Variations in Cell Form and Cytoskeleton in Human Breast Carcinoma Cells in Vitro

B. R. Brinkley, P. T. Beall, L. J. Wible, M. L. Mace, Donna S. Turner, and R. M. Cailleau

ABSTRACT

Cell form and cytoskeletal organization were investigated in 13 human breast carcinoma cell lines in vitro. Using tubulin antibodies and indirect immunofluorescence to detect the arrangement of cytoplasmic microtubules, three distinct cell phenotypes were recognized: (a) cells with extensive arrays of microtubules (type I); (b) cells which were diffusely stained with microtubules apparent only near the cell margins (type II intermediate); and (c) cells in which individual microtubules could not be detected and only diffuse fluorescence was apparent (type II diffuse). Type I cells were flattened epithelial-like cells, much like normal mammary epithelial cells, which when stained with actin antibody displayed many brightly fluorescent parallel cables or "stress fibers." Many microtubules and microfilament bundles were observed in type I cells when examined by transmission electron microscopy. Type II cells were more rounded, often grew in multilayered colonies, and displayed fewer microtubules and microfilament bundles when examined by either immunofluorescence or electron microscopy. Type II cells ranged from very small rounded cells with diffuse tubulin and actin immunofluorescence (type II diffuse) to more flattened cells in which microtubules and actin cables were observed near the flattened cell margins (type II intermediate).

Since all of the cells were derived initially from malignant metastatic lesions and some were tumorigenic when injected into athymic nude mice, we assume that they remained malignant in vitro. Thus, in human breast carcinoma cells in vitro, it is not possible to associate any specific cell morphology or cytoskeletal phenotype with cancer or metastasis in vivo. Whether or not these same conclusions hold for breast tumor cells in situ remains to be determined.

INTRODUCTION

Cell transformation in vitro is often accompanied by alterations in morphology and growth properties which may relate directly or indirectly to cancer. The most frequently cited changes include alterations in cell shape, cell motility, spreading and adhesive properties, loss of density-dependent inhibition of growth, loss of anchorage dependence, and changes in cell surface properties. Recently, evidence has been advanced that some transformed cell properties may be related to alterations within the cytoplasm involving a delicate network of microtubules and microfilaments known collectively as the cytoskeleton (1, 3, 5-7, 19, 21-27, 29, 35, 39, 42). Components of the cytoskeleton appear to play a major role in the regulation of cell shape and form (19, 21, 23, 35, 39), while both microtubules and microfilaments have been shown to be involved in cell motility (1, 22, 42) and anchorage-dependent growth (2, 38). Furthermore, the modulation of cell surface protein is regulated to a large extent by submembranous assemblies consisting of microtubules and microfilaments (4, 15, 29-31, 39, 40, 49-51).

The proposal that the cytoskeleton becomes altered in transformed cells is supported by electron microscopy (17, 20, 24-27), immunofluorescence (6, 16, 24, 29, 37, 46, 47), and biochemical studies (41). Recently, cultured skin fibroblasts both from patients with adenomatosis of the colon and rectum and from their asymptomatic children have been reported to display altered actin patterns (25). Such markers could be useful in screening for preneoplastic and neoplastic transformation of cells in vivo and in vitro.

Several reports have challenged the view that actin and microtubule patterns are altered in transformed cells and show that both microtubules and actin filaments can be found in abundance in malignant cells in vitro (14, 32, 44, 49). In the present study, we have examined 13 established human breast cancer cell lines for variations in morphology and cytoskeletal patterns using immunofluorescence and electron microscopy. Our results show that breast cancer cells in vitro display a wide spectrum of cell shapes and cytoskeletal patterns including small rounded cells with apparently diminished microtubule and microfilament networks, intermediate types with short irregular microtubules and actin filaments, and flattened epithelial cells with extensive arrays of long microtubules and microfilament bundles. Since all of these are derived from malignant lesions and since many are tumorigenic in nude mice, we conclude that cytoskeletal patterns in human breast cancer cells are highly variable and that they correlate more directly with cell shape than with any other aspect of cancer.

MATERIALS AND METHODS

Derivation of Cell Lines

The cells used in this study were from long-term cultures derived from human carcinomas by Cailleau et al. (9-11, 34) at M. D. Anderson Hospital and Tumor Institute. Initially, the cells were collected from pleural effusions (and in one case a brain biopsy) of patients with metastatic tumors. A description of the patient from which each cell line was derived is given below.

Case 1 (MDA-MB-134). This cell line was established first from a pleural effusion obtained on May 1, 1973. Five of 11 effusions yielded morphologically identical cultures.

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tient, age 47, white, had a primary breast cancer treated with mastectomy on May 12, 1970. One of 8 axillary nodes contained tumors. No radiotherapy was given. Recurrent disease in nodes was diagnosed in July 1971. Response to oophorectomy (August 1972) was negative. All the cell cultures were obtained during the postopophorectomy period and prior to giving any chemotherapy. Tumor response to subsequent chemotherapy was equivocal. The patient died in February 1975. Survival after diagnosis: 5 years.

Case 2 (MDA-MB-157). This cell line was established from a right pleural effusion removed on December 29, 1972. The patient, black, age 44, had a left mastectomy in March 1969. This was followed by radiotherapy to the nodal areas. An axillary mass appearing on the right (December 1971) was treated with radiotherapy. The lymphatic spread of tumor throughout the right breast and chest wall that followed failed to respond to androgens and 5-fluorouracil. In November 1972, the right chest wall was treated with radiotherapy just prior to obtaining the cell culture. Cytoxan as a later treatment produced no tumor remission. The patient died in March 1973. Survival after diagnosis: 4 years.

Case 3 (MDA-MB-231). This cell line was grown from a pleural effusion removed on October 17, 1973. The patient, white, age 51, had a mastectomy for an inner quadrant tumor in January 1969. No radiotherapy was given. Recurrence of breast carcinoma was diagnosed in June 1973 from a pericardial effusion containing malignant cells. Oophorectomy in June, 5-fluorouracil in July and August, and a combination of Adriamycin, Cytoxan, and methotrexate given on September 30 and again on October 8 failed to produce tumor regression. Methotrexate, 30 mg, was given 5 days before the cells were obtained for this culture. The patient died in January 1974. Total survival following diagnosis: 5 years.

Case 4 (MDA-MB-330). This cell line was grown from a pleural effusion removed on October 17, 1973. The patient, white, age 43, had had a right mastectomy on July 20, 1973. The carcinoma involved most of the breast, invaded the lymphatics of the breast, and was present in all 23 axillary nodes removed. Radiotherapy, 5000 rads, was given to the right chest wall and nodal areas. Disseminated carcinoma, made manifest by pleural effusion, was diagnosed in October 1974 and was the source of cells for this culture. Subsequent therapy included radiation to the ovaries and multiple chemotherapeutic agents. No regression was obtained. The patient died on August 23, 1975. Total tumor history from diagnosis: 2 years.

Case 5 (MDA-MB-331). This cell line was isolated on October 23, 1974, from a pleural effusion. (Pleural fluid from several thoracenteses done between October 1974 and April 1975 yielded 4 cell lines which were essentially identical.) The patient, age 49, had had a right mastectomy in July 1971. Radiotherapy, 2400 rads, was given to nodal areas only. In January 1973, recurrent tumor appeared on the right chest wall. Throughout 1973, 1974, and 1975, systemic treatment, used in sequence, consisted of androgens; a combination of 5-fluorouracil and methotrexate; a combination of Adriamycin, Cytoxan, and methotrexate; and, finally, Cytoxan as a single agent. No chemotherapy or hormonal therapy had been given for the 5 months before the cells were obtained for culture. The patient died in April 1975. Total tumor history from diagnosis: 4 years.

Case 6 (MDA-MB-415). This cell line was isolated on October 9, 1975, from the pleural effusion of a white patient, age 38. A right mastectomy had been done in March 1973 and was followed with radiotherapy, 5000 rads, to the nodal areas. Metastatic tumor in a rib was diagnosed in August 1974. Oophorectomy and androgen therapy followed. No chemotherapy was given prior to isolation of the cell line in October 1975. Subsequent treatment, combining Adriamycin with 5-fluorouracil and Cytoxan, is not evaluable. The patient died in January 1977. Total tumor history from diagnosis: 4 years.

Case 7 (MDA-MB-416). This cell line was isolated on October 17, 1975, from the pleural effusion of a white patient, age 66. Her primary breast cancer on the left was treated in January and February 1968, with mastectomy and postoperative radiation of the chest wall and nodal areas. Recurrent tumor was diagnosed in an inguinal node in December 1968. From this date through October 1975, systemic treatment included Stilbestrol, 5-fluorouracil, Halotestin, Cytoxan, and Adriamycin, each given as a single agent and each producing a tumor remission. No chemotherapy had been given for 3 months before cells were obtained for this culture. She died on December 13, 1975. Total tumor history from diagnosis: 8 years.

Case 8 (MDA-MB-431). This cell line was established from 2 samples of pleural effusion taken in December 1975 and in January 1976 from a white patient, age 40. Her past history was of cancer of the left breast (February 1972) treated by mastectomy and postoperative irradiation. The pathology report described invasion throughout the breast and in 12 of 12 high axillary nodes. In April 1974, metastatic disease appeared in the right axilla. Response of the tumor to oophorectomy was negative. No other systemic treatment had been given prior to obtaining this cell culture. Chemotherapy and immunotherapy was subsequently given. The patient died on March 24, 1976. Total tumor history from diagnosis: 4 years.

Case 9 (MDA-MB-435). This cell line was obtained on January 23, 1976, from a pleural effusion in a white patient, age 31. Her tumor history began in October 1975, when a left extended simple mastectomy was done following preoperative irradiation. The pathology showed residual tumor throughout the breast, in the breast lymphatics, and in 2 of 8 axillary nodes. Pleural effusion appeared as the first sign of metastasis in December 1975. No systemic therapy had been given prior to establishing the cell line in January 1975. Subsequent chemotherapy, using Adriamycin, Cytoxan, and methotrexate produced a remission of 5 months. Treatment with 5-fluorouracil was negative for tumor response. The patient died in October 1976. Total survival from first diagnosis: 1 year.

Case 10 (MDA-MB-436). This cell line was derived from pleural fluid obtained on January 23, 1976, from a 43-year-old white patient. She had been treated initially with a left mastectomy in December 1971; no lymph nodes of the axilla contained tumor and no radiotherapy was given. A chest wall metastasis from the internal mammary nodes appeared in October 1972, and comprehensive radiotherapy was then given. From August 1973 through December 1975, tumor remission was achieved with combinations of many cytotoxic drugs. There was no response to hormonal therapy. Eighteen days before the cell line was obtained, Adriamycin, 80 mg, was given. The patient died April 9, 1976. Total tumor history from diagnosis: 4.5 years.

Case 11 (MDA-MB-453). This cell line grew from a pericardial effusion which had been aspirated on June 9, 1976, from
a 46-year-old white patient. A right mastectomy had been done in October 1970. No radiotherapy was given, since the axillary lymph nodes contained no tumor. Recurrent disease appeared in February 1974 in a right supraclavicular node. Following this, treatment consisted of local irradiation; oophorectomy; Halotestin; or combination chemotherapy using vincristine, Adriamycin, Cytoxan, 5-fluorouracil, and methotrexate. At the time of the pericardial tap, the patient was receiving chemotherapy every 4 weeks, but the tumor was not responding. She died on June 10, 1976. Extensive carcinoma was found in the pericardium. Total tumor history from diagnosis: 5.5 years.

Case 12 (MDA-MB-461). This cell line was established on April 6, 1977, from a surgically excised metastasis to the frontoparietal area of the brain in a white woman, age 63. Her primary breast carcinoma was treated with a mastectomy in December 1973. No radiotherapy was given since no tumor was detected in the axillary nodes. The first recurrence was a lung nodule in November 1974. Chemotherapy, using multiple agents in combinations, was given from December 1975 through March 1977. (No radiotherapy had been given to the brain prior to obtaining the surgical specimen.) The patient died on December 15, 1978, with generalized carcinomatosis. Total tumor history from diagnosis: 5 years.

Case 13 (MDA-MB-468). This line was isolated on November 4, 1977, from a pleural effusion in a black female patient, age 51. The original breast carcinoma was diagnosed on the right in March 1976. No treatment was given for 1 year. Beginning in June 1977, the patient received 3 courses of combination chemotherapy (5-fluorouracil, Adriamycin, and Cytoxan). This was followed by a right simple mastectomy and irradiation of the chest wall and nodal areas. One month later, the pleural effusion appeared from which this cell line was obtained. (No chemotherapy had been given for 3 months.) Chemotherapy was resumed, but the patient died in March 1978 with generalized carcinomatosis. Total tumor history from diagnosis: 2 years.

Cell Cultures

Normal human breast epithelial cells were kindly provided by Dr. A. Hackett, Peralta Cancer Research Institute. Freshly explanted tissue obtained by mammoplasty from a premenopausal subject were prepared and cultured by the procedure of Stampfer et al. (43). Coverslip preparations to be processed for immunofluorescence contained exponentially growing cells in their sixth subculture.

Human breast cancer cell lines established at M. D. Anderson Hospital and Tumor Institute (9—11, 34) were grown as monolayers in plastic Corning T-flasks on Leibovitz's L-15 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 15% fetal calf serum, insulin, glutathione, and antibiotics (Geopen, gentamicin). The cultures were maintained by 100% during exponential growth.

Another method of estimating doubling time was based upon the dilution factor (1:3, 1:5, 1:50, etc.) in relation to the time interval between subcultures. Thus, a 1:30 dilution every 6 to 7 days corresponds to a doubling time of approximately 1.5 days.

Factors which could influence the doubling time of rapidly growing cells were: concentration of serum in the media; temperature of the incubator (36.5—38°); type of flask used (plastic versus glass container); pH control or range; and dilution ratio. Nevertheless, some grew very slowly (1:2 dilution every 3 to 4 weeks), and others grew so rapidly as to require a 1:50 or 1:100 dilution every 5 to 7 days.

Indirect Immunofluorescence

For indirect immunofluorescence, cells were grown on 11- x 22-mm glass coverslips to a density of 60 to 75% confluency. After a washing in PBS, the cells were fixed in 3% formaldehyde in PBS for 20 to 30 min. The coverslips were dipped 15 to 20 times in PBS and immersed for 7 min in absolute acetone at −20°. The cells were stained for immunofluorescence using monospecific anti-tubulin or anti-actin antibody according to the procedures described elsewhere (6).

Rabbit antibodies against bovine tubulin were prepared according to the procedure of Fuller et al. (18). Antibodies against actin were a generous gift from Dr. E. Lazarides, California Institute of Technology. The stained preparations were examined in a Leitz Orthoplan microscope equipped with epillumination. Controls for antibody specificity included preparations stained with preimmune rabbit serum instead of the anti-tubulin or anti-actin antibody.

Quantitation of Tubulin and Actin Patterns

The procedure for scoring cell cultures for tubulin and actin patterns was essentially the same as that described by Asch et al. (3). We have chosen to use the classification of De-Brabander et al. (13) to identify tubulin phenotype as described in "Results."

Electron Microscopy

Cell monolayers to be examined by scanning electron microscopy were fixed for 1 hr by gradual replacement of the culture medium with 2% glutaraldehyde buffered in 0.1 M PIPES (pH 7.4), washed thoroughly with the same buffer, and postfixed with 1% osmium tetroxide for 1 hr. After a thorough washing with water, the coverslips were dehydrated through a graded series of acetone, critical-point dried, and sputter coated with gold-palladium. Specimens were examined with a JEOL 100 CX scanning transmission electron microscope.

For transmission electron microscopy, cells were fixed in situ with 3% glutaraldehyde in 0.1 M PIPES buffer and postfixed in 1% osmium tetroxide in PIPES buffer. After dehydration through an ethanol series, the cells were flat-embedded in Epon according to the method of Brinkley et al. (8). Following polymerization for 24 hr at 60°, the culture dish was separated from the Epon wafer. Cells in the Epon wafer were examined with a phase microscope, and selected cells were marked.

3 The abbreviations used are: PIPES, piperazine-N,N'-bis(2-ethanesulfonic acid); PBS, phosphate-buffered saline (0.14 M NaCl, 0.003 M KCl, 0.008 M Na2HPO4, 0.002 M KH2PO4, 1.0 mM CaCl, 1.0 mM MgCl2).
bored out of the disc, and glued to the tip of a blank Epon peg. Thin sections of the selected cells were cut on the Porter-Blum MT2B ultramicrotome using a diamond knife. Sections were picked up on collodion-coated, slotted grids and stained with alcoholic uranyl acetate followed by lead citrate. The sections were then examined and photographed on a Siemens 102 electron microscope.

RESULTS

Patterns of Cytoplasmic Microtubules. When the 13 human breast tumor cell lines used in the present study were examined by immunofluorescence using antitubulin, 3 distinct patterns of staining were apparent. In one pattern, the cells were large and flattened, and the cytoplasm was filled with bright fluorescent filaments presumed to be microtubules. As shown in our previous publications (6) as well as in other investigations (44), these filaments are disrupted by microtubule inhibitors such as Colcemid and colchicine. Osborn and Weber (33) have shown recently that each fluorescent filament corresponds to a single microtubule in the electron microscope. In previous publications, we have described cells with extensive arrays of cytoplasmic microtubules as the full complex or FC phenotype (6). Recently, DeBrabander et al. (13) referred to this pattern as type I immunofluorescence. For comparative purposes, we will also refer to cells with the full cytoplasmic microtubule complex as type I.

A second pattern of fluorescence seen in these cell populations was one in which fewer distinct filaments were observed in the cytoplasm. For the most part, these cells were more rounded than were those of type I and displayed a diffuse fluorescent staining pattern. Occasionally, fine fluorescent filaments were observed near the flattened cell surface. It is possible that this cell type contains just as many cytoplasmic microtubules as does the type I cell but, because the cell is more rounded near the center, it was impossible to resolve individual microtubules in the cytoplasm by indirect immunofluorescence. We have termed this pattern type II intermediate.

The third phenotype observed in human breast cancer cells was a small, very rounded cell with no visible cytoplasmic microtubule. In these cells, the pattern of fluorescence is totally diffuse. If polymerized microtubules are present at all in the cytoplasm, they were extremely short and tightly packed in such a way as to produce a nondistinct fluorescence. We termed this pattern type II diffuse.

Type I tubulin immunofluorescence was the predominant staining pattern seen in normal cells and in cell lines 231, 415, and 436. In these lines, 80 to 99% of the cells in the population displayed an extensive cytoplasmic microtubule complex (Figs. 1 to 5). Other lines such as lines 330 and 331 displayed mixed phenotypes consisting of both type I and type II cells in approximately equal numbers. In type I cells, the microtubules extended from 1 to 2 central foci (centrospheres) near the cell nucleus out to the plasma membrane where they either terminated or were bent and extended back into the cytoplasm or parallel with the cell surface (Figs. 4 and 5).

Examination of ultrathin sections with the transmission electron microscope indicated that the type I cells contained numerous long microtubules along with many microfilament bundles (Figs. 16 and 18). Although we did not attempt to quantitate the density of microtubules and microfilaments in type I cells, almost every section contained numerous tubules and filaments.

The cytoplasm of type I cells displayed many mitochondria and an extensive rough endoplasmic reticulum. Both membrane-bound and free ribosomes were abundant, and many intermediate (10-nm) filaments were present throughout the cytoplasm. Although the cells were more flattened than were type II cells, they displayed many characteristics of epithelial cells with numerous desmosomes connecting adjacent cells (Fig. 1A).

When type I cultures were examined by scanning electron microscopy, they appeared to be composed largely of flattened disc-shaped cells in extensive contact with the substratum. The cell surface displayed numerous rounded blebs and short filopodia (Fig. 6).

Cell lines 134, 157, 416, 435, 461, and 468 generally displayed the type II pattern of immunofluorescence when stained with tubulin antibody. For the most part, these cells were smaller and more rounded than were type I cells (Figs. 7 to 9). As mentioned previously, some lines contained cells with both type I and type II phenotypes in the same population. Those lines with type II fluorescence could be further classified into subclasses identified as intermediate and diffuse depending upon cell shape and expression of the cytoplasmic microtubule complex. Cell lines 435, 453, and 461 displayed predominantly intermediate phenotypes due to their slightly flattened shape (Fig. 10). These cells contained numerous cytoplasmic microtubules, but they appeared shorter and were clearly resolved only at the cell margins. Mostly diffuse staining was apparent in the cell centers. The type II diffuse staining pattern was seen in the small rounded cells or cells which do not form colonies. These included cell lines 134, 157, 416, and 468.

When type II cells were examined by transmission electron microscopy, very few microtubules or microfilament bundles were seen in most cells (Fig. 17). The small rounded cells displayed large nuclei with prominent nucleoli. The cytoplasmic volume was small. Microtubules were confined largely to the centrosome region and were occasionally observed near the cell margins. A few cells, probably of the type II intermediate category, displayed abundant microtubule profiles near the plasma membrane, where they were interspersed with dense submembranous bundles of 6-nm filaments (Fig. 19). Confluent cultures displayed tightly adhering cells with distinct desmosomes (Fig. 17) indicating their epithelial characteristics. Intermediate filaments 10 nm in diameter were abundant in many cells and were especially dense in the centrosome region.

Examination of type II cultures with the scanning electron microscope indicated that most of the cells were either very rounded or slightly disc shaped. The surfaces of type II cells displayed numerous short microvilli (Fig. 11).

Actin Patterns. Actin immunofluorescence was carried out on 6 of the 13 cell lines. As shown in Figs. 12 and 13, some cell lines displayed only diffuse fluorescence when stained with actin antibody, while others exhibited one or more bright fluorescent "cables" or "stress fibers" (Figs. 14 and 15). For convenience of scoring, those cells which displayed one or more actin bundles extending along the length or breadth of the cell or along the cell margin were scored as "positive" for actin, while those which displayed only diffuse fluorescence were identified as "negative." As indicated in Table 1, cell
lines 330 and 331 were actin positive, with most of the cells displaying numerous brightly fluorescent cables. Cell lines displaying type I tubulin fluorescence were not always actin positive (i.e., lines 431 and 436). Thus, a direct correlation between the expression of cytoplasmic microtubules and actin cables was not possible in all breast tumor cells.

Actin-negative cells, like cells with type II tubulin immunofluorescence, were generally more rounded (Figs. 12 and 13) and grew in tightly packed colonies or multilayered foci in the culture dish as shown in the scanning electron micrograph in Fig. 11.

Examination of actin-positive cells in the transmission electron microscope revealed the presence of microfilament bundles in the cytoplasm and dense accumulations of microfilaments along the cell periphery (Figs. 16 and 19). Actin-negative cells displayed few, if any, microfilament bundles, although randomly dispersed 6-nm microfilaments could be seen in the cytoplasm of many cells.

**DISCUSSION**

Can the appearance of the cytoskeletal elements including the state of polymerization and organization of cytoplasmic microtubules and actin-containing microfilaments indicate malignant transformation or metastatic potential of cells in vitro or in vivo? An affirmative answer to this question could have far-reaching implications in the areas of carcinogenesis screening, improved clinical diagnosis, and improved chemotherapy. In addition, a knowledge of the direct or indirect involvement of the cytoskeleton in the expression of tumor cell properties might contribute to a better understanding of the molecular basis of malignancy.

Tumor cells in vitro display a broad spectrum of morphological and growth-related properties which appear to relate directly or indirectly to cancer. Indeed, many tumor cell characteristics including alterations in cell motility, shape, spreading and adhesive properties, loss of density-dependent growth control, changes in cell surface properties, and loss of anchorage dependence could relate directly or indirectly to the cytoskeleton.

The present study suggests that human breast carcinoma cells in vitro may display a variety of morphological forms and a wide range of cytoskeletal expressions. Since all of the cell lines were derived from patients with metastatic tumors and some were found to produce tumors in nude mice after a period of in vitro growth, we assume that they were tumorigenic in vitro. Thus, the expression of organized arrays of microtubules and microfilament bundles or their absence is an unreliable phenotypic marker for malignancy in these cell types. These findings do not suggest, however, that cytoskeletal alterations reported in some transformed cells in vitro are insignificant. Transformed fibroblasts often display diffuse actin immunofluorescence and diminished microtubule networks which may be correlated with other tumor cell properties in vitro (29). Indeed, carcinoma cells in vitro often fail to display many of the properties that are characteristic of transformed fibroblast cells in vitro (2, 12, 36, 45). Medina et al. (28) were unable to find markers which would distinguish between cells in culture derived from mouse primary adenocarcinoma and cells established from normal mammary gland. Asch et al. (3), in an extensive study of the cytoskeletons of short-term cultures of normal, preneoplastic, and neoplastic mouse mammary epithelial cells, found no major alterations in the cytoplasmic microtubules and microfilaments in the neoplastic states. The mouse mammary cells, however, were indistinguishable on the basis of cell morphology. In the present study, human breast cancer cells in vitro displayed a broad range of shapes from small and rounded to flattened and epithelial-like. In every case, cells which were more rounded, clustered, and loosely attached to the culture dish displayed diffuse immunofluorescence and a disorganized cytoskeleton when examined by transmission electron microscopy. Cultures which displayed more flattened cells also contained more highly organized cytoskeletons. Thus, in human mammary carcinoma cells, expression of cytoskeleton appears to correlate more closely with cell shape than with any other parameter of in vitro growth. A similar conclusion was reached by Willingham et al. (48) in studies of a mutant AD6 cell line. These cells have the characteristic rounded appearance seen in many transformed cells, lack microfilament bundles, but are nontumorigenic. Thus, loss of cytoskeletal organization in these cells appears to relate more to cell shape and attachment to substratum than to growth control.

In summary, the findings reported herein suggest that the

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**Table 1**

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<th>Cell line</th>
<th>Race</th>
<th>Passage</th>
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<th>Microtubules (%)</th>
<th>Actin cables (%)</th>
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*Type I, cells with a full cytoplasmic microtubule complex; type II, cells with apparently fewer distinct microtubules including both intermediate and diffuse patterns of immunofluorescence.

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gross organization of cytoskeletal elements in human breast carcinoma cells in vitro may be highly variable. Since all of the cell lines were derived from malignant metastatic lesions, we conclude that cytoskeletal organization of epithelial-derived cells in vitro as detected by immunofluorescent staining and electron microscopy relate more to cell shape and attachment to substratum than to malignancy or metastatic potential. It should be pointed out, however, that these observations were made on cells in vitro after various passages in culture. We have very little information on the cytoskeleton of mammary tumor cells in vivo. Moreover, our observations are qualitative and may have little bearing upon more subtle quantitative changes which occur at the molecular level of cytoskeletal assembly and organization.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Epithelial cells from culture of normal human mammary tissue. Note extensive cytoplasmic microtubule complex.

Fig. 2. Two well-spread epithelial cells from normal human breast. Note individual microtubules which terminate near the plasma membrane.
Fig. 3. Tumor cell showing type I tubulin immunofluorescence. Note surrounding cells with type II immunofluorescence. Cell line 231.

Fig. 4. Numerous cells showing extensive arrays of cytoplasmic microtubules from cell line 330.

Fig. 5. Well-spread cell from line 231 showing microtubules which extend out to the cell surface where they either terminate or bend and extend back into the cytoplasm.

Fig. 6. Scanning electron micrograph of flattened epithelial-like cell from cell line 330. The shape and surface microvilli are typical of type I cells. × 3,000.
Fig. 7. Small rounded cells with type II diffuse tubulin immunofluorescence. Cell line 453.
Fig. 8. Type II cells showing large nuclei and narrow band of cytoplasm with diffuse tubulin fluorescence. Cell line 453.
Fig. 9. Multilayered colony of type II cells showing diffuse cytoplasmic fluorescence. Cell line 416.
Fig. 10. Type II intermediate cell showing diffuse staining near the cell nucleus and a network of microtubules at the cell periphery. Cell line 415.
Fig. 11. Scanning electron micrograph of type II cells much like those in Fig. 7. Note multilayered cells with numerous long microvilli. Cell line 331. × 3,000.
Fig. 14. A few actin cables are seen in these cells near the periphery characteristic of type II diffuse cells. Cell line 431.

Fig. 15. Numerous short actin cables are seen in these type I cells. Cell line 157.

Fig. 16. Transmission electron micrograph of type I cell from cell line 231. Note numerous microtubule (M) profiles in the cytoplasm. Dense bundles of actin microfilaments are seen near the cell surface. × 6,600.

Fig. 17. Cytoplasm of type II cells. Very few microtubules profiled are seen in these cell types. Cell line 157. × 10,000.

Fig. 18. Higher magnification showing microtubules (M) and 6-nm microfilaments of type I cells. Cell line 231. × 26,000. A, desmosome typical of epithelial cells. × 13,000.

Fig. 19. Bundles of 6-nm microfilaments (MF) and several microtubule profiles are seen near the cell surface of these type II intermediate cells. Cell line 416. × 8,300.

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