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ACTION OF FIBRONECTIN ON MORPHOLOGY AND CYTOSKELETON OF HEPATIC EPITHELIAL CELLS IN CULTURE

S. M. Troyanovskii

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Fibronectin is one of the basic surface proteins of different types of cells [1, 3]. There is considerable evidence that it participates directly in the mechanism of adhesion of cells to a substrate. For instance, its addition to certain cultures of both nontumorigenic and tumorigenic fibroblasts, which have weak adhesive properties and do not possess fibronectin on their surface, leads to an increase in their adhesiveness and, evidently as a result of this, to their morphological normalization [5, 7]. Additionally, as a rule in such normalized cells the bundles of microfilaments which are absent in transformed cells are restored [5].

Most investigations into the role of fibronectin in cell biology have been carried out in the past on cultures of mesenchymal origin [7, 11,]3]. The action of fibronectin on epithelial cells has not been investigated. To examine this problem, in the research described below nontumorigenic and tumorigenic lines IAR of hepatic epithelium were used. The morphological features, cytoskeleton, surface proteins, and many other characteristics of these cells have been fully described previously [2, 4, 9].

EXPERIMENTAL METHOD

Hepatic epithelium of line IAR 2 was obtained in culture in 1973 [8] from a primary culture of rat hepatocytes of line BD4. After treatment in culture with N-methyl-N-nitro-N-nitrosoguanidine, tumorigenic line IAR 2-31 was obtained from this line [6, 8]. Line IAR 6-IRT 7A was obtained from a clone grown in methyl cellulose from cells of adenocarcinoma IAR 6-IRT 7. The history of this line has been fully described previously [2]. All cells were grown in Williams E medium (from Flow Laboratories, England), with the addition of 10% embryonic calf serum (from Gibco, England) and 100 units/ml monomycin.

Fibronectin was isolated from the plasma of noninbred albino rats, using two-stage affinity chromatography on gelatin-sepharose and on arginine-sepharose by the method in [12]. The final preparation contained electrophoretically pure fibronectin with a molecular mass of about 250 kilodaltons. It was concentrated to a fibronectin concentration of 1 mg/ml and dialyzed overnight against Hanks' solution.

Fibronectin was added to the cultures in a final concentration of 50 μg/ml, either directly to the medium to the already growing cells, or to the substrate for 30 min before

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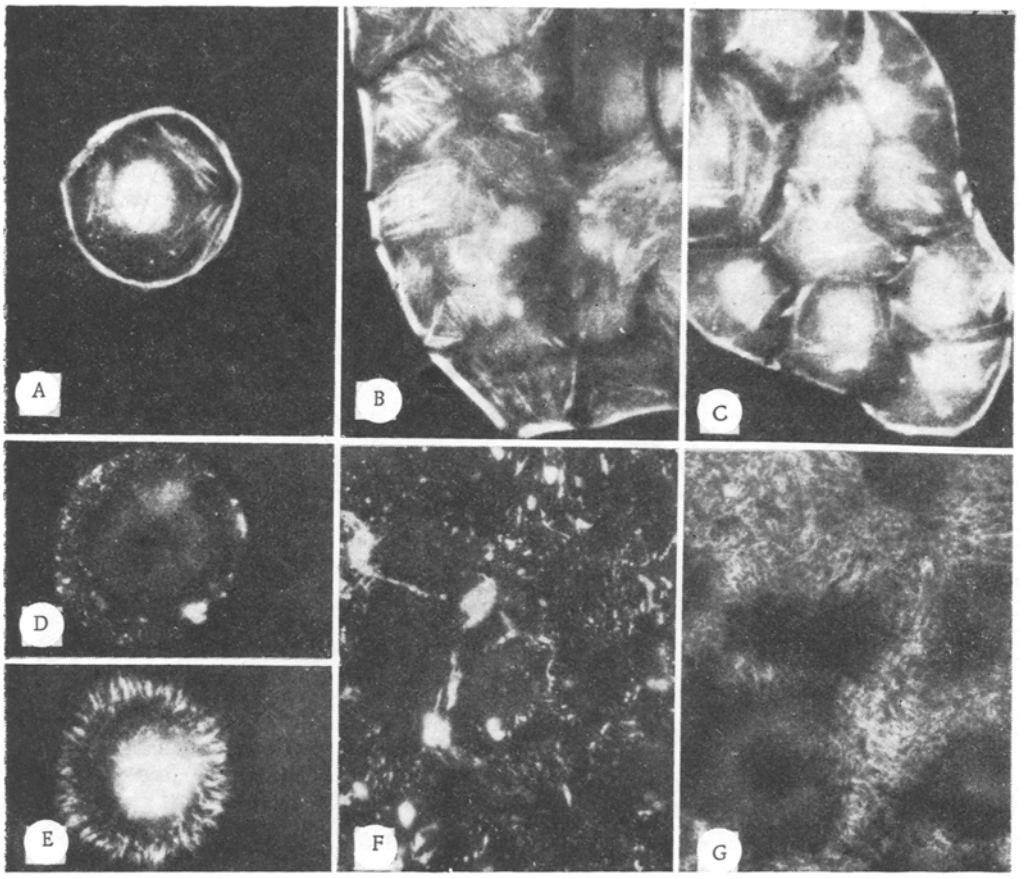

Fig. 1. Action of fibronectin on IAR 2 cells. A-C) Immunofluorescence for actin, D-G) immunofluorescence for fibronectin. A, D) Single cells, B, F) monolayer on control substrates, C, E, G) cells growing on fibronectin. Magnification: A, D, E) 450 ×; B, C, F, G) 400 ×.

passage of the cells. The same quantity of Hanks' solution without fibronectin was added to the control cultures.

Indirect immunofluorescence staining for actin and fibronectin was carried out by the standard method [1, 4, 11].

EXPERIMENTAL RESULTS

Single cells of line IAR 2 spread out well on the substrate and were disk-like in shape. Along the entire border of these cells ran an actin bundle, which we called a marginal bundle (Fig. 1A). In thicker cultures the marginal bundle was preserved only on the active border of the formed epithelial islets (Fig. 1B). Fibronectin in a monolayer of line IAR 2 was localized on the under surface of the cells in the form of dots and short fibrils (Fig. 1D, F) or in the zone of intercellular junctions [4]. Addition of fibronectin to the medium with cells already in culture caused no changes in their morphology, the actin cytoskeleton, or the distribution of fibronectin. Meanwhile the addition of fibronectin to the substrate before seeding of the cells caused loss of the fibronectin dots usually found on their surface, and led to the formation of a large number of radially oriented fibrils of fibronectin on it, which were not found on the control cells (Fig. 1E, G). At the same time, the morphology and the actin cytoskeleton of the IAR 2 cells remained unchanged compared with the control (Fig. 1C).

In cultures of tumorigenic cells of line IAR 2-31 virtually no fibronectin could be discovered by the immunofluorescence method (Fig. 2C). Single cells of this line were highly compressed, irregular in shape, and either without an actin bundle, or such a bundle was found only in some areas (Fig. 2A). Each cell in a monolayer of cells of this line, unlike cells of the parental line IAR 2, had its own marginal bundle (Fig. 2B). On the addition of

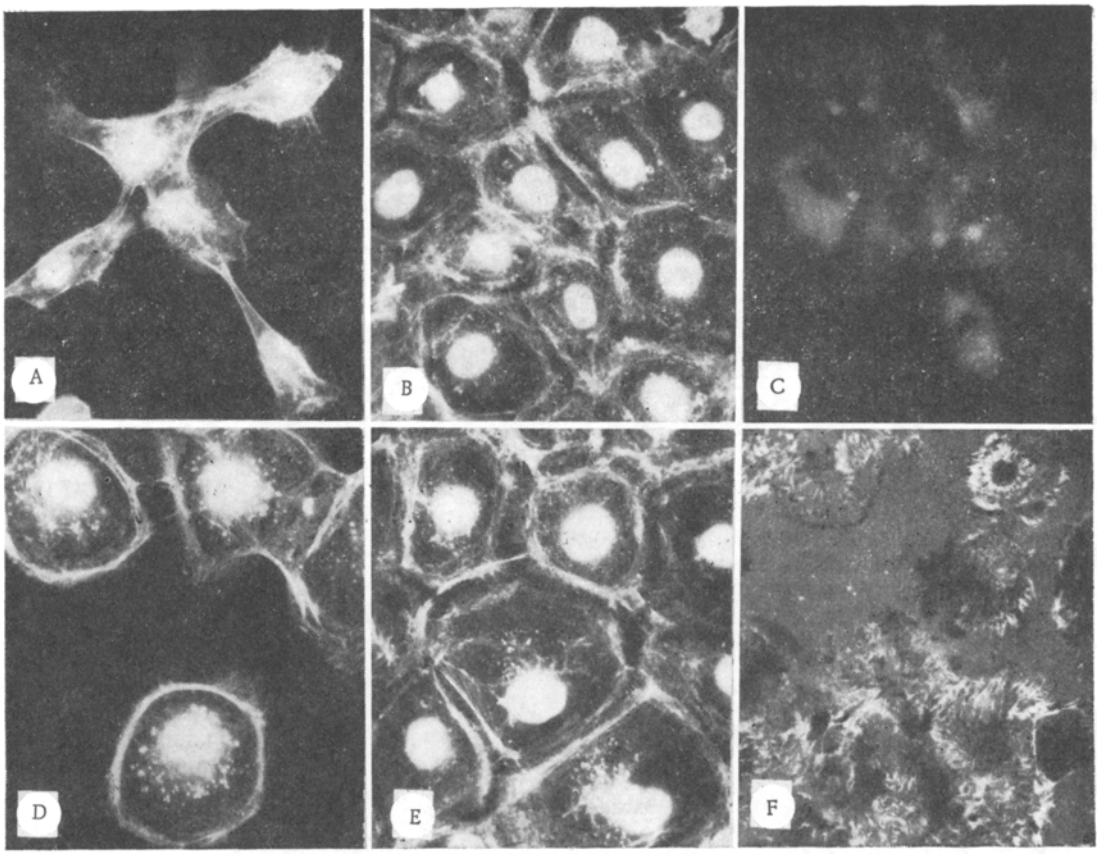

Fig. 2. Action of fibronectin on cells of line IAR 2-31. A, B, D, E) Immunofluorescence for actin; A) single cells; B) layer on control substrate; D) single cells; E) layer of substrate with fibronectin; C) control culture; F) with fibronectin, 450 ×.

fibronectin to medium containing cells already in culture, just as in the case of cells of line IAR 2, no changes were observed compared with the control. On substrate treated beforehand with fibronectin, single cells of line IAR 2-31 became well spread out in a layer and were disk-shaped. The marginal actin bundle also was restored in them (Fig. 2D). By these properties they thus became indistinguishable from single parental cells of line IAR 2 (Fig. 1A). Layers of cells of line IAR 2-31, however, did not differ in principle on substrate with fibronectin and on an ordinary substrate, i.e., both had a marginal actin bundle around each cell (Fig. 2E).

Like cells of line IAR 2, cells of line IAR 2-31 grown on substrate with fibronectin began to form radially oriented fibronectin fibrils on their undersurface (Fig. 2F). These fibrils were found in about equal numbers both on marginal and on inner cells of the monolayer. Cells of line IAR 2-31 formed significantly fewer fibrils than their nontumorigenic precursors — cells of line IAR 2, under the same conditions (Fig. 1G).

Cells of the other tumorigenic line of the IAR series, which under ordinary conditions of culture have no surface fibronectin (IAR 6-IRT 7A) underwent virtually no change in their morphology when grown on substrates with fibronectin. Under these circumstances actin bundles were not present in them, just as in control cells, and only individual fibronectin fibrils appeared on their surface (Fig. 3).

The action of fibronectin on nontumorigenic IAR 2 cells thus produces no morphological changes or changes in the actin cytoskeleton, although it led to the appearance of a network of radially oriented fibronectin fibrils. When this protein acted on tumorigenic IAR-2 cells, normalization of single cells took place as regards both morphology and actin cytoskeleton. Radially oriented fibronectin fibrils also appeared. Cells in monolayers, both in the control and in the normalized form, were characterized by a circular actin bundle, which distinguished them sharply from the nontumorigenic IAR 2 precursor. Cells of the other transformed line IAR 6-IRT 7A lost their ability to be normalized by fibronectin.

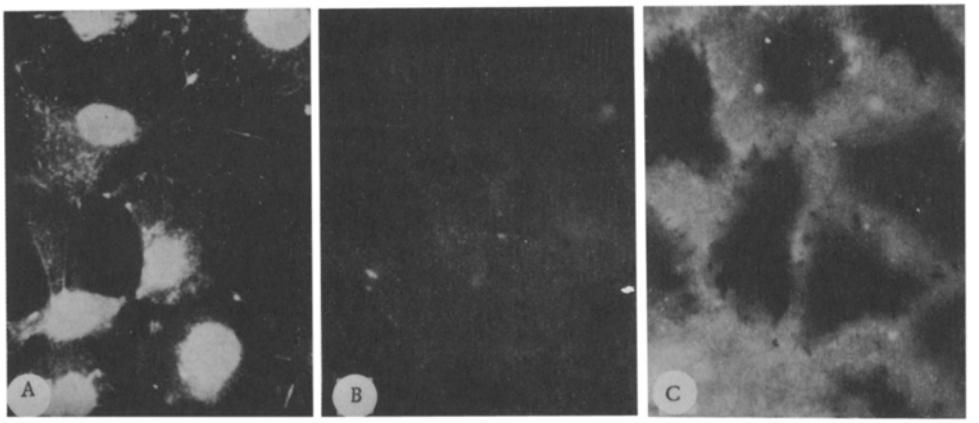

Fig. 3. Action of fibronectin on cells of line IAR 6-IRT 7A. A) Immunofluorescence for actin, control cells; B, C) immunofluorescence for fibronectin: B) control culture, C) culture on fibronectin, 400 ×. Fluorescence of substrate on account of adsorbed fibronectin can be seen. Black zones are sites beneath cells to which antibodies did not penetrate during staining.

The main result of this investigation is that at least some transformed epithelial cells, like many transformed fibroblasts, can be morphologically "normalized" by fibronectin. It is very probable that the mechanisms of "normalization" are the same in these two types of cells. This is shown, first, by the fact that preliminary adsorption of fibronectin on the substrate of the culture is necessary for "normalization" of certain mesenchymal cells [7, 10], just as for IAR 2-31 cells. In the case of addition of fibronectin to cells already in culture, this adsorption, as has been shown for BHK/21 cells [7], is blocked by serum albumin and "normalization" does not take place.

A second property common to the "normalization" of fibroblasts and epithelial cells is the formation of radially oriented fibronectin fibrils on "normalized" transformed cells [5]. It must be noted that similar fibrils also appear on nontumorigenic IAR 2 cells and that they affect neither the morphology nor the cytoskeleton of these cells. It is not yet clear whether these fibrils have any role to play in the "normalization" of transformed cells.

Compared with its effect on mesenchymal cells, the action of fibronectin on IAR 2-31 cells shows some distinguishing features. For instance, the addition of fibronectin to transformed fibroblasts potentiates their polarization [13] but, in the case of IAR 2-31 epithelial cells, fibronectin, on the other hand, leads to the appearance of practically nonpolarized cells. In other words, in both cases the ability of the cells to spread into a monolayer is increased, but the morphology is determined by the tissue type of the cells. Another difference is that "normalization" for epithelial line IAR 2-31 is well marked only for single cells. Normalization for transformed fibroblasts by fibronectin is virtually identical whatever the density of the culture [5, 13].

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