Comparison of Polypeptide Profiles in Normal and Transformed Kidney Cell Lines Derived from Control, DimethylNitrosamine-treated, and Renal Tumor-bearing Rats, with Particular Reference to Contractile Proteins

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ABSTRACT

The polypeptide profiles of three malignantly transformed kidney cell lines of mesenchymal type, established in culture from rats treated 2 hr, 24 hr, and 4 days previously with a single carcinogenic dose of dimethylnitrosamine were compared on the one hand with the profiles of three cell lines derived from advanced renal mesenchymal tumors which had been induced by the same carcinogenic regimen and on the other with cultures of normal mesenchymal cells isolated from the kidneys of untreated rats. Polycrylamide gel electrophoresis and quantitative gel densitometry provided both qualitative and quantitative biochemical data demonstrating that the polypeptide profiles of the transformed kidney cells closely resembled those of the tumor cells. This confirms previously reported evidence based on morphology and growth behavior which links these cells of chronologically disparate isolation to a common origin. Thus, the evidence strongly suggests that the mesenchymal cells, which are derived from rat kidneys very shortly after the carcinogenic insult and which express transformation in vitro, represent the same target cell populations as those transforming in vivo to produce macroscopic renal mesenchymal tumors some 6 months later. Individual samples of fibroblast-like mesenchymal cells grown from the kidneys of normal rats were characterized by polypeptide profiles of consistent pattern regardless of whether they were assayed at subcultures 1, 2, or 3. However, there were distinct points of difference between these and the common profiles of the tumor and transformed cell lines. The most prominent differences involved those bands observed at M.W. 220,000, M.W. 200,000, M.W. 42,000, and a doublet at M.W. 18,000 to 20,000, corresponding respectively to fibronectin, myosin (heavy chain), actin, and calmodulin. Actin and myosin contents of tumor and transformed cells were significantly decreased when compared with normal rat kidney cells, although the actin/myosin ratios did not vary. Fibronectin was also decreased in the neoplastic state, whereas calmodulin showed a pronounced increase in comparison with the normal rat kidney cell counterparts.

INTRODUCTION

The single i.p. administration of DMN to young rats which have been preconditioned with a diet high in carbohydrate but lacking in protein has proved to be a potent model in the study of the biology of chemical carcinogenesis, since both mesenchymal (connective tissue) and epithelial tumors of the kidney are induced, the former in up to 100% incidence (13). Studies tracing the sequence of morphological events which occur in the rat kidney following the carcinogenic insult and which lead to the presence of macroscopic neoplasms (14-16) have facilitated the development of an in vivo-in vitro system of chemical carcinogenesis (1, 12). In the latter, cells are removed for establishment in culture after the animal has been treated in vivo with the carcinogen. The in vivo-in vitro system thus provides an opportunity for observations to be made on transformed target cell populations which may be directly correlative with known events triggered in vivo by the carcinogen.

In the first place, cells isolated from advanced mesenchymal tumors have been established as continuously growing cell lines in vitro, and the structural and behavioral patterns have been characterized (9, 10). Secondly, the isolation of kidney cells into primary culture from rats within a few hr or days of the single, carcinogenic dose of DMN results in the expression of morphological transformation, usually at the fifth subculture, as piled-up colonies of densely crowded cells in random orientation (11, 18). The assumption is that the transformed mesenchymal rat kidney cell lines derived very shortly after the carcinogenic insult represent the same malignant populations of cells as those that are derived many months later from the DMN-induced renal mesenchymal tumors. This is supported in a qualitative sense by morphology and altered growth parameters. Both transformed and tumor cell lines consist of pleomorphic mesenchymal cells of similar appearance (10, 11), and they possess common in vitro properties characteristic of neoplastic cells including enhanced DNA-synthetic activity (30), loss of anchorage dependence as indicated by colony formation in semisolid media, agglutinability by concanavalin A (1, 9, 18), and ability to produce tumors of mesenchymal type upon implantation into suitable hosts (8).

The purpose of this study was 2-fold. The polypeptide profiles of representative kidney lines were assessed on the one hand as a possible basis for quantitatively consolidating the suspected homology of cell lines derived from the DMN-animal. 

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3 The abbreviations used are: DMN, dimethylnitrosamine; PBS, phosphate-buffered saline (in g/liter: KCl, 0.20; KH2PO4, 0.20; NaCl, 8.00; Na2HPO4, 7H2O, 2.16), SDS, sodium dodecyl sulfate.
model at temporally distinct periods. On the other hand, cell lines isolated from DMN-treated rats (tumor and transformed) were contrasted with counterpart target cell populations taken from control rats in order to characterize differences in polypeptide profiles between normal and neoplastic kidney cells, with particular reference to their relative contents of individual cytoskeleton-associated proteins. A number of recent reports have suggested that there may be a relationship between the altered growth characteristics of neoplastic cells and the changed expression of contractile and cytoskeletal components within these cells. Immunofluorescent studies, for example, have established a marked alteration in the organization of the major contractile proteins actin and myosin in neoplastic cells in vitro (27, 33), while other investigations have indicated significant changes in the content of a number of other cytoskeleton-associated proteins such as fibronectin or large, external transformation-sensitive protein (22) and the calcium-sensitive modulator protein, calmodulin (37). In the case of the present cell lines, the tumor and transformed cells have previously been distinguished from normal rat kidney cells in culture on the basis of their antiactin immunofluorescent staining patterns (32).

MATERIALS AND METHODS

Origin of Cell Lines

Normal Rat Kidney Cells. Monolayers of normal mesenchymal cells were prepared from the kidneys of 6-week-old rats of Porton albino Wistar stock by removing the renal capsule and mincing cortical tissue in warm Hanks' balanced salt solution (in g/liter: KCl, 0.40; KH2PO4, 0.06; NaCl, 8.00; NaHCO3, 0.35; Na2HPO4-7H2O, 0.09; glucose, 1.00; phenol red, 0.01). The tissue was subjected to repeated washings and agitation in 10-ml aliquots of 0.125% trypsin in calcium- and magnesium-free PBS. The supernatants from each trypsinization were collected in Waymouth's MB 752/1 medium supplemented with 10% fetal calf serum and antibiotics (penicillin, 10 units/ml, and streptomycin, 10 μg/ml) in Falcon 3012 flasks. The cultures were held in an atmosphere of 5% CO2 in air at 37°. Medium was replaced every 2 to 3 days up to confluence when monolayers were subcultured by stripping from the flask with trypsin Versene in PBS.

Preparation of Cell Samples for Assay

The cells were removed from the tissue culture flasks with the aid of a plastic policeman. After resuspension in PBS, a small sample was taken for cell counting by hemocytometry. The cells were collected by low-speed centrifugation (500 x g for 5 min). Under the conditions of culture (see below), the primary isolate from normal rat kidney was always a mixture of fibroblast-like mesenchyme and islands of epithelial cells. The latter did not survive passage, however, and intermediate subcultures consisted of homogeneous monolayers of mesenchymal cells. Consequently, for the purpose of serving as normal counterparts of transformed and tumor cell lines in this study, polypeptide estimations were performed only on the first (7 samples), second (17 samples), and third (2 samples) subcultures of normal rat kidney cells.

Renal Mesenchymal Tumor Cell Lines. Continuously growing mesenchymal cell lines, designated RRMT2, RRMT8, and RRMT9, had been derived from 3 rapidly growing renal mesenchymal tumors of a characteristic histological pattern previously described in detail (9, 13, 16). The tumors were initiated by a single i.p. injection of DMN (60 mg/kg body weight) administered at least 6 months prior to tumor cell harvest. Eight samples of RRMT2 (subcultures ranging from 70 to 99), 11 of RRMT8 (subcultures 84 to 130), and 7 of RRMT9 (subcultures 18 to 33) were examined in this study.

Transformed Rat Kidney Cell Lines. Three transformed mesenchymal cell lines, designated TRKM5, TRKM7, and TRKM12, which had been derived from the kidneys of Porton albino Wistar rats treated with a carcinogenic dose (60 mg/kg body weight) of DMN were used for comparison with the normal rat kidney cells and renal mesenchymal tumor cell lines. TRKM5 (11 samples examined, ranging from subcultures 92 to 127) had been isolated into primary culture 24 hr after DMN administration, TRKM7 (7 samples at subcultures 77 to 93) was derived from the kidneys of a rat treated 2 hr previously with carcinogen, and TRKM12 (7 samples at subcultures 5 to 20) was derived from the kidneys of a rat at 4 days after the DMN dose.

Maintenance of Cell Cultures

All rat kidney cell lines were maintained in Waymouth's MB752/1 medium supplemented with 10% fetal calf serum and antibiotics (penicillin, 10 units/ml, and streptomycin, 10 μg/ml) in Falcon 3012 flasks. The cultures were held in an atmosphere of 5% CO2 in air at 37°. Medium was replaced every 2 to 3 days up to confluence when monolayers were subcultured by stripping from the flask with trypsin Versene in PBS.

Protein Estimation

Total protein concentrations of the solubilized cell extracts were determined (24) using bovine serum albumin in 2.5% SDS as standard. To ensure that complete solubilization of cell protein had been achieved, solubilized extracts were centrifuged at 12,000 x g for 20 min, and the protein content of the resulting supernatants was compared with that of the original extract. The efficiency of the solubilization procedure was indicated by the fact that less than 3% of the total protein of the extracts sedimented under these conditions.

Quantitative Polyacrylamide Gel Electrophoresis

Electrophoresis in the presence of SDS was performed in 1-mm-thick 10 or 12.5% acrylamide slab gels using the discontinuous buffer system of Laemmli (23). Prior to electrophoresis, samples of the extracts mixed with an equal volume of a sample buffer containing 0.125 M Tris-Cl (pH 6.8), 5% β-mercaptoethanol, 5% SDS, and 10% glycerol, were heated at 100° for 1 min to ensure complete dissociation. For actin quantitation, samples of cell extracts were loaded on a cell number basis so that each slab contained the protein derived from an equal number of cells (usually 1 to 2 x 10⁴). On occasion, the cell extracts were loaded on an equal protein basis. Four to 6 aliquots (0.25 to 2.0 μg) of rabbit skeletal muscle actin were...
applied to each slab gel to serve as internal standards for quantitation of actin in the cell extracts. The gels were calibrated for molecular weight estimation using the following standard mixture (Pharmacia Fine Chemicals, Uppsala, Sweden): thyroglobulin, M.W. 450,000; ferritin, M.W. 220,000; myosin, M.W. 200,000; phosphorylase, M.W. 94,000; bovine serum albumin, M.W. 68,000; catalase, M.W. 60,000; ovalbumin, M.W. 45,000; lactate dehydrogenase, M.W. 36,000; carbonic anhydrase, M.W. 30,000; trypsin inhibitor, M.W. 21,500; and a-lactalbumin, M.W. 14,000.

Following electrophoresis, the gels were stained in 0.25% Coomassie Blue in 50% methanol/10% acetic acid for 16 hr and completely destained by extensive washing in 5% methanol/10% acetic acid. The gel patterns were then scanned using a Kipp-Zonen densitometer. The integrated areas of the actin standards were used to construct a standard curve for actin calibration on each gel run. The relationship between the area under the peak and the amount of actin loaded on the gel was linear over the range examined. The amount of actin in each cell extract was read directly from this calibration curve. It was assumed (a) that kidney cell actin bound the same amount of dye as did standard rabbit muscle actin, a reasonable assumption in view of the remarkable conservation of actin structure (35) and (b) that the band at M.W. 42,000 contained only actin and no other polypeptides (evidence in support of this is presented below). The myosin content of the cells was estimated in a similar manner, using 7.5% polyacrylamide gels and skeletal muscle myosin as standard. A good estimate of the relative amount of the other polypeptide bands in the cell extracts for comparative purposes was obtained by expressing the area of their peaks as a percentage of the total area of the scan.

Protein Preparations

Rabbit skeletal muscle was used for the preparation of actin (29), myosin (2), and troponin and its subunits, troponin T, troponin I, and troponin C (4). The concentrations of G-actin and myosin were estimated as ε values at 280 nm of 1.11 mg/ml (21) and 280 nm of 0.543 mg/ml (2), respectively. Fibronectin was prepared in the form of cell-free matrices from rabbit skin fibroblasts (3).

Peptide Mapping

One-dimensional peptide maps in polyacrylamide gels were obtained essentially by the method of Cleveland et al. (5), using the M.W. 42,000 band proteins extracted from gels as described by Djondjurov and Holtzer (6).

Identification of Modulator Protein-Calmodulin

Calmodulin was identified by electrophoresis of cell extracts in polyacrylamide gels in the presence of 6 M urea with and without calcium (7). Briefly, cell extracts were prepared by homogenizing the cells in 6 M urea/1 M mercaptoethanol/1 mM CaCl₂/50 mM Tris-Cl (pH 8.0) followed by centrifugation at 40,000 × g for 30 min. Samples of the extracts were electrophoresed in 7.5% polyacrylamide gels in 25 mM Tris (7). Briefly, cell extracts were prepared in 6 M urea/1 mM mercaptoethanol and electrophoresed in 7.5% polyacrylamide gels in 25 mM Tris/80 mM glycine (pH 8.3) containing 6 M urea. Either 10 mM ethyleneglycol-bis(β-aminopropylether)-N,N′-tetraacetic acid or 2 mM CaCl₂ was added to the samples prior to electrophoresis. When cell homogenates are electrophoresed in polyacrylamide gels containing 6 M urea in the absence of Ca²⁺ ions, calmodulin can be seen as the most acidic (fastest migrating) band (7). In the presence of Ca²⁺ ions, this band disappears due to complex formation with other cellular components, analogous to the well-established Ca²⁺-dependent interaction of troponin C with troponin I (20).

RESULTS

Chart 1 shows typical densitometric scans of the polypeptide patterns obtained after analysis of normal rat kidney, renal tumor (RRMT8), and transformed (TRKM7) cell lines by SDS/polyacrylamide gel electrophoresis. The most important feature of these electrophoretic analyses was the striking similarity in the polypeptide profiles of the tumor and transformed cell lines. The other tumor (RRMT2, RRMT9) and transformed (TRKM5, TRKM12) cell lines examined in this study also gave profiles very similar to those shown in Chart 1 for RRMT8 and TRKM7. On the other hand, normal rat kidney cells gave rise to polypeptide profiles which, although presenting no individual sample variation regardless of subculture number (1, 2, or 3), differed significantly in several respects from the common profiles of the tumor and transformed cell lines. Among the polypeptide bands which displayed the most obvious qualitative differences between the normal and the neoplastic state were those at M.W. 220,000, M.W. 200,000, M.W. 42,000, and a doublet M.W. 18,000 to 20,000 (Chart 1, arrows). Visualisation of these characteristic polypeptide profiles was independent of whether the loading of the gels was made on the basis of equal cell number or equal protein. Of particular reference to consideration of the intracellular contractile apparatus were the bands at M.W. 42,000 and M.W. 200,000 which comigrated with skeletal muscle actin and the myosin heavy chains, respectively. The identity of the M.W. 42,000 band as actin was supported by peptide analysis (Fig. 1) which clearly indicated that the relevant bands on gels of the various cell lines corresponded to actin. Differences in actin content for each cell line were quantitated by gel densitometry and expressed as pg per cell (Table 1). These results demonstrated that normal rat
followed by analysis of fragments in a 18% SDS/polyacrylamide gel. Sample 1. generated as described by Cleveland et al. (5) by partial hydrolysis of the protein from normal, transformed, and tumor cells. Rabbit skeletal muscle actin and the K, molecular weight in thousands. transformed cell M.W. 42,000 band; Sample 4. normal cell M.W. 42,000 band. rabbit skeletal muscle actin; Sample 2, tumor cell M.W. 42,000 band; Sample 3. M.W. 42,000 bands of cell extracts were cut from gels, and the protein was extracted as described in "Materials and Methods." The peptide maps were tent between normal and neoplastic cells were related to cor

with the heavy chain of skeletal muscle myosin was also
the neoplastic cells in general displayed a remarkable similarity to the normal cell than did the other tumor and transformed lines (p > 0.005), transformed cell line, TRKM7, had significantly more actin per
to 1.5 times) than did the tumor or transformed cells, which were found to have a slightly greater total protein content (1.2
for each cell line and the myosin/protein and actin/protein
content of the tumor and transformed cells. Again, the neo
plastic cells displayed equivalent amounts of this protein. To
determine whether these differences in myosin and actin content between normal and neoplastic cells were related to corresponding differences in total cell protein, the protein content for each cell line and the myosin/protein and actin/protein ratios were determined (Table 2). In general, the normal cells were found to have a slightly greater total protein content (1.2 to 1.5 times) than did the tumor or transformed cells, which displayed very similar amounts of total protein. The myosin/protein and actin/protein ratios which effectively express the
content of these contractile proteins as a percentage of the total cellular protein confirmed their decreased presence in the transformed and tumor cells. However, the actin/myosin ratio of tumor and transformed lines closely resembled the actin/myosin ratio of normal cells (Table 2, Column 5).

The protein with a molecular weight of 220,000 (Chart 1) which differed in relative content between control and neoplastic cells comigrated with fibronectin isolated from rabbit fibroblast matrices (3). The bands were quantitated by densitometry of the gel patterns. The area under the peak expressed as a percentage of the total area of the trace provided a reasonable estimate for comparative purposes. The percentages of the total Coomassie Blue stain present in the presumed fibronectin band were 2.0 ± 0.8 (S.D.) and 1.7 ± 0.8 in tumor and transformed cells compared to 3.5 ± 1.0 in normal rat kidney cells (9 determinations), thus denoting a significant decrease in the expression of this protein in the neoplastic cell lines.

A characteristic feature of the polypeptide profiles of the tumor and transformed cell lines (Chart 1) was the presence of a prominent doublet in the M.W. 18,000 to 20,000 region which was virtually absent from the profiles of normal rat kidney cells. The apparent molecular weight of the M.W. 18,000 band comigrated with calmodulin. As shown in Fig. 2, when analyzed in 6 M urea polyacrylamide gels in the absence of Ca²⁺ ions, the rat kidney cell lines displayed a fast-migrating band which comigrated with skeletal muscle troponin C. This band was
further characterized as calmodulin by its disappearance in the presence of Ca²⁺ ions (Fig. 2). The calmodulin band was very evident in extracts of tumor and transformed cell lines but was virtually absent from normal rat kidney cells. The relative content of calmodulin in each of the cell lines was quantitated from the percentage of the total Coomassie Blue stain associated with the bands after densitometry of the urea gels. Such estimates, although useful for comparative purposes, were approximate, because no allowance was made for the different Coomassie Blue-binding capacities of different proteins. On this basis, calmodulin comprised 1.5% and 1.4% of the total protein in tumor and transformed cell lines, compared to 0.3% for normal rat kidney cells.

### DISCUSSION

The results provide qualitative and quantitative biochemical data to demonstrate a striking similarity in the protein composition of transformed and tumor cell lines which have been

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<th>Table 1</th>
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<td><strong>Tumor cell lines</strong></td>
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<td><strong>Transformed cell lines</strong></td>
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<td><strong>Normal rat kidney cells</strong></td>
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a Mean ± S.D.
b Numbers in parentheses, number of determinations.
c p < 0.0005.
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**Table 2**

<table>
<thead>
<tr>
<th>Tumor cells</th>
<th>Myosin* (pg/cell)</th>
<th>Protein* (pg/cell)</th>
<th>Myosin/ protein</th>
<th>Actin**/ protein</th>
<th>Actin/ myosin</th>
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<td>7.0 ± 3.3**d (9)*</td>
<td>370 ± 56 (3)</td>
<td>0.019</td>
<td>0.096</td>
<td>5.06</td>
</tr>
<tr>
<td>Transformed cells</td>
<td>10.2 ± 1.9**d (9)</td>
<td>406 ± 89 (3)</td>
<td>0.025</td>
<td>0.091</td>
<td>3.66</td>
</tr>
<tr>
<td>Normal rat kidney cells</td>
<td>27.2 ± 8.1 (9)</td>
<td>530 ± 60 (3)</td>
<td>0.051</td>
<td>0.181</td>
<td>3.54</td>
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* Data from the 3 tumor cell lines and the 3 transformed cell lines were pooled in these calculations, inasmuch as no significant difference were found between cells within these groups.
** The average actin contents of the tumor and transformed cell lines (Table 1) were used in these calculations.
* Mean ± S.D.
<sup>d</sup> p < 0.005.
<sup>e</sup> Numbers in parentheses, number of determinations.
<sup>f</sup> p < 0.01.

![Fig. 2. Identification of calmodulin in cell extracts. Samples of cell extracts, troponin I, troponin C, and troponin I/troponin C mixtures that were prepared in 6 M urea/1 mM β-mercaptoethanol were electrophoresed in 7.5% polyacrylamide gels containing 6 M urea/25 mM Tris/80 mM glycine (pH 8.3). Samples 1 and 2, transformed cell extracts; Samples 3 and 4, tumor cell extracts; Samples 5 and 6, troponin I/troponin C mixtures; Samples 7 and 8, troponin C; Samples 9 and 10, troponin I. CaCl<sub>2</sub> (2 mM) was added to Samples 1, 3, 5, 7, and 9 prior to electrophoresis, while 10 mM ethyleneglycol-bis(β-aminoethyl)-N,N'-tetraacetic acid was added to Samples 2, 4, 6, 8, and 10. Comparison of Samples 5 and 6 illustrates the Ca<sup>2+</sup>-dependent formation of the troponin I/troponin C complex. In the cell extracts, note the appearance of calmodulin (arrow) only in the presence of ethyleneglycol-bis(β-aminoethyl)-N,N'-tetraacetic acid.](Image)

Derived from the kidneys of rats exposed to DMN in vivo. In particular, comparison of the densitometric traces in Chart 1 clearly support the close identity of the tumor and transformed cells in terms of polypeptide profile. In addition to the overall similarity in total polypeptide complement, quantitative equivalence in terms of individual polypeptide components has also been established. Such consistent biochemical resemblance between the neoplastic cells supports the view that these lines are identical populations despite their widely differing derivations. The tumor cells have been established in culture from advanced- or terminal-stage renal mesenchymal tumors induced in protein-deprived rats by a single injection of DMN (13). On the other hand, the transformed cells have been derived from cells that were removed from rat kidneys only a few hr or days after the administration of the same carcinogenic dose of DMN, i.e., at the very earliest stage in the induction process, preceding overt tumor formation by some 5 to 6 months (1, 18). These latter cells express morphological transformation after approximately 65 days in culture or usually at subculture 5, beyond which they persist, like the tumor-derived cells, as continuously growing mesenchymal lines with malignant properties (8–11). Sequential in vivo studies on the evolution of the renal mesenchymal tumors indicate their origin from an interstitial mesenchymal cell resident in the outer zones of the kidney (14, 15). The probability that the putative target cell is programmed very early in the process to express this malignant potential is implied by the rapid disappearance of DMN or its labeled metabolites in this system within 19 hr of administration (31). The in vivo-in vitro system, in which mesenchymal cells express morphological transformation in vitro after isolation from recently carcinogen-treated animals, presumably is selecting out very early clones of these programmed target cells. If this is the case, then it would be expected that the transformed cell lines and cell lines grown from renal mesenchymal tumors would represent one and the same basic population, despite the extensive chronological gap in their derivations. The biochemical case presented here is supported also by previous qualitative studies on the morphology and in vitro growth characteristics of these cell lines (11, 18) and by the recent demonstration of the histological conformity between implantation tumors produced by the transformed cells and the primary tumor induced in vivo by the carcinogenic dose of DMN (8).

It has become evident in recent years that conversion to the neoplastic state is accompanied by alterations in the expression of contractile and cytoskeleton-associated elements. Decreased expressions of actin (28), myosin (25), and fibronectin (for reviews, see Refs. 22 and 34) have been found to characterize various malignantly transformed cell lines. Most of the studies have utilized isolated cell populations which have been transformed by viral agents in vitro. The in vivo-in vitro system also permits meaningful comparison of neoplastic and normal cell populations in vitro but at the same time maintains a direct link with chemical carcinogenesis in the whole animal. Using the rat kidney model, mesenchymal cells persist as homogeneous monolayers for several subcultures following primary isolation from the outer zones of the rat kidney before reaching senescence at approximately subculture 4. These cells are morphologically identical to the pretransformation subcultures of carcinogen-treated rats (11) and appear to represent the normal counterparts of the transformed mesenchymal and tumor-derived lines. Comparison of the polypeptide profiles of the neoplastic cell lines with their normal counterparts from untreated rats reveals a number of focal points of difference,
the major variations involving mainly contractile or cytoskeletal components. With the transformation by DMN from normal to neoplastic kidney cells, there is a quantitative decrease in the content of the basic contractile proteins, actin and myosin, and in fibronectin. Consideration of relative cell size, however, suggests that variations in actual intracellular concentrations may be less than the differences shown in absolute amounts. For instance, planimetric measurements of the spread outlines of normal rat kidney mesenchymal cells on substrate are substantially greater than those of the renal mesenchymal tumor lines, and the nucleus/cytoplasm ratio is lower (9). How this translates to the possibility of cell volume differences in these cells is unclear. It is known that, in general, normal cells flatten out on substrate to a much greater degree than do transformed counterparts, an effect which appears to be related at least in part to fibronectin content (26, 36). However, certain evidence indicates that the degree of spread on a flat surface may reflect little, if any, difference in cross-sectional area, and thus in size, when comparison is made between normal and neoplastic cells which are rounded up in a suspended form (33). The diminished total protein of neoplastic kidney cells relative to the normal kidney cells may suggest a small volumetric difference, but this would not account for the full extent of the decrease in actin, myosin, or fibronectin that was found in the tumor and transformed cells. Certainly, our previous observations on the indirect immunofluorescent staining of rat kidney cell populations both in vivo and in vitro with antiaction antibody, which implied an enhanced expression of this contractile protein in DMN-induced neoplastic cells (19, 32), was not confirmed by the biochemical analysis. Such a discrepancy suggests that differences in the organization of the contractile apparatus in normal and neoplastic cells, as affecting immunofluorescence visualization, depend not on the relative concentrations of the basic proteins per se, but rather on prevailing behavioral states (e.g., anchorage, cell shape, and locomotion) which imply regulation of the contractile protein assembly into functionally distinct entities.


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