Similarities and Differences between the Fibronectins of Normal and Transformed Hamster Cells*

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Fibronectins from normal and virally transformed hamster cells were compared by several criteria. The fibronectin from transformed cells was similar to that from normal cells in being an intact dimeric glycoprotein with the ability to bind to gelatin, activated thiol-Sepharose, and cells. No evidence was found for proteolytic cleavages or abnormalities in disulfide bonding of transformed cell fibronectin. This fibronectin was also shown to be active in promoting cell attachment, elongation, and alignment. Therefore, the fibronectin produced by transformed cells is not defective. However, it was shown that the transformed cells were partially deficient in their capacity to bind fibronectins from either normal or transformed cells. This deficiency has implications for the significance of the loss of fibronectin on oncogenic transformation.

Partial proteolysis of the fibronectins from normal and transformed cells gave rise to the same fragments. However, the glycosylated fragments from transformed cell fibronectin appeared somewhat larger than those from normal cell fibronectin. Analysis of fibronectin glycopeptides showed that transformation leads both to more branches per core and to a higher sialylation of the asparagine-linked complex carbohydrate side chains.

Oncogenically transformed cells generally have reduced levels of the glycoprotein, fibronectin (reviewed in Refs. 1-5). This change is one of the most consistent biochemical alterations associated with transformation and has also been correlated with tumorigenicity and/or malignancy in vivo (see reviews above). The consequences of the reduced levels of fibronectin have been analyzed. Fibronectin affects cell adhesion and a variety of other properties probably related to adhesion.

The reasons for the loss of fibronectin have also been investigated but a clear picture has not emerged. Some transformed cells show reductions in the rate of synthesis of fibronectin (6-9), but the reductions are not sufficient to account for all the loss, and other transformed cells appear to show no depression in biosynthesis of fibronectin (10, 11). Since fibronectin is very sensitive to proteolytic digestion, the possibility of transformation-related proteolysis has been investigated (12-16). These earlier studies provided no clear evidence for proteolysis of fibronectin in transformed cell cultures. However, fibronectin is a very large protein and minor cleavages could have been missed. Recent analyses have defined the structure of fibronectin in some detail (17-22), making it worthwhile to reinvestigate the possibility of proteolysis. Also various activities of fibronectin have been characterized, including binding to gelatin, collagen, heparin, and cells, disulfide binding, and effects on cellular phenotype (see reviews in Refs. 1-5).

Since biosynthetic depression does not appear to provide a sufficient explanation for reduction in surface levels of fibronectin and since increased turnover rates (6, 14, 23, 24) and decreased binding of fibronectin (8, 25), have been reported in transformed cell cultures, we have investigated the possibility that the fibronectin produced by transformed cells might be defective in some aspect of its structure or function. We report here the results of these studies which suggest that the fibronectin of transformed cells is not defective but, rather, that the ability of the cells to bind fibronectin is reduced. These results have implications for the involvement of fibronectin in the transformed phenotype in vitro and in vivo.

**EXPERIMENTAL PROCEDURES**

*Cells and Culture Conditions—The cells used were the normal hamster cell line, NIL8, and its virally transformed counterpart, NIL8 HSV. The NIL8 cells produce large amounts of fibronectin and lay it down in a fibrillar matrix whereas the NIL8 HSV cells have extremely low levels of cell surface fibronectin (52, 53). Cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum (Flow Labs). For labeling experiments, the levels of methionine or glucose were reduced to 10% of normal and 

[17-22]

The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; kd, kilodaltons; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate.
**Transformed Cells Secrete Intact Dimeric Fibronectin**

Earlier studies have shown that transformed cells do secrete fibronectin into the culture medium even when they have greatly reduced levels on their cell surfaces (7, 10). Fig. 1 shows this for the transformed cells under study here. While the levels of secretion of fibronectin by transformed NIL8.HSV cells are much lower than those of their normal counterparts, NIL8 (compare tracks 1 and 2), fibronectin can be isolated from the culture medium of the transformed cells. It appears intact on reducing gels (tracks 4 and 6) and migrates as a dimer on nonreducing gels (tracks 10 and 11).

The large fragments characteristic of partial proteolytic digests of fibronectin (see Figs. 3 and 4) are not observed in the transformed cell medium either after immune precipitation (tracks 6 and 10) or after purification on gelatin-Sepharose (tracks 3 and 4). Note that the large proteolytic fragments do bind to gelatin (17, 18, 20).

From these results, we conclude that NIL8.HSV-transformed cells secrete dimeric fibronectin which is competent to bind to gelatin. This fibronectin does not become extensively degraded by proteolysis in the culture medium over the course of a 24-h incubation. In other experiments not shown here, we have observed a greater tendency of the fibronectin in transformed culture medium to become degraded during purification unless care was taken to add protease inhibitors as soon as the culture medium was harvested as in the experiments reported in this paper.

Comparisons of reduced fibronectin from normal and transformed cells were performed on low percentage gels to investigate the possibility of small size differences such as might arise by limited proteolysis (Fig. 1, tracks 3 and 4). Contrary to the prediction of transformation-related proteolysis, the fibronectin from transformed cells appeared somewhat larger than that from normal cells. These results argue against, but do not completely rule out, limited proteolysis of transformed cell fibronectin (see "Discussion").

**Transfected Cell Fibronectin Has Free Sulfhydryls**

Since we had shown that fibronectin from normal cells contained free sulfhydryls, blockage of which prevented binding of fibronectin to cell layers (20, 21), we investigated whether fibronectin from transformed cells might lack these sulfhydryl groups. Analysis on activated thiol-Sepharose showed clearly that this was not the case (Fig. 2). All the fibronectin purified from transformed cells bound to the column and was eluted with reducing agents. We have shown earlier that this binding is due to free sulfhydryls on the protein and not to free sulfhydryls on the column (20). Thus, fibronectin from transformed cells is capable of disulfide bonding.

**Partial Proteolysis of Normal and Transformed Cell Fibronectins Reveals Differences**—To investigate further the apparent size difference between normal and transformed cell fibronectins, we subjected both proteins to limited tryptic digestion to generate fragments. Fig. 3, left, shows the results of digestion with 1 μg/ml of trypsin which produces 200-kd and 25-kd fragments (20, 21) from both normal and transformed cell fibronectins. On 10% gels, the fragments from the two sources co-migrate reduced or nonreduced showing that the proteolysis-sensitive sites and overall disulfide-bonding patterns are similar in both fibronectins. On 5% gels, the 200-kd fragments derived from the normal cell fibronectin appeared somewhat larger than those from normal NIL8 cells (Fig. 3, right).

More extensive trypsinization (10 μg/ml) resulted in further digestion of the 200-kd fragment although the 25-kd fragment remained resistant (Fig. 4). Fragments of 200, 145, 125, 100, and 40 kD are generated from the normal cell fibronectin.
Corresponding fragments appear in the digests of the transformed cell fibronectin but most appear to be slightly larger in size. This is especially noticeable for the 100-kd and 40-kd fragments and is less obvious for the two larger fragments (Fig. 4). All these fragments, with the possible exception of the 40-kd fragment, are derived from the 200-kd fragment which, as shown in Fig. 3, also differs in its migration between normal and transformed cell fibronectins. Since the 200-kd fragments and is less obvious for the two larger fragments the NH2 or COOH terminus of the intact molecule (20, 21).

The Glycopeptides of Normal and Transformed Cell Fibronectins Are Different—[3H]Mannose-labeled or [3H]gluco-

samine-labeled fibronectins were purified by gelatin-Sepharose affinity chromatography. The mannose-labeled samples were radiochemically pure and were used without further purification. The glucosamine-labeled preparations had variable amounts of contamination especially at the top and interface of gels and were further purified by SDS-gel electrophoresis. The preparations were extensively digested with pronase and the resulting glycopeptides were analyzed by chromatography on Bio-Gel P-4 columns. All the carbohydrate side chains of mature fibronectin are of the "complex" type (26-28). Comparison of the glycopeptides of the NIL8 and NIL8.HSV fibronectins showed that those from the transformed cells were significantly larger (Fig. 5). Much of this difference in apparent size, but not all, was eliminated on treatment of the glycopeptides with mild acid which removes sialic acid residues (data not shown).

In order to analyze the differences in greater detail, the glycopeptides from each source were fractionated on concanavalin A-Sepharose which separates glycopeptides on the basis of numbers of branches attached to the mannose core. Those with two or fewer branches bind and are eluted with hapten whereas those with three or more branches do not bind (29). Bound and unbound glycopeptides were separated from NIL8 and NIL8.HSV fibronectin-derived glycopeptides. Each of the four fractions was split in two parts, one of which was treated with mild acid, and all eight samples were analyzed on Bio-Gel P-4 (Fig. 6).

The results showed that the proportion unbound by the concanavalin A-Sepharose (i.e. three or more branches) was much greater for the transformed than for the normal glycopeptides (66 versus 20%). When the glycopeptides were desialylated by mild acid hydrolysis, it was possible to see that the large majority of the bound glycopeptides in each case was of a single size class, presumably with two branches, whereas in each case the desialylated concanavalin A-unbound glycopeptides were larger (three or four branches; see Fig. 6, C and D). The ratio of tetraantennary to triantennary glycopeptides was higher in the transformed case than in the normal (Fig. 6, C and D). Thus, the transformed cell fibronectin has more highly branched carbohydrate side chains than does the normal.

Furthermore, comparison of the sialylated with the desialylated glycopeptides (Fig. 6, compare A with C and B with D) shows that the degree of sialylation of the branches is also higher in the transformed cells. Thus, for the concanavalin A-
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Fig. 3. Digestion of fibronectins from normal and transformed cells by low doses of trypsin. Both sides show time courses of digestion with 1 μg/ml of trypsin; numbers represent minutes of digestion. Left, analysis on a 10% gel to show all fragments. Right, analysis on a 5% gel to compare migrations of larger fragments. The tracks in the center were run without reduction; all others were reduced. Note that the course of digestion is similar for both fibronectins. The 25-kd fragments from both sources co-migrate on high percentage gels (left) but those from transformed cells appear larger on lower percentage gels (right). All large fragments run essentially the same whether reduced or not.

Fig. 4. Digestion of fibronectins from normal and transformed cells by higher doses of trypsin. Time course of digestion with 10 μg/ml of trypsin; numbers indicate minutes of digestion. Left, analysis on a 10% gel to display all fragments. Right, analysis on a 6% gel to show differences in migration between corresponding fragments from normal and transformed cells. Arrows indicate 200-, 145-, 125-, 100-, 40-, and 25-kd fragments.

bound biantennary side-chains (Fig. 6, A and B), a large fraction from the normal cells appears unsialylated (tubes 82 and 83), an equal fraction appears monosialylated (tubes 74 and 75) and only a small fraction disialylated (tubes 66-74). In contrast, the biantennary glycopeptides from the transformed cell source show very few unsialylated side chains, with many monosialylated and more disialylated.

The comparison is harder for the tri- and tetraantennary side chains since nonsialylated tetraantennary and monosialylated triantennary side chains co-migrate on the Bio-Gel P-4 columns. However, inspection of the profiles in Fig. 6 with solid symbols in Fig. 6, A and B, shows that the degree of sialylation is again higher in the transformed cell case.

Thus, the fibronectin from transformed cells differs from the normal in having larger glycopeptides. This size difference arises from two sources, more extensive branching and more extensive sialylation of the branches.

Fibronectin from Transformed Cells Is Active in Binding to Cell Layers—To test directly the interaction of transformed cell fibronectin with normal and transformed cells, radiolabeled fibronectin was purified from both normal and transformed cells by gelatin affinity chromatography (Fig. 1), and
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FIG. 5. Comparison of glycopeptides of fibronectins from normal (NIL8) and transformed (HSV-NIL) cells. Glycopeptides obtained by pronase digestion of purified \[^{3}H\]mannose-labeled fibronectins were separated on a Bio-Gel P-4 column.

FIG. 6. Analysis of concanavalin A-bound and unbound glycopeptides. A and B show size separations of bound (○—○) and unbound (●—●) fibronectin glycopeptides from normal (NIL) and transformed (HSV) cells. The shaded area in the bound profile corresponds with the nonsialylated biantennary glycopeptides. C and D show size separations of glycopeptides which have been desialylated by mild acid treatment. The profiles of concanavalin A-unbound glycopeptides (○—○) are composed primarily of biantennary side chains whereas the concanavalin A-bound profiles (●—●) consist of tri- and tetraantennary side chains.

the time courses and dose dependencies of binding of the two types of fibronectin to normal and transformed cells were studied (see “Experimental Procedures”). No consistent differences were observed between the two types of fibronectin. However, we consistently observed that transformed cell cultures were less effective in binding either fibronectin than

FIG. 7. Recovery of intact fibronectins from culture media of readdition experiments. Aliquots of culture medium were removed after 24 h of incubation of \[^{35}S\]methionine-labeled fibronectins with cells and analyzed on a 6% gel. \[^{35}S\]Fibronectin from NIL8.HSV cells incubated with NIL8 cells (A) or NIL8.HSV cells (B). \[^{35}S\]Fibronectin from NIL8 cells incubated with NIL8 cells (C) or NIL8.HSV cells (D). There is no indication of degradation of fibronectin by either cell type.

FIG. 8. Cell attachment promoted by fibronectins from normal (NIL8) or transformed cells (NIL8.HSV). 2.5·10^5 NIL8.HSV cells were seeded in 35-mm dishes; 24 h later, purified fibronectins were added to the 2-ml medium. After a further 24-h incubation, the numbers of floating cells in each dish were counted. FN, fibronectin.

were the normal cell cultures. Table I presents the normalized data from three separate experiments, each involving multiple samples, in order to show, on the one hand, the consistent similarity between the fibronectins and, on the other, the consistent difference between the cells. This difference was not due to degradation of the added fibronectin as shown in
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compared for each cell type at each dose of fibronectin (experiments of binding efficiencies of mal morphology under the influence of the added fibronectins.

In each experiment, each of the two different types of fibronectin were incubated with each cell type in replicate dishes. In experiments 1 and 2, the amounts of fibronectin were varied; in experiment 3, the time of incubation was varied. For each condition, the counts per minute of fibronectin bound/μg of cell protein were determined. These values were then compared pairwise in two ways: A, the ratio of binding efficiencies of NIL8 and NIL8.HSV fibronectins were compared for each cell type at each dose of fibronectin (experiments 1 and 2) or at each time (experiment 3); and B, the ratio of binding efficiencies of NIL8 and NIL8HSV cells were compared for each fibronectin. Results are expressed as mean ± S.D. for each experiment. The results show that there is no consistent difference in binding of the two fibronectins but that the NIL8 cells consistently bind more of each fibronectin than do the NIL8.HSV cells.

### Table 1

**Binding of fibronectins to cells**

| Ratio of binding | Experiment | Experiment | Experiment |
|------------------|------------|------------|------------|
| A. NIL8.fibronectin/ NIL8.HSV.fibronectin | 0.89 ± 0.03 | 1.56 ± 0.09 | 0.96 ± 0.11 |
| NIL8 cells | 0.64 ± 0.05 | 1.02 ± 0.21 | 1.13 ± 0.12 |
| NIL8.HSV cells | | | |
| B. NIL8 cells/NIL8.HSV cells | 2.81 ± 0.25 | 5.11 ± 1.01 | 4.85 ± 0.94 |
| NIL8.fibronectin | 2.01 ± 0.21 | 3.25 ± 0.18 | 4.07 ± 0.59 |

Fig. 9. Cell morphology affected by fibronectins from normal (NIL8) or transformed (NIL8.HSV) cells. The procedure was as in Fig. 8. At the end of the incubation with fibronectin, attached cells were photographed. A, control culture with 900 μl of CAPS buffer only. Note refractile appearance of cells and their lack of ordered arrangement. B, 36 μg/ml of fibronectin purified from NIL8.HSV cells. C, 48 μg/ml of fibronectin purified from NIL8 cells. In B and C, cells have acquired flattened, elongated, and aligned morphology under the influence of the added fibronectins.

Fig. 7. In all four combinations of cells and fibronectins, intact fibronectin was recovered from the culture. Therefore, transformed cell fibronectin is as competent to bind to cells as is the fibronectin secreted by normal cells. However, transformed cell cultures are reduced in their ability to bind fibronectin from either source.

**Fibronectin from Transformed Cells Is Active in Promoting Cell Adhesion, Elongation, and Alignment**—Since the fibronectin secreted by transformed cells was active in binding to cell layers, we investigated whether higher doses were able to induce cell adhesion and morphological changes as previously demonstrated for the fibronectin secreted by normal cells (30). The transformed cell fibronectin was isolated from cultures grown in serum free of fibronectin to ensure that serum-derived fibronectin did not contribute to the results.

We first compared the efficiency of the fibronectins isolated from normal and transformed cultures in causing adhesion of unattached NIL8.HSV cells (30). The results are shown in Fig. 8. Both fibronectin preparations showed equal abilities to cause attachment of the floating cells.

Microscopic examination of the cultures showed that both preparations were also effective in causing flattening, elongation, and alignment of the cells (Fig. 9) with similar dose dependencies.

Therefore, fibronectin secreted by transformed cells, when purified, concentrated, and added back to the same cells at higher concentrations than those to which the cells are normally exposed, is capable of reverting the cellular behavior toward normal just as is fibronectin similarly purified from the culture medium of normal cells.

**DISCUSSION**

We have shown that the fibronectin secreted by transformed cells is similar to that secreted by normal cells in the following respects. It is intact and dimeric (Fig. 1); it retains the ability to bind to gelatin-Sepharose (Fig. 1), to activated sulfhydryl columns (Fig. 2), and to cell layers (Table I); it is also capable of promoting cell adhesion (Fig. 8) and causing flattening, elongation, and alignment of cells (Fig. 9).

These results argue against the existence of several possible defects of transformed cell fibronectin. The possibility of proteolysis of fibronectin by transformation-induced proteases is addressed by several of the experiments. Straightforward analysis of the fibronectin molecules on gels failed to show any evidence for degradation (Fig. 1). In fact, the chains of transformed cell fibronectin appeared somewhat larger than those of normal cell fibronectin. Other results make even stronger the conclusion that proteolytic cleavage of fibronectin can play only a minor role in affecting the ability of the cells to retain fibronectin. There cannot be significant digestion near the NH2 terminus since the NH2-terminal 25-kd fragments from both fibronectins appear identical (Figs. 3 and 4) whereas even a difference of 1–2 k全资 detectable. Equally, since the interchain disulfide bonds are very close to the COOH terminus (21) and both types of fibronectin are dimeric, there cannot be much, if any, proteolysis occurring at the COOH-terminal end. Finally, exogenously added fibronectin remains intact (Fig. 7). Therefore, over the time course of these experiments, up to 24 h, there is no indication that proteolysis of fibronectin is significant. We have observed, however, that if transformed cell cultures are allowed to secrete fibronectin for several days, the fibronectin purified from such cultures has a greater tendency to be degraded (data not shown). Also, it is important to add protease inhibitors to prevent degradation during preparation of the fibronectin from transformed cell culture medium. These observations presumably reflect a higher level of proteolytic enzymes in transformed cell cultures. Perhaps, as cultures age, continued secretion of proteases eventually exceeds the ability of serum protease inhibitors to prevent proteolysis. Be that as it may, proteolytic digestion of fibronectin does not provide a sufficient explanation for the reduced surface levels of fibronectin. Over 24 h, the transformed cells studied here secrete ~10% as much fibronectin as do the normal cells, all apparently intact, but do not accumulate on their surfaces any detectable amounts of fibronectin (<1%), whereas the parent normal cells incorporate an amount equivalent to 33% of what they secrete (28). It remains possible that proteolysis of other molecules may play a role in the reduced surface levels of fibronectin (see below). Another possible area of difference between the fibronectins of normal and transformed cells is in their disulfide bonding. Since disulfide bonds play a role in connecting the two chains of the dimer, in binding fibronectin into disulfide-bonded aggregates (20, 21, 31–34), and in the ability of fibronectin to bind to gelatin (17, 20), alterations in this aspect of fibronectin structure would be significant. However, the results presented provide no evidence for such alterations. Transformed cell fibronectin retains interchain disulfide bonds and free sulfhydryl groups and still binds to gelatin. Furthermore, the large
proteolytic fragments lack interchain disulfides as they do in normal cell fibronectin (Fig. 3) and the NH₂-terminal 25-kd fragment, which contains extensive interchain disulfide bonding, retains its anomalous migration on nonreducing gels (Fig. 3). While these results cannot rule out subtle alterations in disulfide bonding, they provide no evidence for the existence of any such defects in fibronectin from transformed cells.

Some of our experiments did detect differences between fibronectins of normal and transformed cells. The intact chains and many of the larger partial proteolytic fragments appear larger in the transformed case (Figs. 1, 3, and 4). All of the fragments which differ between the two sources contain carbohydrate whereas the 25-kd fragment which does not (20) appears identical from both fibronectins. This suggests that the differing fragments, all of which are internal and glycosylated, probably differ because of altered glycosylation. The analysis of glycopeptides (Figs. 5 and 6) confirms that the carbohydrate side chains of transformed cell fibronectin are larger, having both more branches and a higher degree of sialylation. This difference reflects similar overall differences in glycosylation by transformed cells (29, 35, 36).

There is no evidence that increased glycosylation of fibronectin from transformed cells has any functional consequences. Fibronectin lacking any carbohydrate functions normally in a variety of assays (37) although the carbohydrates could be important in other aspects of function which have not yet been tested. Finally, the reconstitution experiments (Figs. 8 and 9, Table I) indicate that fibronectin from transformed cells is completely competent in binding to cells and affecting their adhesion and morphology. A recent report by Hayman et al. (11) also indicates that the fibronectin released by transformed NRK cells is active in cell adhesion. These results suggest that there is nothing defective about the fibronectin produced and secreted by transformed cells and force one to look elsewhere for the defect in the cells' ability to retain fibronectin.

The binding experiments indicate directly that the transformed cultures are deficient in binding fibronectin purified from either normal or transformed cells (Table I). These observations extend our earlier less extensive reports of this phenomenon (8, 25). The nature of the experiments, binding to confluent layers of cells, does not allow one to deduce very much about the nature of the binding. The results would be consistent with a reduced number of binding sites of a single class, with loss of one or more classes of binding site from among several classes, with reduced affinity of binding sites, or with any combination of these alterations. Fibronectin binding to cell layers could be by binding either to cell surface molecules such as (glyco) proteins, or glycolipids (38) or to extracellular matrix components such as proteoglycans, or collagen, or to fibronectin itself. Alterations in any of these components are possible and, indeed, differences between normal and transformed cells have been reported for all these classes of molecule (39). Further progress in understanding the defect in fibronectin binding will require more information on the other alterations in cell surface molecules, be they caused by altered synthesis, altered degradation, or kinase-induced changes.

The conclusion that a major cause of reduced surface fibronectin is reduced ability of the transformed cells to bind it has been demonstrated here for a single cell line. Indirect results consistent with this idea have been reported for other transformed cells (6, 10, 11, 23, 24). A similar conclusion was drawn for differentiated chondrocytes (40) which continue to synthesize fibronectin but do not retain it as do their undifferentiated counterparts. Reduced ability of cells to interact with fibronectin has significant implications for the phenotype of cells both in vitro and in vivo, since fibronectin affects several aspects of cell behavior. A number of transformed cell types respond to addition of fibronectin by partial restoration of normal phenotype (3, 4, 8, 30, 41). However, as shown here, some of these cells are partially deficient in binding fibronectin. Therefore, these alterations in their phenotype which are affected by fibronectin presumably arise from a combination of reduced synthesis and reduced binding, neither one alone being sufficient. Presumably, if cells were completely deficient in the ability to bind fibronectin they would not respond to its addition even in large amounts. Therefore, different transformed cells could have low surface levels of fibronectin in vitro because of reduced synthesis, reduced binding, or a combination of both.

Such different causes for reduced fibronectin levels are likely to have different consequences for behavior of tumor cells in vivo. If the defect were in synthesis alone, fibronectin could be provided by surrounding cells (effectively an in vivo readdition experiment) and the cells would not be expected to show much alteration in behavior, at least until the tumor mass became large. In contrast, if there were a defect in the ability of the cells to interact with fibronectin, then exogenous supplies of fibronectin would have less effect, none if the binding defect were absolute. Therefore, the tumorigenicity and malignancy would be more likely to reflect the cells' ability to bind fibronectin than ability to make it. The levels detected in vitro are a consequence of reduced levels both of synthesis and of binding, the relative importance probably varying for different cells. Such considerations need to be taken into account when making correlations between in vivo behavior and in vitro levels of fibronectin detected by surface labeling or immunofluorescence. While the correlations between tumorigenicity and malignancy and fibronectin loss are quite good in some studies (42-49), some exceptions have been noted (50, 51). These correlative studies need extending to analyze separately the biosynthetic rate and the binding of fibronectin because of their likely different consequences.

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