatin. The sample was eluted with either 1 M NaBr, 0.02 M Na acetate, pH 5.0, or with 6 M urea in PBS. Samples were concentrated by precipitation with 4 vol of cold acetone and precipitates isolated by centrifugation in a Beckman centrifuge model J2-21 at 30,000 *g* for 15 min. Precipitates were resuspended in reducing sample buffer (3% SDS, 0.66 M Tris, pH 6.8, 15% glycerol, 0.01% Bromophenol Blue + 5% beta-mercaptoethanol) and run on SDS PAGE.

RESULTS

The cell matrix was isolated from A431 carcinoma cells. SDS PAGE analysis of the matrix and Coomassie Blue staining showed one major band with a nonreduced M_r of 53,000 (Fig. 1*b*) and reduced M_r of 70,000 (Fig. 1*c*). Metabolic labeling of cells with [^{35}S]methionine showed incorporation into the 70,000-mol-wt protein, indicating that cells were synthesizing this molecule rather than passively adsorbing it from serum (Fig. 2*b*). Residual material left after urea extraction was solubilized with reducing sample buffer. This residue had decreased intensity of the ^{35}S-labeled 70,000-mol-wt band compared to a number of other bands (Fig. 2*d*). In addition to the 70,000-mol-wt band in the matrix, a 70,000-mol-wt, [^{35}S]methionine-labeled molecule was secreted into the culture medium (Fig. 2*c*). The fact that other radiolabeled bands appear in Fig. 2*b* while only one Coomassie Blue band appears in Fig. 1*c* suggests limited methionine incorporation into the protein, while its observed accumulation over time is seen in Fig. 1*c*.

Antibodies to the 70,000-mol-wt protein were made in rabbits but showed additional reactivity to BSA presumably present in trace amounts (from serum in the culture medium) at the approximate M_r of the 70,000 protein used to immunize the rabbits. Therefore, BSA affinity columns were used to absorb out these antibodies. The remaining antibodies, which had activity against the 70,000-mol-wt protein, did not react with albumin by ELISA (data not shown).

Immunofluorescence using the purified anti-70,000-mol-wt antibodies showed the 70,000-mol-wt protein to have a pericellular distribution in cultures of A431 cells grown in vitro (Fig. 3). Two pericellular staining patterns were observed: a plasma membrane linear reaction and dense punctate extracellular accumulations (Fig. 3, *a* and *c*). The intensity of this globular extracellular reaction varied within the cell population; some areas of the culture stain intensely, while others lacked these extracellular globular deposits and had only the membrane staining. Staining with normal rabbit serum showed nominal reactivity and did not produce this pattern (Fig. 3*e*). Immunofluorescent staining of A431 cells with antibodies to laminin and fibronectin also showed minimal reaction (data not shown). Additional immunofluorescence studies were performed to determine the presence of SSF or epibolin. No reactivity was observed against these proteins (data not shown).

The 70,000-mol-wt pericellular protein shows no reaction with antibodies to fibronectin, laminin, SSF, epibolin, and human keratin by ELISA or immunodot analysis (Fig. 4). To further show that the 70,000-mol-wt band is distinct from these molecules and that antibody raised against 70,000-mol-wt is specific for 70,000-mol-wt molecule, gel-transfer immunolocalization (Western blots) were performed. The anti-70,000-mol-wt antibody reacts against the pericellular material derived from A431 cells and not to the other previously defined matrix molecules or attachment proteins such as laminin or fibronectin. Controls in these experiments showed reactivity of other antibodies to their respective antigens, i.e., antilaminin with laminin (data not shown).

A 70,000-mol-wt glycoprotein isolated by gelatin-Sepharose affinity chromatography from the culture medium of various cell types has been described (23). To show that the 70,000-mol-wt–derived glycoprotein from A431 cells was distinct from this gelatin-binding glycoprotein, we ran approximately equal amounts of the A431 derived "matrix" preparation and fibronectin over a gelatin-Sepharose column. As shown in Fig. 5*b*, the 70,000-mol-wt protein did not bind and came through in the void volume, while the fibronectin bound to the column and could be eluted with urea (Fig. 5*c*).

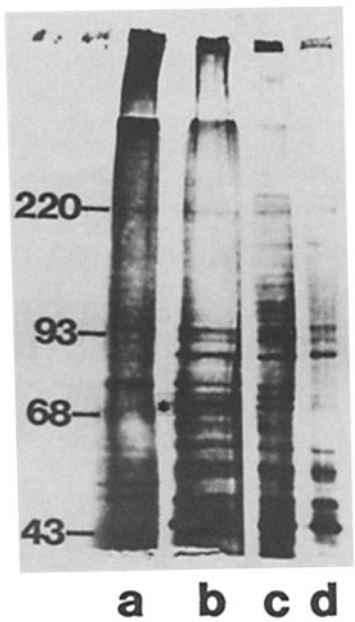

FIGURE 2 Fluorograph of 7.5% SDS polyacrylamide gel. All lanes show [^{35}S]methionine labeled proteins. (*a*) Urea-extracted matrix: unreduced. (*b*) Urea-extracted matrix: reduced. (*c*) Conditioned medium: reduced. (*d*) Posturea extract. Residue solubilized in reducing sample buffer. Asterisk indicates 70,000-mol-wt, epinectin molecule. 9,000 cpm per lane.

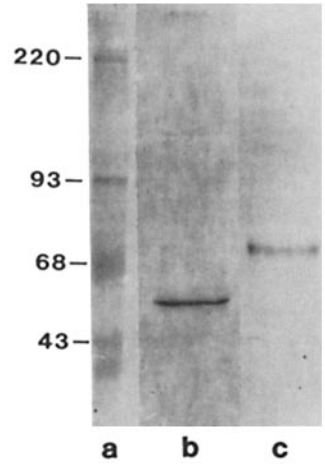

FIGURE 1 7.5% SDS polyacrylamide gel: Coomassie Blue stain. (*a*) Molecular weight standards (× 10^{-3}): fibronectin (220), phosphophorylase A (93), BSA (68), and ovalbumin (43). (*b*) Urea-extracted cell matrix A431 cells: unreduced. (*c*) Urea-extracted cell matrix A431 cells: reduced. Approximately 50 μg protein per lane.

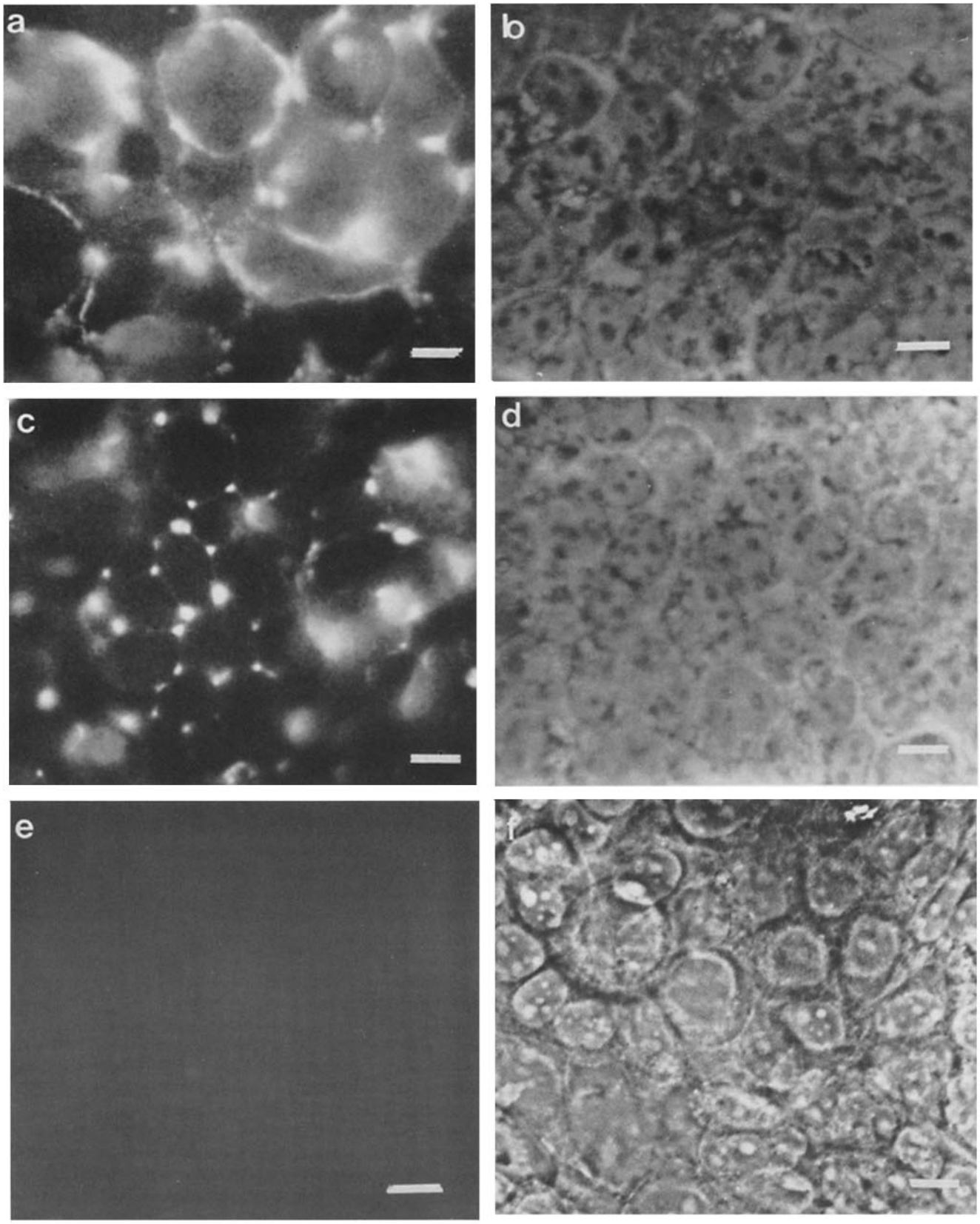

FIGURE 3 Phase-contrast and indirect immunofluorescence microscopy of A431 cells. Cells were grown to postconfluence on glass coverslips and fixed in 3% formaldehyde. (*a*) Immunofluorescent staining with antiepinectin antisera. × 500. (*b*) Phase-contrast micrograph of *a*. × 500. (*c*) Immunofluorescent staining with antiepinectin showing globular extracellular deposits. × 500. (*d*) Phase contrast of (*d*). × 500. (*e*) Immunofluorescent staining control with preimmune normal rabbit serum. × 500. (*f*) Phase-contrast micrograph of *a*. × 500. Bar, 5 μm.

	Epinectin	SSF	Epibolin	Laminin	Fibronectin
α Epinectin	+	−	−	−	−
α SSF	−	+			
α Epibolin	−		+		
α Laminin	−			+	
α Fibronectin	−				+

FIGURE 4 Summary of ELISA and gel-transfer immunolocalization analysis. All antibody reactions are at 1:100 dilution. α indicates antibodies to the various proteins. *SSF*, serum-spreading factor.

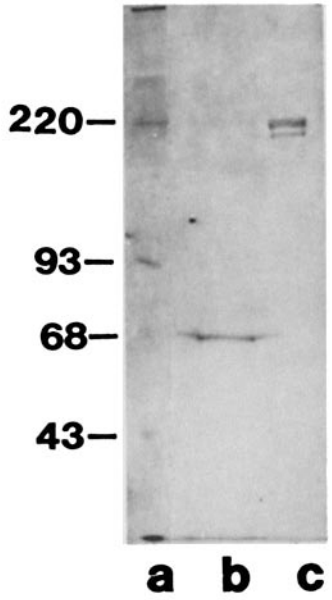

FIGURE 5 Gelatin-Sepharose affinity chromatography using similar concentrations of fibronectin and urea extracted matrix from A431 cells containing epinectin. 7.5% SDS polyacrylamide gel. (*a*) Molecular weight standards ($\times 10^{-3}$): fibronectin (220), phosphorylase A (93), BSA (68), and ovalbumin (43). (*b*) Unbound materials: containing mainly epinectin in the void volume. (*c*) Bound material: eluted with 8 M urea: mainly fibronectin.

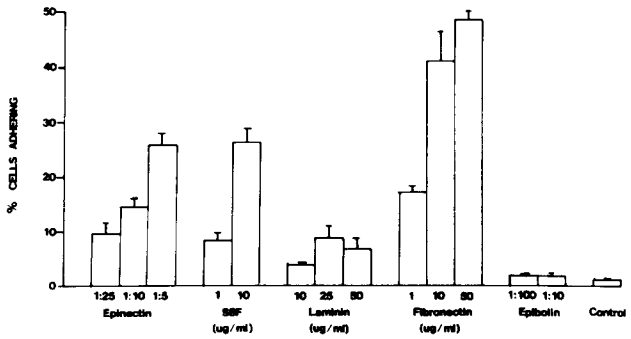

FIGURE 6 Cell attachment assay using A431 cells. Cells were labeled for 1 d with ^{3}H amino acids. Tissue culture plates were coated for 2 h with the test protein, washed, and then coated for an additional 3 h with 5 mg/ml BSA. Plates were washed and cells were incubated on coated wells for 45 min. All tests were done in triplicate. Bars represent one standard deviation and data represents percent of cells added that adhered.

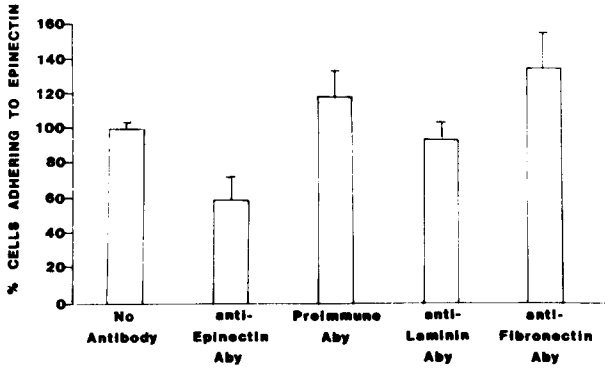

FIGURE 7 Antibody-mediated inhibition of cell adhesion on epinectin coated plates. Tissue culture plates were incubated as in Fig. 6 with the 70,000-mol-wt matrix protein and then with BSA. Plates were then incubated for 1 h with the appropriate antibodies. Cell binding to the 70,000-mol-wt protein alone with no antibody added was termed 100%. All cell binding in the presence of antibodies is expressed as a percent of this value. Bars represent one standard error of the mean. *Aby*, antibody.

Cell Attachment

The ability of the matrix extract to promote A431 cell attachment was compared to the known attachment molecules: laminin, fibronectin, SSF, and epibolin. Various concentrations of extract were coated in test wells and then the number of cells adhering was measured after 45 min. The exact concentrations of the 70,000-mol-wt extract and of epibolin were difficult to precisely ascertain as these preparations have not been purified to absolute homogeneity. However, these as well as the other proteins were assayed at or near their maximal adhesion-promoting activity. Therefore, Fig. 6 shows that the efficacy of the 70,000-mol-wt matrix extract in promoting cell adhesion is similar to that of 1–10 μg/ml SSF but somewhat less effective than fibronectin. Laminin was less efficient than the 70,000-mol-wt protein in promoting attachment of A431 cells, and epibolin was totally ineffective. It is important to note that the 70,000-mol-wt extract used in this assay has no reactivity with antibodies to fibronectin, laminin, epibolin, or SSF by ELISA (Fig. 4). Experiments were performed by preincubating cells with 10 μg/ml cycloheximide for 3 h. Then cell-adhesion assays were performed in the presence of cycloheximide. These studies showed that the adhesion of cycloheximide-treated cells to epinectin or fibronectin was slightly >50% of the adhesion to either protein in the absence of cycloheximide (data not shown). Therefore, these data suggest that epinectin can function directly as a cell-adhesion protein for these cells. Fig. 7 shows that antibodies to epinectin inhibit 40% of cell adhesion on the 70,000-mol-wt matrix extract, whereas immunoglobulins from preimmune serum, and antibodies to laminin and fibronectin do not block cell adhesion on the 70,000-mol-wt matrix extract.

DISCUSSION

This study defines a new cell surface and pericellular molecule produced by human epidermoid squamous carcinoma cells, the A431 carcinoma cell line. This protein was extracted from cell layers/cell matrices by 6 M urea and had a M_r of 70,000 on SDS PAGE under reducing conditions. The 70,000-mol-wt protein was synthesized de novo by cells as shown by [^{35}S]methionine incorporation in metabolic labeling studies. Antibodies to this 70,000-mol-wt protein were used for immunofluorescence studies and showed two staining patterns: (*a*) a thin line virtually tracing the plasma membrane, and (*b*) dense pericellular punctate accumulations adjacent to the cells. Studies were performed that showed that the 70,000-mol-wt molecule was extremely potent in promoting cell adhesion. This 70,000-mol-wt molecule promoted attachment

of A431 cells at levels less than that of fibronectin, equal to that of SSF, and substantially better than laminin or epibolin. Adhesion-promoting activity in the presence of cycloheximide provided evidence that epinectin was acting directly as a cell-adhesion molecule, rather than releasing endogenous stores of adhesion factors. Preliminary studies of human tissue sections show specificity of antiepinectin antibodies for squamous epithelium (for example, tongue and skin) as opposed to other tissues.

It was important to show whether there was any relationship between this 70,000-mol-wt molecule and any other known cell adhesion, basement membrane, or cell-matrix glycoproteins. Antibodies to the 70,000-mol-wt protein showed no cross reactivity by ELISA, dot immunoassay, or gel-transfer immunolocalization to the following cell-adhesion and/or matrix molecules: laminin, fibronectin, SSF, and epibolin. Correspondingly, antibodies to laminin, fibronectin, SSF, and epibolin did not react with the 70,000-mol-wt protein by ELISA, dot immunoassays, or gel immunolocalization. The 70,000-mol-wt protein did not seem to be a proteolytic product of larger cell-matrix glycoproteins because polyclonal antibodies possessing numerous specificities did not react with the 70,000-mol-wt protein. On the basis of the above studies it would appear that the 70,000-mol-wt adhesion-promoting molecule is distinct from other known adhesion molecules. Because this 70,000-mol-wt molecule promotes cell adhesion and is an epithelial cell membrane and pericellular molecule, we would term it epinectin.

Cell surface molecules with M_r ~70,000 have been isolated by several groups by producing antibody to either whole neural retina cells (24), neural cell surface (25), or conditioned medium fractions from mammary epithelium cells (80,000 mol wt) (26). In these cases the molecules can then be isolated by their ability to bind to these antibodies. These molecules in the ~70,000–80,000-mol-wt range have been implicated in cell-cell rather than cell-substratum adhesion.

A number of molecules have been isolated from cell matrices or cell surfaces with M_r ~70,000. We have been able to distinguish some of these from epinectin, while others await further experimentation. A 70,000-mol-wt glycoprotein has been isolated by gelatin affinity chromatography from conditioned medium of a variety of cells in culture (23). Epinectin from the A431 carcinoma cells seems distinct from this molecule as it does not bind to gelatin. Antibodies to epinectin did not react with keratin, another protein of potential concern that has components of 70,000-mol-wt (27), and correspondingly, antibodies to keratin did not react with epinectin. Early antibody preparations to epinectin were contaminated with antibodies to BSA. Presumably this represented trace amounts of BSA migrating with a M_r ~68,000 that was contaminating the original immunogen. Anti-BSA antibodies were removed by passing antisera over a BSA affinity column. The antibody that passed through in the void volume of this column contained anti-epinectin antibodies that reacted by ELISA with epinectin but not with BSA. Also, it should be noted that BSA and epinectin are functionally quite distinct, in that BSA does not promote cell adhesion and is commonly used to inhibit nonspecific cell-substratum adhesion.

It is clear from other systems that some cells may produce more than one noncollagenous glycoprotein involved in cell attachment. For example, fibrosarcoma cells (9), NRK cells (28), Schwannoma cells (19), among others produce both laminin and fibronectin. Particular cells may use or produce one molecule preferentially during different stages of development or differentiation, in response to injury or in pathological processes. The studies above describe a glycoprotein, epinectin, which is very effective in promoting attachment of A431 epithelial tumor cells. Consideration for this and other yet undescribed glycoproteins should be given in examining the attachment of cells of epithelial origin to various substrata or basement membranes. The distribution of epinectin in vivo, potential molecular interactions with other basement membrane components, and possible biological roles other than cell adhesion are currently under investigation.

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