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. Kurknen, K. Alitalo, A. Vahti, S. Johansson, and M. H66k, 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 [35S]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, but 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 glycopolypeptide, including fibronectin (1), laminin (2), epibolin (3), serum-spreading factor (SSF) (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-immunosorbent assay; PBS, phosphate-buffered saline; SSF, serum-spreading factor.
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 immunofluorescence 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). The cell line was originally isolated from a vulvar carcinoma (13). Cells were grown in 75-cm² Falcon flasks (Falcon Labware, Oxnard, CA) in Dulbecco's modified Eagle's medium (DMEM) (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) with 6% fetal calf serum (Flow Laboratories, Rockville, MD) at 37°C, 5% CO₂, 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 cells using a modification of the urea extract procedure described (15, 16). Cells were grown for 4 d (until confluence) in DMEM with 6% fetal calf serum, rinsed three times with serum-free DMEM, 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 0.3% 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) at 37°C, 5% CO₂, and then reacted with antibodies for 3 h. Well plates were coated for 2 h at 37°C with 0.05 mg/ml of antibody (anti-fibronectin, anti-laminin, anti-streptokinase or anti-Sigma). Cells were rinsed five times in PBS-Ca²⁺ to remove unattached cells. 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'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 Furth (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 no 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 antiserum 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 "immunoblot" 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 Ca²⁺ (0.56 mM CaCl₂), then reacted with a 1:20 dilution of antibodies for 1 h in a humidified atmosphere. Cells were rinsed five times in PBS-Ca²⁺ and reacted with rhodamine conjugated goat anti-rabbit immunoglobulin G (Cappel Laboratories) followed by five rinses in PBS-Ca²⁺. Fluorescence was observed on a Zeiss Universal microscope with an epifluorescence attachment and photographed with 50x ISO 1000 color slide film (3M, St. Paul, MN).

Cell Adhesion: A431 cells were radiolabeled for 1–2 d with 25 μCi/ml of ['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 x 10⁴ cells/ml in DMEM 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 N 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-Ca²⁺ 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, immunoabsorptions of anti-fibronectin and anti-laminin were performed at 1:100 dilutions using antigens from fibrinogen and laminin from larval Aedes aegypti (22). To prepare samples for antifibronectin and antialbumin antibodies and the residual antibody not binding albumin was used in all further studies.
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-Sepharose column made as described (22). Preparation of the 70,000-mol-wt protein was applied to the column (2 x 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 Mr of 53,000 (Fig. 1b) and reduced Mr of 70,000 (Fig. 1c). Metabolic labeling of cells with [35S]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. 2b). Residual material left after urea extraction was solubilized with reducing sample buffer. This residue had decreased intensity of the [35S]-labeled 70,000-mol-wt band compared to a number of other bands (Fig. 2d). In addition to the 70,000-mol-wt band in the matrix, a 70,000-mol-wt, [35S]methionine-labeled molecule was secreted into the culture medium (Fig. 2c). The fact that other radiolabeled bands appear in Fig. 2b while only one Coomassie Blue band appears in Fig. 1c suggests limited methionine incorporation into the protein, while its observed accumulation over time is seen in Fig. 1c.

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 Mr 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. 3a 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. 3e). 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. 5b, 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. 5c).
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. X 500. (b) Phase-contrast micrograph of a. X 500. (c) Immunofluorescent staining with antiepinectin showing globular extracellular deposits. X 500. (d) Phase contrast of (d). X 500. (e) Immunofluorescent staining control with preimmune normal rabbit serum. X 500. (f) Phase-contrast micrograph of a. X 500. Bar, 5 μm.
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.

**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 M₀ of 70,000 on SDS-PAGE under reducing conditions. The 70,000-mol-wt protein was synthesized de novo by cells as shown by [35S]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 anti-epinecin 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 $M_r \sim 70,000$ have been isolated by several groups by producing antibody to either whole neural retina cells (24), neural cell surface (25), and 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 $\sim 70,000-80,000$-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 $M_r \sim 70,000$. We have been able to distinguish some of these from epinecin, 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 epinecin were contaminated with antibodies to BSA. Presumably this represented trace amounts of BSA migrating with a $M_r \sim 68,000$ 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-epinecin antibodies that reacted by ELISA with epinecin but not with BSA. Also, it should be noted that BSA and epinecin 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 epinecin 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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REFERENCES

1. Furcht, L. T. 1983. Structure and function of the adhesive glycoprotein fibronectin. Med. Cell Biol. 1:53-117.

2. Terranova, V. P., D. H. Rohrbach, and G. R. Martin. 1980. Role of laminin in the attachment of PAM 212 (epithelial) to basement membrane collagen. Cell. 22:119-126.

3. Stein, K. S. 1981. Epibolin, a protein of human plasma that supports epithelial cell movement. Proc. Natl. Acad. Sci. USA. 78:9007-9011.

4. Barnes, D. W., and J. Sihvert. 1983. Isolation of human serum spreading factor. J. Biol. Chem. 258:12548-12552.

5. Carter, W. G. 1982. The cooperative role of the transformation-sensitive glycoprotein GP140 and fibronectin in cell attachment and spreading. J. Biol. Chem. 257:3249-3257.

6. Yamada, K. M. 1983. Cell surface interactions with extracellular materials. Annu. Rev. Biochem. 52:761-799.

7. Pierschbacher, M. D., E. G. Hayman, and E. Ruoslahti. 1981. Location of the cell-attachment site in fibronectin with monoclonal antibodies and proteolytic fragments of the molecule. Cell. 26:259-267.

8. Tibble, R., H. Rohde, P. G. Robery, S. I. Renard, J. M. Foidart, and G. R. Martin. 1979. Laminin-A glycoprotein from basement membranes. J. Biol. Chem. 254:9933-9937.

9. Chung, A. E., R. Jaffe, I. I. Freeman, J.-P. Vergnes, J. E. Braginski, and B. Caffin. 1979. Properties of basement-membrane-related glycoprotein synthesis in culture by a mouse embryonal carcinoma-derived cell line. Cell. 16:277-287.

10. Vlodavsky, I., and D. Gospodarowicz. 1981. Role of basement and fibronectin in adhesion of human carcinoma and sarcoma cells. Nature (Lond.) 290:304-306.

11. Couchman, J. R., M. Hock, D. A. Rees, and R. Furcht. 1983. Adhesion, growth, and matrix production by fibroblasts on laminin substrates. J. Cell Biol. 96:177-183.

12. Oliver, N. R. F., Newby, L. T. Furcht, and S. Bourgeois. 1983. Regulation of fibronectin biosynthesis by glucocorticoids in human fibrosarcoma cells and normal fibroblasts. Cell. 33:287-296.

13. Fahey, R. N., J. E. DeLarco, and G. J. Todaro. 1977. Nerve growth factor receptors on human melanoma cells in culture. Proc. Natl. Acad. Sci. USA. 74:565-569.

14. Barnes, D. W. 1982. Epidermal growth factor inhibits growth of A431 human epidermoid carcinoma in serum-free culture. J. Cell Biol. 91:1-4.

15. Hedman, K., M. Kurkinen, K. Alitalo, A. Vaheri, S. Johansson, and M. Hock. 1979. Isolation of the pericellular matrix of human fibroblasts. J. Cell Biol. 81:83-91.

16. Yamada, K., and J. A. Weston. 1974. Isolation of a major surface glycoprotein from fibroblasts. Proc. Natl. Acad. Sci. USA. 71:3492-3496.

17. Baintner, P., P. Garnier, K. Van Slyke, and A. Bradley. 1972. Quantitative electrophoresis in polyacrylamide gels. J. Chromatogr. 65:147-155.

18. Larsson, O., and J. Orner. 1979. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (Lond.) 227:680-685.

19. Palm, S. L., and L. T. Furcht. 1983. Production of laminin and fibronectin by Schwannoma cells: cell-protein interactions in vitro and protein localization in peripheral nerve in vivo. J. Cell Biol. 96:1218-1226.

20. Engvall, E., and P. Pearlman. 1972. Enzyme-linked immunosorbent assay, ELISA. J. Immunol. 109:129-135.

21. Tovbin, H., T. Staehelin, and J. G. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and applications. Proc. Natl. Acad. Sci. USA. 76:4350-4354.

22. Kowalczuk, P., and C. B. Asleson. 1979. Affinity chromatography. Methods Enzymol. 22:345-378.

23. Vartio, T., and A. Vaheri. 1981. A gelatin-binding 70,000 dalton glycoprotein synthe-
sized distinctly from fibronectin by normal and malignant adhered cells. J Biol. Chem. 256:13085-13090.
24. Grumwald, G. B., R. S. Pratt, and J. Lilien. 1982. Enzymatic dissection of embryonic adhesive mechanisms. III. Immunological identification of a component of the calcium-dependent adhesive system of embryonic chick neural retina cells. J Cell Sci. 55:89-93.
25. Thiery, J.-P., R. Brackenbury, U. Rutishauser, and G. M. Edelmann. 1977. Adhesion among neural cells of the chick embryo. II. Purification and characterization of a cell adhesion molecule from neural retina. J Biol. Chem. 252:6841.

26. Damsky, C. H., J. Richa, D. Solter, K. Knudsen, and C. A. Buck. 1983. Identification and purification of a cell surface glycoprotein mediating intercellular adhesion in embryonic and adult tissue. Cell. 34:455-466.
27. Moll, R., W. W. Franke, D. L. Schiller, B. Geiger, R. Krepler. 1982. The catalogue of human cytokeratins: patterns of expression in normal epithelial, tumors and cultured cells. Cell. 31:1-24.
28. Hayman, E. G., E. Engvall, and E. Ruoslahti. 1981. Concomitant loss of cell surface fibronectin and laminin from transformed rat kidney cells. J Cell Biol. 88:89–157.