Tumor Types Derived from Epithelial and Myoepithelial Cell Lines of R3230AC Rat Mammary Carcinoma

Anna Sapino, Mauro Papotti, Brunella Sanfilippo, Patrizia Gugliotta, and Gianni Bussola

ABSTRACT

Epithelial and myoepithelial cell lines were derived from the R3230AC mammary carcinoma in Fischer rats. They were obtained by transplanting the tumor into the dorsal region of female Fischer 344 inbred rats. Approximately 1 mm³ of tumor (or 1-3 x 10⁶ neoplastic cells) was used for serial s.c. transplantations. The tumor consistently grew to a 2-cm mass within 20 days. Animals were killed by decapitation every 25-35 days; tumor tissue was in part inoculated in recipient rats as described above, in part fixed in absolute ethanol or methacarn for histology and immunohistochemistry (antibodies listed in Table 1), and in part processed for cell culture lines (see below).

In order to evaluate the response to estrogen stimulation, tumor-bearing animals were given for 1 month weekly s.c. injections of estradiol valerate (20 mg/kg body weight) according to the method of Hilf (14).

Establishment of Primary Stabilized and Cloned Cultures: The tumor tissue, removed aseptically, was minced into small pieces (~1 mm³) with scalpels and digested with 125 units/ml collagenase (type II) (Worthington Biochemical Corp., Freehold, NJ) in RPMI (GIBCO-BRL, Grand Island, NY), 10% FCS, 200 units/ml penicillin, 200 μg/ml streptomycin (EUROBIOS), and 2.5 mg/ml fungizone (GIBCO) for 2 h at 37°C.

Single cell suspensions and several small tumor fragments were washed twice in RPMI supplemented with 10% FCS without collagenase and plated in T25 flasks (Falcon Plastics, Los Angeles, CA) at a concentration of about 3 x 10⁵ cells/flask.

Incubation of the flasks for 24 h at 37°C in a 5% CO₂ humidified atmosphere allowed most of the fragments and free cells to attach. In order to obtain an epithelial cell line (EPI) from the primary culture, elongated cells were removed following trypsin-EDTA treatment (15) with a minor modification proposed by Kuzumaki et al. (16). Briefly, the mixed cell sheet was rinsed once with Ca²⁺- and Mg²⁺-free PBS and incubated for 3 min at 37°C in the presence of fresh trypsin-EDTA. The less rapidly detaching cells, mostly islands of epithelium-like cells, were rinsed and supplied with fresh medium. This procedure was repeated every 3 days until epithelial-like cells covered more than 90% of the culture surface.

The stabilized EPI cells are presently at the 100th passage. Subcultures were routinely carried out in RPMI, 10% FCS, and antibiotic.

Cell Cloning: The mixed population obtained from primary cultures of R3230AC tumor as well as the stabilized (EPI) epithelial cells were cloned following the dilution plating technique proposed by Sato et al. (17). A single cell suspension was appropriately diluted and 96-well microtest plates (Falcon) were seeded; each well was inoculated with only one cell as ascertained by microscope inspection were considered available for clone establishment; the others were discarded.

Conditioned media were prepared by adding to the routine medium 10% of the supernatant obtained from confluent cultures of epithelial cells filtered through 0.22-μm Millipore filters and stored at 4°C before use. We obtained 3 clones (I, H, L) from the mixed population of primary culture and 4 clones (A, C, D, E) from the EPI cell line.

The cell lines, at early passages, were reinfected into syngeneic animals and subcultured until passage 80.

1 The abbreviations used are: FCS, fetal calf serum; PBS, phosphate-buffered saline; NTEN, 50 mM Tris, pH 7.4-0.15 mM NaCl-2 mM EDTA-0.1% Nonidet P-40; sm, smooth muscle.
In Vitro Hormonal Stimulation. Primary cultures, stabilized EPI cells, and clones D and E were cultured in Petri dishes and on glass coverslips for 9 days in a basal medium containing 10^{-4} M 17β-estradiol (Merck, Darmstadt, Germany) and 10% of charcoal-adsorbed FCS (18).

The medium was changed every 3 days.

α-Lactalbumin production was estimated immunocytochemically.

Light Microscopy. The morphological appearance of living cultures in flasks was observed on a Leitz Labovert inverted microscope fitted with phase contrast. Mixed cells from primary cultures, EPI cells, and clones were also grown on glass coverslips; fixed in methanol; and stained by the Papanicolaou method for cytological examination.

For immunohistochemical tests, cells were grown on glass coverslips, fixed in methanol for 5 min at −20°C, permeabilized in acetone for 5 s at −20°C, and rehydrated with pig normal serum (diluted 1:50 in PBS for 20 min). Cells were incubated at 37°C for 60 min with the primary antibodies listed in Table 1 and then with the appropriate fluorochrome-labeled secondary antisera (Sera-Lab, Ltd., Sussex, England) diluted 1:10 in PBS for 30 min at room temperature, following a standard indirect immunofluorescence procedure.

Electron Microscopy. EPI cells and cells from clone I were fixed in a solution of 1−2% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.3) and 2% aqueous osmium tetroxide for 1 h at 4°C, dehydrated through a solution of 1−2% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.3) and 2% aqueous osmium tetroxide for 1 h at 4°C, dehydrated through graded alcohols, cleared in propylene oxide, and embedded in Epon-Araldite. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 1 or a Philips EM 401 electron microscope.

Cell Extracts. The established cell line EPI and the clones (C, D, H, I, L) were homogenized in a buffer 20 mM Tris (pH 7.4), 0.1 M NaCl, 5 mM MgCl{2}, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1 mM 2-mercaptoethanol, and 2000 IU/ml Trasylol. Extracts were stored at −80°C until used. Protein concentration was determined by the method of Lowry as modified by Markwell et al. (19).

Immunoblotting. Total proteins (100 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 8% acrylamide gels and transferred to nitrocellulose filters in a Ployblot apparatus (ABN, Emeryville, CA) according to the manufacturer’s instructions.

Nitrocellulose was then saturated in 3% bovine serum albumin in NTEN for 3 h at 37°C, incubated in the same buffer containing the anticytokeratin 19 monoclonal antibody 1165 (Amersham International, England) diluted 1/100 for an α-sm-actin monoclonal antibody diluted 1:800 (Sigma Chemical Co., St. Louis, MO) for 16−18 h at 4°C, washed twice for 10 min each in NTEN, incubated with the secondary rabbit anti-mouse antibody (Dako) diluted 1:500 for 3 h at 4°C, washed twice for 10 min each in NTEN, incubated with [125I]protein A (0.1 μCi/ml) (Amersham), and then washed as before, air dried, and exposed for 2 days at −80°C to Hyperfilm MP autoradiographic films (Amersham).

Transplantation of Cell Lines into Animals. Cultured cells (approximately 0.8−4 × 10⁶) were injected s.c. into female Fischer rats. The majority (80%) of the tumor stained positively for α-sm-actin in the elongated basal cells, thus confirming their myoepithelial nature (Fig. 1a).

Epithelial secretory cells were stained with different antibodies to keratin. AE1, a monoclonal recognizing acidic cytokeratins, was positive only in single squamous cells while AE3, a monoclonal to basic cytokeratins, recognized a few epithelial cells and the majority of basal myoepithelial cells. Cytokeratin 8 (M, 52,000−55,000) recognized by 1166 monoclonal was found in 70% of epithelial cells (Fig. 1b) while cytokeratin 19 (M, 40,000), marked by 1165 monoclonal, was in less than 10% of epithelial cells. The basal membrane was positively stained by anti-type IV collagen antibodies.

Primary and Stabilized Cell Cultures and Clones. Primary cell cultures, large flat cells with epithelial appearance, were surrounded by thick bundles of fusiform cells, that tended to overgrow the epithelial-like elements.

Cytokeratins were consistently detected in large epithelial-looking cells, while only 10% of elongated cells were positive for the anti-sm-actin monoclonal. The majority (80%) of the cells were vimentin positive. Collagen type IV was present as small dots on the surface of the fusiform cells.

Stabilized EPI cells formed closely packed islands of large cuboidal cells, sometimes surrounded by elongated cells (Fig. 2a). Isolated single cells were fusiform with fibroblast-like appearance (Table 2). All the cells were intensely positive for the 1165 and 1166 monoclonals against cytokeratins. Bundles of intermediate filaments at the periphery of the cytoplasm and in a perinuclear arrangement were stained by the AE3 mono-

Table 1 Reagents used in immunohistochemistry and immunofluorescence

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Reagent</th>
<th>Source</th>
<th>Dilution*</th>
<th>Target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 19 (M, 40,000)</td>
<td>1165</td>
<td>Amersham International, England</td>
<td>1/10−undiluted</td>
<td>Epithelial</td>
</tr>
<tr>
<td>Cytokeratin 8 (M, 52,000−55,000)</td>
<td>1166 (MAB)</td>
<td>Amersham International, England</td>
<td>1/10−undiluted</td>
<td>Myoepithelial</td>
</tr>
<tr>
<td>Cytokeratin 19-16-15-14-10</td>
<td>AE1 (MAB)</td>
<td>Cambridge Research Laboratory, United States of America</td>
<td>1/100−1/10</td>
<td>Epithelial</td>
</tr>
<tr>
<td>Cytokeratin 8-7-6-5-4-3-2-1</td>
<td>AE3 (MAB)</td>
<td>Cambridge Research Laboratory, United States of America</td>
<td>1/40−1</td>
<td>Epithelial</td>
</tr>
<tr>
<td>α-Smooth muscle actin</td>
<td>LA4 (MAB)</td>
<td>Sigma</td>
<td>1/800−1/100</td>
<td>Myoepithelial</td>
</tr>
<tr>
<td>Collagen type IV</td>
<td>Antiserum</td>
<td>Heyl, Berlin, Germany</td>
<td>1/350−1/50</td>
<td>Basal membrane</td>
</tr>
<tr>
<td>Vimentin</td>
<td>V9 (MAB)</td>
<td>Dakopatts, Glostrup, Denmark</td>
<td>1/300−1/10</td>
<td>Mesenchymal</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>Anti-serum</td>
<td>Vonderhaar, Bethesda, MD</td>
<td>1/400−1/20</td>
<td>Secretary</td>
</tr>
<tr>
<td>Rat MFGM</td>
<td>Anti-serum</td>
<td>Our laboratory</td>
<td>1/5000−1/50</td>
<td>Secretory</td>
</tr>
</tbody>
</table>

* Data referred to dilutions in immunoperoxidase and immunofluorescence, respectively. MFGM, milk fat globule membrane; MAB, monoclonal antibody.
clonal in 80% of the cells. The AE1 monoclonal was positive in only 40% of the cells.

Clones A, C, D, and E (obtained from EPI cells) were characterized by rounded clusters of cuboidal cells and isolated cells with large cytoplasm (Fig. 2, b and c). Immunohistochemically, these clones showed a pattern similar to the EPI cells (Table 2).

Cells of Clones I, L, and H (all derived from primary cultures of R3230AC tumor) were elongated or star shaped (Fig. 2d). The cells formed an irregular network on the Petri dish. Generally, the cells were proliferating intensely.

Clone L grew as islands of large star-shaped cells. Keratins were moderately detectable with the 1165 monoclonal antibody directed against keratin 19; actin microfilaments were clearly detectable in some elements (Fig. 2e). Production of type IV collagen was shown in Clone H, I, and L cells. Specific immunolocalization was focally revealed inside the cytoplasm and patchy deposits were detectable in the intercellular spaces (Fig. 2f).

The immunocytochemical results were further checked in immunoblot. All clones, as well as the EPI line, when tested by Western blotting for the presence of cytokeratin 19 with the antibody 1165, showed a M, 43,000 band, characteristic of this intermediate filament; only Clone H was always negative for keratin (Fig. 3).

Electron Microscopy. The EPI cells showed typical features of epithelial cells with desmosomes and numerous microvilli; bundles of intermediate filaments were present in the cytoplasm of some cells. On the contrary Clone I cells had an elongated shape with large mitochondria and numerous ribosomes; no desmosomes were observed but tight junctions were occasionally present. Microfilaments were detected, often arranged in bundles close to the cell membrane. Dense bodies along such bundles were observed (Fig. 4).

Growth Patterns of Transplanted Clones. EPI cells injected into Fischer rats generated approximately 2-cm tumors within 30 days. Histologically, polygonal or round cells were arranged in gland-like or trabecular structures, delimited by thin stromal bundles (Fig. 5). No actin-rich cells were observed. Necrosis and foci of squamous metaplasia were present. Cytokeratins and rat milk fat globule membrane were abundant in all tumor cells. Type IV collagen and vimentin were not detectable.

All seven clones were injected into animals and generated tumors in a time range of 20 days to 7 months (Table 3). Animals were killed when tumors grew up to 2 cm or more (largest diameter) and the tumor material was reinjected into other syngeneic rats for one to three passages.

Clone A, D, and E tumors had frank epithelial carcinomatous features with solid nests or gland-like structures haphazardly intermingled with a spindle-shaped cell component. Foci of squamous metaplasia were seen in Clone A and E tumors (Fig. 6). Keratins were found in a variable proportion of carcinomatous cells, while vimentin stained the stromal component consistently. Few cells reacted with rat milk fat globule membrane antiserum. Type IV collagen was present in limited areas of these tumors; cell membranes of single cells were outlined and some neoplastic nests were marked at the basal membrane level. No actin reactivity was observed.

Tumors obtained from Clones H, I, and L had a sarcomatous appearance with spindle-shaped neoplastic cells mixed with liposarcoma-like areas (Fig. 7). A second generation tumor, obtained by previous injection of clone L cells, showed keratin-positive adenocarcinomatous foci while in a tumor originated from Clone H, areas of chondromatous metaplasia were present. Clone I had a peculiar morphology, strongly resembling cystosarcoma phylloides (Fig. 8, a and b). The epithelial component was strongly positive for cytokeratins and was supported by a highly cellular stromal proliferation reactive to vimentin only. A basal layer of myoepithelial cells lined the ducts, as confirmed by positive actin staining (Fig. 8c).

Type IV collagen was positive in myoepithelial cells but not in the basal membrane of the phylloides-like tumor from clone I. Liposarcoma-like tumors were positive for this marker.

In Vitro and In Vivo Hormonal Stimulation. Estrogen treatment of control R3230AC tumor induced typical lactational
Fig. 2. In vitro cultures of the cell lines obtained from R3230AC rat mammary carcinoma. In a, a stabilized cell line (EPI), observed in phase contrast, forms closely packed islands of elongated and cuboidal cells. In b, Clone D cells, observed in phase contrast, grow as small roundish colonies of cuboidal cells. In c, immunofluorescence for cytokeratins (monoclonal 1165) in Clone D cells gives a strong positive reaction. In d, cells of Clone H, examined in phase contrast, present a star-like shape. In e, intense reaction for α-smooth muscle actin displayed by cells of clone L. In f, immunocytochemical detection of type IV collagen in clone H reveals mainly extra- and pericellular deposits. a, b, and d, × 150; c and e, × 240; f, × 480.

Table 2 Morphological and immunochemical features of R3230AC mammary carcinoma cell lines

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Cytokeratins</th>
<th>Coll. IV</th>
<th>Vim.</th>
<th>α-sm-actin</th>
<th>Rat MFGM</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>Roundish + spindle-shaped</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Clone A</td>
<td>Roundish</td>
<td>nt</td>
<td>nt</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Clone C</td>
<td>Roundish</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Clone D</td>
<td>Roundish</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Clone E</td>
<td>Roundish</td>
<td>nt</td>
<td>nt</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Clone H</td>
<td>Star-like</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Clone I</td>
<td>Spindle-shaped</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Clone L</td>
<td>Star-like</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Primary culture</td>
<td>Roundish + spindle-shaped</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

*AE1, AE3, 1165, 1166, cytokeratins (see references); Coll. IV, type IV collagen; Vim., vimentin; α-sm-actin, α-smooth muscle actin monoclonal; rat MFGM, serum against rat milk fat globule membrane antigens; EPI, stabilized cell line; nt, not tested.

changes and α-lactalbumin production, demonstrated by immunocytochemistry and immunoblotting. In contrast, estrogen stimulation did not affect the morphology of any of the cultured cell lines, and production of α-lactalbumin was never detectable by immunocytochemical procedures.

In vivo estrogen stimulation, started as soon as a nodule was palpable, did not induce milk protein production or change the histological pattern.

DISCUSSION

We recently observed that the architectural organization of the R3230AC tumor parallels that of the lactating gland since, in addition to the luminal epithelial (secretory) cells, myoepithelial cells are present (13). The neoplastic myoepithelium shows: (a) typical peripheral arrangement against the basement membrane; (b) typical ultrastructural cytoplasmic organization; and (c) the presence of immunocytochemically detectable α-smooth muscle actin. This isoform of actin is present, within the epithelial domain, only in myoepithelial cells of the breast and salivary glands (22, 23).

Similar biphasic patterns involving both myoepithelial and epithelial neoplastic components have been described in dimethylbenz[a]anthracene-induced mammary tumors in mice (24). Two alternative hypotheses can be advanced to explain the observed phenomena: myoepithelial and epithelial cell components represent differentiated terminal stages stemming from...
Fig. 3. Sodium dodecyl sulfate-gel electrophoresis of protein extract of the clone cell lines obtained from R3230AC rat mammary adenocarcinoma. Total extracts, run on 12% polyacrylamide gel, were then transferred on nitrocellulose paper and reacted with anti-cytokeratin 1165 monoclonal antibody as described in "Materials and Methods." A strong positive reaction is observed at M, 43,000 (43 Kd) in Clones C and D and EPI, while Clones I and L give a weak positivity and Clone H is negative.

a common uncommitted precursor; or they constitute interactive but independent neoplastic populations. To test these hypotheses, we obtained separate clones of the epithelial and the myoepithelial cells.

Primary and stabilized cell populations as well as seven clones were derived from the R3230AC carcinoma; they presented either epithelial or myoepithelial-like features. Transplantation s.c. gave rise to carcinomas, to sarcomas, or to mixed (carcinosarcomatous) neoplasms (Table 3).

Epithelial cell lines (EPI, A, D, E) generated in vivo carcinomas lacking both the myoepithelial component and the hormone responsiveness of the parent tumor; α-lactalbumin production was not stimulated by estrogen administration. A possible interpretation of these findings is that the complex structural organization of the primary tumor is necessary to complete functional differentiation. Moreover, a number of investigators have shown that the matrix on which the mammary epithelial cells attach in vitro modulates their response to hormones (25-29).

Myoepithelial-like cells (lines H, I, and L) were elongated; all cells were producing collagen type IV, which is selectively produced in the mammary gland by myoepithelial cells (30-32). In addition, tight junctions and subplasmalemmal bundles of microfilaments with interposed dense bodies (characteristic features of myoepithelial cells) (33) were observed in Clone I and α-sm-actin was detected in Clone L. The presence of this isoform of actin in cultured cells is not per se proof of this myoepithelial nature, since it was recently demonstrated in mammary stromal cells evolving into myofibroblasts (34). However, in line with the myoepithelial nature of the cloned cells, we detected cytokeratin of basal type by both immunofluorescence and immunoblotting procedures in Clone I cells. All cell lines contained vimentin, but in cultured cells this latter finding is not proof of nonepithelial nature (35) and coexpression of both keratin and vimentin intermediate filaments has been reported in numerous epithelial tumors (36).

Tumors generated from cell lines with myoepithelial features showed mainly sarcomatous patterns. Vimentin and collagen type IV were demonstrated in these tumors; the latter finding seems at variance with the behavior of human liposarcomas which do not produce collagen type IV (37). Focal keratin positivity and chondroid metaplasia were found in single tumors; of major interest was, however, the peculiar association

Fig. 4. Electron microscopic study of Clone I. In A, the cells show elongated shape and lack desmosomes; the nuclei are irregular in outline. At higher magnification (B) a selected area (arrows, A) shows subplasmalemmal bundles of microfilaments (arrowheads), with interposed dense bodies. A, × 2,000; B, × 16,000.
Table 3 Morphological and immunochemical features of in vivo growth of R3230AC cell lines

<table>
<thead>
<tr>
<th>Main component</th>
<th>Cytokeratins</th>
<th>Coll. IV*</th>
<th>Vim.</th>
<th>α-sm-actin</th>
<th>Rat MFGM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AE1</td>
<td>AE3</td>
<td>Coll 1165-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>Carcinomatous</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Clone A</td>
<td>Carcinomatous</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>nt</td>
</tr>
<tr>
<td>Clone C</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone D</td>
<td>Carcinomatous (adenocarcinoma)</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Clone E</td>
<td>Carcinomatous (anaplastic)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Clone H</td>
<td>Sarcomatous (lipoblasts, with chondromatous metaplasia)</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Clone I</td>
<td>Sarcomatous (area phylloides-like)</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Clone L</td>
<td>Sarcomatous (lipoblasts, with carcinomatous areas)</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Original R3230AC carcinoma</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>±</td>
<td>++</td>
</tr>
</tbody>
</table>

* Coll. IV, type IV collagen; AE1, AE3, 1165, 1166, cytokeratins (see references); Vim., vimentin; α-sm-actin, α-smooth muscle actin monoclonal; Rat MFGM, serum against rat milk fat globule membrane antigens; EPI, stabilized cell line; *, not grown after injection; nt, not tested.

The literature provides examples of fibrosarcomas, rhabdomyosarcomas, and other types of sarcoma originating in animals transplanted with fragments of spontaneously developed mouse mammary tumors and with long term cultures of mouse mammary cancer cells (16, 40, 41). Peculiarly, mammary tumors seem to be the only ones for which this phenomenon (of a sarcoma originating by serial transplantation or by culture of an epithelial tumor) has been described. This peculiarity of experimental mammary tumors has some relationship with certain well known features described in human breast neoplasms. Areas of sarcomatous (osteosarcoma, chondrosarcoma) metaplasia are known to occur in carcinomas of the human breast (42); a similar phenomenon is seen rather commonly in malignant mammary tumors of female canines, in which chondroid elements are regarded as being of myoepithelial derivation (43). Our studies support the hypothesis that these sarcomatous areas might be related to proliferation of neoplastic myoepithelial cells.

Previously, other authors (44, 45) tried to establish cultures of myoepithelial cells from dimethylbenz(a)anthracene-induced rat mammary tumors and from the mammary gland of a neonatal rat. The line called RAMA 25 showed a peculiar spontaneous transformation in vitro from cuboidal to elongated fusiform cells which have been regarded as the equivalent of the

and provides experimental evidence of derivation of sarcomatous and mixed carcinomasarcomatous tumors of the breast from neoplastic myoepithelial-like cells.

in Clone I tumors of liposarcomatous areas with features of cystosarcoma phylloides, a lesion with mixed epithelial and stromal growth regarded as the malignant counterpart of the more common fibroadenoma of the breast. Liposarcomatous differentiation is rather common in human malignant phylloides tumors (38). Ultrastructural and immunocytochemical evidence of myoepithelial derivation of the stromal sarcomatous component in cases of human cystosarcoma phylloides has been reported in the literature (39). Our study confirms these data...
normal myoepithelial cells (32, 46, 47). Formation of fusiform cells has also been described in cell lines derived from BALB/Cf3H mouse mammary tumors (48) and from nitrosomethylurea-induced mammary tumors in WF rats (49). According to the observations of Dulbecco et al. (49) and Warburton et al. (32) these fibroblast-like cells did not exactly correspond to myoepithelial cells but showed immunocytochemical features common to both stromal cells (presence of vimentin and of Thy-1–1 antigen) and myoepithelial cells (production of type IV collagen). The biphasic behavior of myoepithelial cells in skin and salivary gland tumors, where these cells seem to be able to form a stromal matrix, has been described and discussed by several authors (50, 51). The nature of the myoepithelial cell itself is indefinite: while its content of basic-type keratins suggests an epithelial nature, the presence of vimentin (52) and its contractile ability, testified to by the presence of α-smooth muscle actin (a unique feature among epithelial cells), indicate its affinity to mesenchyme-derived cells. Even the assumption that myoepithelial cells are terminally differentiated cells (53, 54) seems untenable, since we observed (55) that in the developing mouse mammary gland myoepithelial cells can proliferate.

All these data, in line with the reported experimental observation in R3230AC rat tumor and related cell lines, support the hypothesis that mixed epithelial-stromal tumors of the mammary gland can originate from the myoepithelial cells.

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Tumor Types Derived from Epithelial and Myoepithelial Cell Lines of R3230AC Rat Mammary Carcinoma

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