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Distribution of a Major Surface-Associated Glycoprotein, Fibronectin, in Cultures of Adherent Cells

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Fibronectin was present in media and cell layers of cultures of adherent cells from human skin, kidney, lung, chest wall, liver, and heart. Cell-surface fibronectin, visualized by immunofluorescence, was in dense fibrillar (cultures from lung), discrete fibrillar (e.g., cultures from skin), or punctate (some cultures from kidney) structures. The subunit sizes of cell-surface fibronectin and fibronectin soluble in medium appeared identical in sodium dodecyl sulfate-polyacrylamide gels. To explain the polymorphism of cell-surface fibronectin, there must be chemical differences among the fibronectins synthesized by different cell strains or factors in the cell layer which influence fibronectin binding and aggregation.

Key words: binding, fibroblasts, fibronectin, immunofluorescence, receptor, secretion

Fibronectin is a glycoprotein composed of large subunits and is present in the blood, basal lamina, and blood vessels of vertebrates (1, 2). It is synthesized in fibroblast cultures, where it is found in conditioned medium, on cell surfaces (large, external, transformation sensitive protein), and intracellularly (3). Fibronectin is generally missing from the surfaces of transformed cultured cells (reviewed in Refs. 3 and 4). We estimate the subunit size of fibronectin circulating in plasma (cold-insoluble globulin) to be 2.0×10^5 daltons (5), 2.0×10^4 daltons less than the size of the subunit of cell-surface fibronectin (6, 7). However, as described below, the subunit sizes of fibronectin secreted or shed by fibroblasts into the medium and fibronectin associated with fibroblast cell surfaces appear identical. The factors that determine the distribution of fibronectin in cell cultures or within the body are not understood.

As one approach to this problem, we studied the distribution of fibronectin in cultures of a variety of adherent cells. We found that, although the amounts and subunit sizes of fibronectin were similar in cultures of adherent cells from different tissues, the arrangements of cell-surface fibronectin, visualized by immunofluorescence, were tissue specific.

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MATERIALS AND METHODS

The materials and methods for lactoperoxidase-catalyzed iodination of cell-surface proteins (8), separation and detection of proteins in sodium dodecyl sulfate-polyacrylamide gels (8), radioimmunoassay of fibronectin in conditioned media and cell extracts (9; D. F. Mosher and A. Vaheri, submitted for publication), indirect immunofluorescent detection of fibronectin in fixed coverslip cultures (9; D. F. Mosher and A. Vaheri, submitted for publication), and double antibody immunoprecipitation of fibronectin intrinsically labeled with [^{35}S]-l-methionine (D. F. Mosher, O. Saksela, and A. Vaheri, submitted for publication) are described elsewhere. The antifibronectins used in the various double antibody procedures were monospecific for human plasma fibronectin (by double immunodiffusion and immunoelectrophoresis of plasma) and were adsorbed with fetal calf serum to remove cross-reactions with bovine fibronectin in the growth media (which contained 10% fetal calf serum).

Primary cultures of human embryonic tissues were prepared by standard techniques. The cells grew to confluency in 3–4 days and were subcultured (1:2 split) twice weekly. The state of fibronectin was monitored over the first few passages by radioimmunoassay of media and cell extracts and indirect immunofluorescence of cell layers. Strains of adult skin fibroblasts were established locally. Other established strains and lines of the human cells described in Table I were obtained from the American Type Culture Collection, Rockville, Maryland.

RESULTS

Newly established adherent cell strains from human embryonic skin, heart, chest wall, lung, kidney, and liver were compared to one another and to established strains from adult skin and embryonic lung and lines of rhabdomyosarcoma cells and SV-40 transformed embryonic lung cells (Table I).

All nontransformed strains had a 2.2×10^5 dalton external polypeptide which was prominently labeled by lactoperoxidase-catalyzed iodination. Extracts of 12 newly-established strains were analyzed, and the patterns of stainable polypeptide in sodium dodecyl sulfate-polyacrylamide slab gels were remarkably similar. In particular, extracts all contained a 2.2×10^5 dalton polypeptide which was bracketed by polypeptides of 2.0×10^5 and 2.5×10^5 daltons (6). The 2.2×10^5 dalton iodinated bands and protein-staining bands were of higher molecular weight (dimers and multimers) when the extracts were analyzed by electrophoresis without prior reduction (6).

All cultures had fibronectin in media and associated with cell layers. The newly established strains were robust producers of fibronectin (Table I) and secreted or shed more fibronectin into the medium than older established strains. The older established strains, in turn, secreted or shed more fibronectin into the medium than transformed lines (Table I).

The patterns of cell-associated fibronectin, visualized by indirect immunofluorescence, varied among strains derived from different tissues (Fig. 1). Cells from skin had copious amounts of patchy intracellular fibronectin in a perinuclear distribution as well as discrete surface fibrils (Fig. 1a). Cells from chest wall and liver looked much like the skin cells.

TABLE I. Fibronectin Content of Cultures 24 h After Subculture*

Cells	Passage	Protein in cell layer	Fibronectin in medium	Fibronectin in cell layer
		(μg)	(μg)	(μg)
Embryonic				
skin	6	200	14.0	1.0
kidney	6	168	10.3	0.7
heart	6	167	27.3	0.7
lung	6	274	25.5	1.8
chest wall	6	233	41.5	0.9
Mean ± SD		208 ± 41	23.7 ± 11.0	1.0 ± 0.4
ES adult skin	16	103	2.1	0.32
JKO adult skin	18	95	5.0	0.40
WI-38 embryonic lung	22	104	1.9	0.42
MRC-5 embryonic lung	19	156	6.5	0.49
Mean ± SD		115 ± 24	3.9 ± 2.0	0.40 ± 0.07
RD rhabdomyosarcoma	41	161	2.0	0.18
HT 1080 rhabdomyosarcoma	18	187	3.4	0.29
VAH transformed lung	58	149	0.7	0.12
VA13 transformed lung	132	168	1.5	0.21
RSA transformed lung	16	186	1.0	0.20
Mean ± SD		170 ± 15	1.72 ± 0.95	0.20 ± 0.05

*Values represent the mean of duplicate 20-cm^2 dishes (each containing 5 ml growth medium) sampled 24 h after subculture (1:2 split of a previously confluent culture). The differences among early-passage strains, older established strains, and transformed lines persisted through the 6th day in culture (D. F. Mosher and A. Vaheri, submitted for publication).

Heart cells had more fusiform fibrils. Cultures from lung looked similar to cultures from skin until the cultures reached confluency, and a dense network of fine extracellular fibronectin fibrils appeared (Fig. 1b). Some cultures from kidney consisted of a homogeneous population of large, round, flat cells on which fibronectin was present in punctate structures (Fig. 1c). Most of the punctate structures appeared to be in the plane of focus between the cell and substratum. Several types of fibronectin-containing cells were in secondary cultures of human kidney (Fig. 1d). In the lower left of Fig. 1d is a large round cell with both punctate and fibrillar immunofluorescence. In the upper left of Fig. 1d is a portion of an elongated cell with fibronectin fibrils oriented along the cell body. At the right of Fig. 1d are 2 cells with perinuclear (probably intracellular) fluorescence and small amounts of fibrillar fluorescence.

The older established strains of adult skin and embryonic lung fibroblasts had patterns of surface fibronectin identical to the patterns seen in the newly established strains of embryonic skin and lung, respectively. Cell-surface fibronectin was missing from the surfaces of the rhabdomyosarcoma cells and SV-40 transformed embryonic lung cells (9), and only intracellular fibronectin was seen.

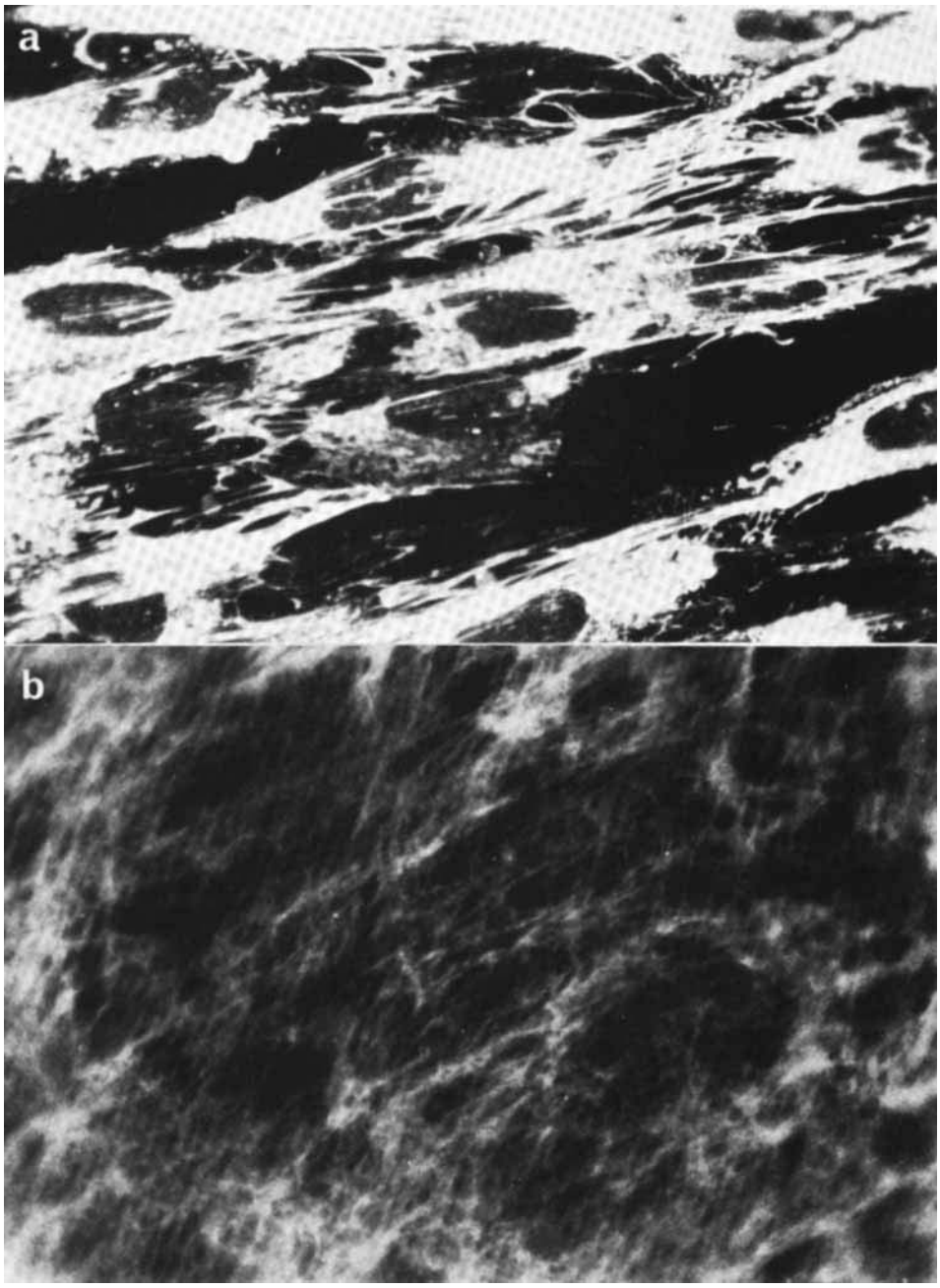
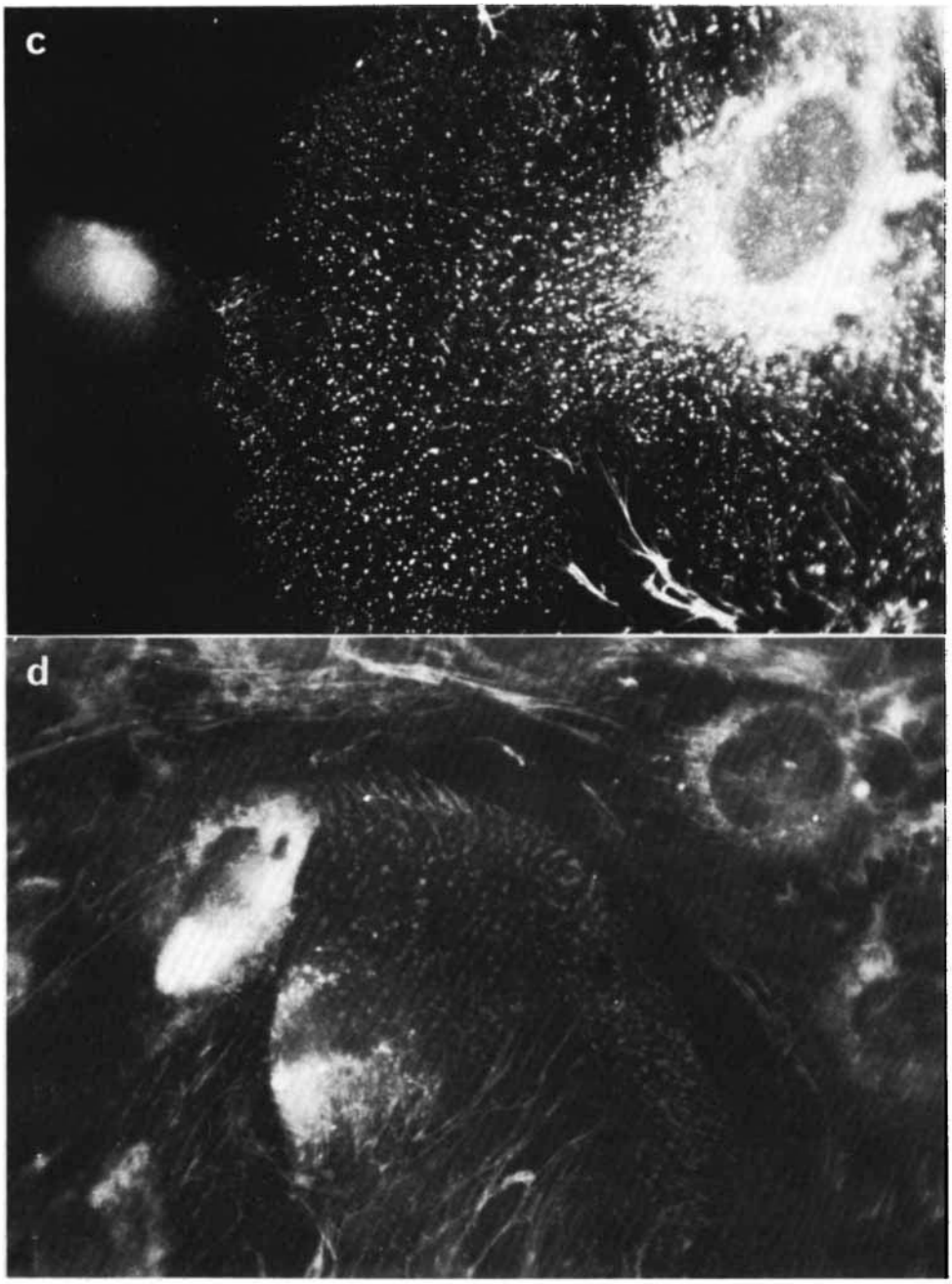


Fig. 1. Fibronectin immunofluorescence of different human embryonic cell strains. Coverslip cultures were studied 3 days after subculture. Cells were fixed with formaldehyde-acetone and stained by indirect immunofluorescence: a) embryonic skin, 4th passage; b) embryonic lung, 4th passage; c) embryonic kidney, 4th passage; and d) embryonic kidney, secondary culture. Magnification × 512.



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DISCUSSION

The amounts of fibronectin in culture media of the newly established strains was much greater than the amounts of fibronectin in the cell layers (Table I). Fibronectin in the media is probably in equilibrium with a portion of the fibronectin on cell surfaces. Yamada and Weston demonstrated that fibronectin (cell-surface protein) extracted from cell surfaces with low concentrations of urea could reassociate with cell surfaces (10). Hynes et al. reported that fibronectin in medium was capable of associating with cell surfaces (11). We have performed many experiments trying to demonstrate molecular weight differences between fibronectin in the medium and cell-surface fibronectin. The fibronectins were metabolically labeled with [^{35}S]-l-methionine, isolated by immunoprecipitation, and analyzed by sodium dodecyl sulfate-polyacrylamide slab gels combined with autoradiography. In every case, the molecular weights of fibronectins from the 2 sources were the same (12; our unpublished results).

A large fraction of cell-surface fibronectin, however, is apparently bound together by disulfide bonds (6, 13) or other types of covalent bonds (14). Most of the fibronectin-positive fibrils seen by immunofluorescence appear ultrastructurally as aggregates which are either tenuously associated with the cell surface or clearly part of the pericellular matrix (K. Hedman, A. Vaheri, and J. Wartiovaara, submitted for publication). These aggregates, if bound together by covalent bonds, could only be broken apart by proteases.

The immunofluorescence studies indicate that the distribution of fibronectin on cell surfaces varies among cell strains in a tissue-specific manner. The studies raise the question: How do cells control the distribution of cell-surface fibronectin? We have considered 4 possibilities: i) Specific surface receptors for fibronectin are arranged differently in different cell strains. ii) There are chemical differences among fibronectins synthesized by different cell strains which cause the fibronectins to bind and aggregate differently. iii) Distribution of fibronectin is influenced by other macromolecules secreted by the various strains. iv) Much of the fibronectin in the cell layer is deposited as the cells move, and cells move in a tissue-specific manner. At present, we favor the possibility of specific fibronectin receptors since it accounts for the fact that cells with different patterns of cell-surface fibronectin can exist in the same culture dish (Fig. 1d). Binding to receptors, however, would be expected to primarily influence the distribution of reversibly-bound fibronectin. The formation of fibronectin aggregates may be influenced by chemical differences among fibronectins, by interactions with other molecules of the pericellular matrix, and by cell movement. Analysis of the factors that influence the binding of fibronectin to normal cells may lead to an understanding of why most transformed cells, which do secrete or shed fibronectin (9; Table I), fail to bind it to their surfaces.

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