Lack of Production of Myoepithelial Variants by Cloned Epithelial Cell Lines Derived from the TMT-081 Metastasizing Rat Mammary Tumor

Damien J. Dunnington, Untae Kim, Christine M. Hughes, Paul Monaghan, and Philip S. Rudland

ABSTRACT

A series of cell lines was isolated from the metastasizing rat mammary tumor cell strain TMT-081.MS by single-cell cloning. Feeder cells were required for development of single tumor cells into clonal colonies. The rate, pattern, and incidence of metastases following injection of cells into the mammary fat pads of syngeneic rats were relatively similar for the various cell lines, with dissemination to the lungs and axillary and paraaortic lymph nodes. When a representative cell line termed Rama 800 was subcloned, one subline was nontumorigenic, and another gave a lower incidence of lung metastases, but the remainder had similar in vivo properties to the parental Rama 800 cells. The metastatic properties of Rama 800 cells were not affected by passage in vitro through 60 cell generations. No production of myoepithelial-like variants from Rama 800 cells was observed at the ultrastructural level. Antisera to keratin, actin, laminin, and fibronectin, which normally stain myoepithelial cells and basement membrane, failed to stain Rama 800 cells, either in cultures or in tumor sections. Heterogeneous staining of Rama 800 tumor cells with antisera to epithelial cell-specific milk fat globule membrane antigens was seen in tumor sections but not in culture. Abundant microvilli and membrane blebs were observed on the surface of cultured Rama 800 cells, but no lumen formation, desmosomes, or tonofilaments were seen, either in vivo or in vitro. The results suggest that the metastatic epithelial-derived cell lines lack the ability to express features of myoepithelial cells, in contrast to cell lines isolated previously from nonmetastasizing rat mammary tumors.

INTRODUCTION

The majority of deaths from breast cancer are associated with metastases. The organs affected are, in decreasing order of prevalence, lymph nodes, bone, lung, liver, and brain (6). A series of transplantable rat mammary tumor systems has been developed (10), with metastatic properties similar in many respects to those of human breast carcinomas. One such transplantable tumor is the TMT-081 (10) from which an uncloned cell strain designated TMT-081.MS was derived. The cell strain was cloned by picking single cells (1) and transferring them to individual wells of a 96-well tissue culture tray (Grand Island Biological Co., Uxbridge, Middlesex, United Kingdom). Each well contained 100 μl of cloning medium (17) and approximately 10⁴ mitomycin C-inactivated Rama 29 feeder cells (3). Tumor cell colonies arising in the wells were detached by trypsinization and transferred successively through 1.5-cm- and 5-cm-diameter tissue culture vessels in the absence of extra feeder cells and finally plated in 200-ml flasks. During these passages, cells were grown in routine medium (Dulbecco’s modified Eagle’s medium) plus 5% fetal calf serum and 50 ng/ml each of cortisol and insulin. Stocks of cells were frozen in liquid nitrogen at this stage. One representative cell line was designated Rama 800 and was itself subcloned in a similar manner.

MATERIALS AND METHODS

Animals. Female Ludwig-Wistar WF rats were obtained from Olac Ltd., Banbury, Oxfordshire, England, and maintained on Expanded Rat and Mouse Diet No. 1 (British Petroleum Ltd., Witham, Essex, United Kingdom) and tap water ad libitum.

Tissue Culture. The derivation of an uncloned cell strain designated TMT-081.MS from the TMT-081 transplantable metastasizing rat mammary tumor (10) has been described elsewhere (5). The cell strain was cloned by picking single cells (1) and transferring them to individual wells of a 96-well tissue culture tray (Grand Island Biological Co., Uxbridge, Middlesex, United Kingdom). Each well contained 100 μl of cloning medium (17) and approximately 10⁴ mitomycin C-inactivated Rama 29 feeder cells (3). Tumor cell colonies arising in the wells were detached by trypsinization and transferred successively through 1.5-cm- and 5-cm-diameter tissue culture vessels in the absence of extra feeder cells and finally plated in 200-ml flasks. During these passages, cells were grown in routine medium (Dulbecco’s modified Eagle’s medium) plus 5% fetal calf serum and 50 ng/ml each of cortisol and insulin. Stocks of cells were frozen in liquid nitrogen at this stage. One representative cell line was designated Rama 800 and was itself subcloned in a similar manner.

Serological Characterization of Cells. Rabbit antisera to MFGM, laminin, keratin, and fibronectin were prepared and characterized as described previously (21). The reactivity of these antisera with histological sections (21) of normal rat mammary glands, rat mammary tumors (4), and cell lines derived from nonmetastasizing tumors (3) have been described elsewhere. Rabbit antiserum was obtained from Miles Ltd., Slough, Buckinghamshire, United Kingdom. This antiserum stains myoepithelial cells in tissue sections of normal rat mammary glands and sections of collagen gel cultures of myoepithelial-like cells derived from 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors. Rama 800 cells at passage 6 were embedded in collagen gels as

Received March 14, 1984; accepted July 20, 1984.

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Mammary Tumor Cell Differentiation and Metastasis

detailed elsewhere (22) because they do not adhere to the gel surface. Each gel culture contained 5 ml of routine medium, and the cells were allowed to grow within the gel matrix for 4 days, after which they were fixed overnight in methacarn (16), and processed for histology (21). Histological sections, either of tumors or collagen gels, were stained with antisera to MFGM, laminin, fibronectin, keratin, or actin using the immunocytochemical method described previously (21). The sections were dewaxed, treated with 1.5% acetic acid to block endogenous alkaline phosphatase, washed with PBS, and incubated for 1 hr with antiserum to the marker of interest, usually diluted 1:100 in PBS containing 0.5% bovine serum albumin. Treated sections were then washed 3 times in PBS containing 0.01% Tween 80 and incubated for 1 hr with 100 μl of the relevant antigen per ml of a solution of antibody diluted 1:100 in PBS containing 0.5% w/v bovine serum albumin.

The specificity of the staining for each antiserum was checked by the following controls: (a) sections from normal mammary glands from 80-day-old female rats were stained in parallel with the immunocytochemical reagents and compared with previous results for consistency of staining (21); (b) complete abolition of staining was achieved by prior incubation of each antiserum with the requisite antigen; (c) tumor cells that failed to stain were only recorded as negative providing that a normal rat cellular structure could be identified as staining on the same section (e.g., blood vessels for myosin or laminin antisera, myoepithelial cells in sebaceous glands for keratin antisera); and (d) finally, 5 fields from 2 sections of each lesion for each antibody were examined by 2 independent observers, and the average result was recorded.

In Vivo Studies. The tumorigenic and metastatic properties of the TMT-081-MS cell strains and derived cell lines were examined by injection of cells into the right posterior abdominal mammary fat pads of syngeneic recipients. Cells were harvested from 500-ml tissue culture flasks by trypsinization of attached cells and centrifugation (200 x g for 5 min) of suspended cells, pooled, and washed once in PBS. The washed cells were suspended at 10⁷ cells/ml in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-buffered Dulbecco's modified Eagle's medium (pH 7.4), and 0.2 ml of cell suspension (>90% viability as assessed by trypan blue exclusion, was injected directly into the mammary fat pads of syngeneic rats as described previously (3). Tumor-bearing animals were left as long as possible before autopsy, to maximize the degree of tumor dissemination. The tumors frequently became necrotic, and the resultant ulceration and subsequent risk of death of the animals through infection placed an upper limit of approximately 160 days on the posttreatment observation period. Non-tumor-bearing animals were also killed at this stage. Samples of the primary tumor, the lungs, the axillary lymph nodes, and any other lesions of abnormal appearance were fixed in methacarn and processed for histology.

A cell strain designated TMT-081.P2 was obtained by culturing tumor cells from a lymph node metastasis of a TMT-081-MS-induced tumor. The lymph node was excised, chopped with scalpels blades, homogenized with a Teflon pestle, and digested with collagenase (2 mg/ml; Sigma Chemicals, Ltd., Poole, Dorset, United Kingdom) in routine medium at 37° for 2 hr. The digest was allowed to sediment at 1 x g for 1 min, and the supernatant centrifuged at 200 x g for 5 min. After removal of collagenase by washing twice in routine medium, the cells were resuspended in routine medium and plated in 5-cm-diameter tissue culture vessels. The uncloned cells were subsequently grown in routine medium and passed and frozen as described above.

Electron Microscopy. Collagen gel cultures of Rama 800 cells and fragments (8 cu mm) of Rama 800-induced tumors were processed for transmission electron microscopy as described elsewhere (14). For scanning electron microscopy, cells were grown on glass cover slips and fixed in 2% glutaraldehyde in PBS for 3 hr, and in 1% osmium tetroxide for 18 hr. The cells were washed with 2 changes of PBS at 4° and dehydrated for 5 min in each of a series of aqueous acetone solutions in the following order of concentrations (v/v): 6%; 10%; 30%; 40%; 70%; 80%; 90%; and finally in 2 changes of anhydrous acetone. The cells were critical point-dried in a Polaron (Watford, Herts, United Kingdom) critical-point drying apparatus, sputter coated with gold in a Emscope (Ashford, Kent, United Kingdom) sputter coater, and examined in a Phillips 501 Scanning Electron Microscope with an accelerating voltage of 15 kV.

RESULTS

Isolation and Morphology of Rama 800 Cell Lines. No colonies were observed in any of 24 wells seeded with single TMT-081-MS cells in the absence of Rama 29 feeders. Of a further series of 24 wells containing approximately 10⁴ feeder cells/well, 6 contained viable colonies at 2 weeks after seeding with single TMT-081-MS cells, and these colonies were designated Rama 800 to Rama 805 (Table 1). The Rama 800 cell line was itself single-cell cloned in the presence of feeders, and 8 of 24 wells yielded viable colonies (Table 2). The morphology of each of the Rama 800 cell lines and sublines was similar to that of the parental TMT-081-MS cell strain. Cells attached to the culture surface, but tended to remain rounded (Fig. 1A) and failed to spread on the substratum. Many cells were observed free-floating in suspension and when replated, these "floaters" were

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Metastasis of TMT-081-MS single-cell clones in syngeneic rats</th>
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<tbody>
<tr>
<td>Cell line</td>
<td>Tumor incidence</td>
</tr>
<tr>
<td>Rama 800</td>
<td>18/18</td>
</tr>
<tr>
<td>Rama 801</td>
<td>4/6</td>
</tr>
<tr>
<td>Rama 802</td>
<td>5/6</td>
</tr>
<tr>
<td>Rama 803</td>
<td>5/6</td>
</tr>
<tr>
<td>Rama 804</td>
<td>1/6</td>
</tr>
<tr>
<td>Rama 805</td>
<td>6/6</td>
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<td>Rama 806</td>
<td>6/6</td>
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</table>

Cells (2 x 10⁶) at passage 4 were injected into the right posterior abdominal mammary fat pads of Ludwig-Wistar rats.

The incidence of metastases in the various treatment groups did not differ significantly (χ² test; p > 0.05) from those of the Rama 800 passage 4 group.

axILN, axillary lymph node; palLN, paraaortic lymph node.

Numbers in parentheses, range.

One rat cannibalized.

All rats were killed at this time.

Injected at in vitro passage 24.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Metastasis of Rama 800 subclones in syngeneic rats</th>
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<tbody>
<tr>
<td>Subclone</td>
<td>Tumor incidence</td>
</tr>
<tr>
<td>Rama 800-B3</td>
<td>6/6</td>
</tr>
<tr>
<td>Rama 800-C2</td>
<td>6/6</td>
</tr>
<tr>
<td>Rama 800-B5</td>
<td>6/6</td>
</tr>
<tr>
<td>Rama 800-A6</td>
<td>6/6</td>
</tr>
<tr>
<td>Rama 800-B6</td>
<td>6/6</td>
</tr>
<tr>
<td>Rama 800-C1</td>
<td>6/6</td>
</tr>
<tr>
<td>Rama 800-A3</td>
<td>6/6</td>
</tr>
<tr>
<td>Rama 800-A2</td>
<td>0/6</td>
</tr>
</tbody>
</table>

Cells (2 x 10⁶) at passage 4 were injected into the right posterior abdominal mammary fat pads of Ludwig-Wistar rats.

axILN, axillary lymph node; palLN, paraaortic lymph node.

All rats were killed at this time.

Significantly lower (χ² test; 0.05 > p > 0.01) than the corresponding value for Rama 800 passage 4 cells.

Numbers in parentheses, range.
of similar appearance to the original cultures with some cells attaching to the substratum and others remaining in suspension. It was not possible to select cells showing enhanced adherence to the substratum by repeatedly washing away the floating cells, nor to select nonadherent cells by replating floaters. Production of stable elongated cells was not observed in cultures of uncloned TMT-081.MS cells, and no elongated cell clones were obtained from them, in contrast to the Rama 25 and Rama 37 cuboidal cells described previously (1, 3). However, some of the attached cells in all the cloned cell lines and the uncloned cell strain assumed a more elongated morphology, with adhesion to the substratum occurring at the apical points of the cell (Fig. 1B). At these adhesion points, spreading of the cells' membranes occurred, giving rise to structures that resembled lamellipodia (18). Abundant microvilli were present on the cell surfaces, and extensive blebbing of the membranes was observed (Fig. 1, A and B). Occasionally, these blebs detached from the cells and were observed floating in the medium.

**Behavior of Cell Lines In Vivo.** The uncloned TMT-081.MS cell strain induced palpable tumors in over 80% of treated rats at approximately 42 days after injection (Table 3). Two tumorbearing rats killed at 70 days after injection were devoid of gross or microscopic metastases, but at approximately 110 days, the remainder exhibited lymph node and nodular lung metastases (Table 3). No attempt was made to count the lung nodules in these experiments, because the presence of micrometastases and metastatic deposits internal to the lung tissue, fusion of adjacent nodules, and the possibility of heterogeneity in the growth rates of disseminated cells into overt nodules all contribute to the unreliability of such estimates. Furthermore, some animals exhibited axillary lymph node metastases in the absence of any gross or microscopic lung nodules. In most animals with lung involvement, multiple small (2- to 4-mm) nodules were observed. When one TMT-081.MS local tumor was passaged by injection of a homogenate of tumor tissue into the mammary fat pads of syngeneic recipients, tumors were detected in all the treated animals. Lung and lymph node metastases were detected in most of the tumor-bearing rats at approximately 55 days, roughly one-half the time required for metastasis of the original cell strain (Table 3).

Rats given injections of 2 x 10⁶ uncloned TMT-081.P2 cells at the third in vitro passage also developed tumors (Table 3). These tumors became palpable at approximately 30 days, and by 63 days, most animals were found to have gross metastases (Table 3). The pattern of dissemination was similar to that of the TMT-081 cell strain, with involvement of axillary and paraaortic lymph nodes and nodular lung deposits. A similar pattern of metastases was observed in rats bearing tumors induced by injection into the mammary fat pad of a homogenate of an axillary lymph node metastasis from a TMT-081.P2 tumor (TMT-081.P3; Table 3).

Cell lines prepared by single-cell cloning the TMT-081.MS cell strain varied in their ability to induce tumors following injection into syngeneic rats (Table 1). Whereas cell lines such as Rama 800 and Rama 805 induced tumors in 100% of the animals, others such as Rama 804 gave a much lower tumor incidence (Table 1). Nevertheless, Rama 804 cells retained the metastatic properties of the parental cell strain (Table 1). The patterns of metastasis of the tumors induced by the various cell lines were similar to that of the parental TMT-081.MS tumor. No significant differences were seen in the incidences or patterns of metastases between the different clones (Table 1). Sublines of Rama 800 induced tumors in 100% of recipient animals except Rama 800-A2, which was apparently nontumorigenic (Table 2). Metastases were detected in most of the animals bearing tumors induced by the various sublines, and the pattern for each subline was similar to that of the parental Rama 800 cells (Table 1). Significant differences between early- and late-passage Rama 800 cells were observed in the rate or pattern of metastasis, nor in the degree of tissue involvement at autopsy.

### Table 3

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Tumor incidence</th>
<th>Time of autopsy (days)</th>
<th>axLN⁶</th>
<th>paLN⁶</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT-081.MS</td>
<td>7/8</td>
<td>110 (70–121)</td>
<td>4/7</td>
<td>3/7</td>
<td>3/7</td>
</tr>
<tr>
<td>TMT-081.P2⁶</td>
<td>6/6</td>
<td>51 (33–63)</td>
<td>4/6</td>
<td>3/6</td>
<td>2/6</td>
</tr>
<tr>
<td>TMT-081.P3⁷</td>
<td>6/6</td>
<td>74 (59–98)</td>
<td>6/6</td>
<td>5/6</td>
<td>3/6</td>
</tr>
</tbody>
</table>

⁶Tumor cells (2 x 10⁶) were injected into the right posterior abdominal mammary fat pads of Ludwig-Wistar rats.

⁷The incidence of metastases in animals receiving TMT-081.P1, -2, and -3 treatments did not differ significantly (χ² test; p > 0.05) from those of rats given injections of TMT-081.MS cells.

The histological appearance of tumors induced by the various cell lines described above, 2 x 10⁶ Rama 800 cells were injected into the mammary fat pads of syngeneic rats. Tumors were detected in all animals at approximately 100 days after injection, and these tumors metastasized to the lungs and lymph nodes as did the early passage Rama 800 cells (Table 1). Significant differences between early- and late-passage Rama 800 cells were observed in the rate or pattern of metastasis, nor in the degree of tissue involvement at autopsy.

**Histology and Immunocytochemistry of Rama 800 Cells and Tumors.** The histological appearance of tumors induced by the TMT-081.MS cell strain and all cell lines and sublines derived from it were similar, and Rama 800 tumors have been chosen as a representative example. Tumors consisted of tightly compacted cuboidal cells of varying sizes with highly pleomorphic nuclei (Fig. 2A). Occasional multinucleate and giant cells were identified, but there was no evidence of cytoplasmic filaments. Much invasion of surrounding tissue was evident, in some animals to the extent of penetration of the tumor through the body wall. No spindle cell component was observed (3, 4). Lung metastases were nodular (Fig. 2B) and histologically similar to the primary tumor, as were lymph node metastases (not shown).

Sections of Rama 800-induced tumors and of collagen gel cultures of Rama 800 cells were treated with antisera to fibronectin, laminin, actin, MFGM, and keratin using an immunocytochemical procedure. No staining of tumor cells or cells in collagen gel cultures with antisera to laminin, actin, fibronectin, or keratin was observed (e.g., Fig. 2, C and D), but heterogeneous cytoplasmic staining of tumor cells with antisera to MFGM was seen (Fig. 2E). Rama 800 cells in collagen gel cultures failed to stain with anti-MFGM serum (Fig. 2F). Sections of normal rat mammary glands processed in parallel with the collagen gel sections.
stained with all the antisera as described previously (Fig. 3, A to D). The staining of tumor sections with anti-MFGM serum was not due to incorporation of host material because: (a) cells that stained were morphologically identifiable as Rama 800 cells; (b) lymph node metastases showed a similar staining pattern; and (c) primary Rama 800 tumors grown in the interscapular fat pads of syngeneic rats stained in a similar manner.

Ultrastructure of Rama 800 Cells. When growing as tumors, Rama 800 cells were cuboidal with microvilli-like membrane processes, but no tonofilaments, lumen formation, or desmosomes were observed. Irregularly shaped nuclei, abundant endoplasmic reticulum, and cytoplasmic thin filaments were present in most of the cells (Fig. 4A). Similar features were seen in Rama 800 cells embedded in collagen gels, but in regions where the cell membranes were not in contact with other cells, large blebs were present (Fig. 4B). These blebs were devoid of organelles, but there was no evidence of a mesh of microfilaments acting as a filter at the neck of the bleb (18).

DISCUSSION

The present system of metastasizing cell lines differs in a number of respects from that described by Neri et al. (12, 13). Tumors induced by the Rama 800 cell lines grow and metastasize more slowly, although the pattern of metastasis is similar to that of the previous system. When cells prepared from a lymph node metastasis of the uncloned TMT-081.MS strain are injected into recipient animals, the resulting tumors grow and metastasize more rapidly than the parental strain, a finding also noted by Neri et al. (13). The slow rate of growth and metastasis of the Rama 800 tumors are probably due to poor adaptation of the initial TMT-081.MS cell strain to growth in our animals, since both rates can be markedly increased by serial transplantation either of TMT-081.MS tumors or their metastases. The marked stimulation of growth of single TMT-081.MS cells by Rama 29 feeder cells suggests that factors associated with the feeder cells may influence the growth of these tumor cells in vitro. Hewitt et al. (9) showed that addition of irradiated cells to an inoculum of non-irradiated tumor cells decreased the number of viable cells necessary to initiate tumor growth. Although the mechanisms of these feeder effects are not known, it is possible that local environmental factors may influence the growth of Rama 800 cells in vivo.

Although the incidences and patterns of metastasis are relatively uniform among clones derived from the TMT-081.MS cell strain, the Rama 800 cloned cell line does display significant intrinsic heterogeneity. This heterogeneity is evident from the pattern of MFGM antigen expression in Rama 800 tumors and from the findings that Rama 800-A2 cells are nontumorigenic, and Rama 800-B3 cells induce a lower incidence of lung metastases than the parental Rama 800 cells. Generation of such variants during passage of Rama 800 cells in vitro does not appear to affect the overall behavior of the Rama 800 population as shown by the lack of effect of growth in vitro on the tumorigenic and metastatic properties of Rama 800 cells, in contrast to the findings of Neri et al. (13). The proportion of variants generated during 60 cell doublings is presumably too low to significantly alter the tumorigenic and metastatic properties of the predominant Rama 800 cell population. Previous studies have demonstrated stability of the metastatic phenotype during in vivo (20) and in vitro (19) passage; however, these investigators used fibrosarcoma cells which are not necessarily comparable to the mammary epithelial cells used in the present study and to those of Neri et al. (13).

Previous studies using the independently isolated rat mammary tumor cell lines Rama 25 (1) and Rama 37 CL-A3 (3), both of which are tumorigenic but poorly metastatic, showed that these cells have the capacity to convert from a cuboidal epithelial to an elongated, myoepithelial-like morphology. Associated with this conversion are changes in the expression of markers such as laminin, myosin, and actin. These markers are detectable using immunocytochemical methods in rat mammary myoepithelial cells (21) and in elongated cells derived from Rama 25 and Rama 37 CL-A3, but not in their cuboidal epithelial precursors. Cells that stain with antisera to these markers are present in nonmetastasizing rat mammary tumors (4) and benign human breast lesions (7) but are absent in more malignant rat and human mammary tissues. Both the Rama 37 CL-A3 and Rama 800 cell lines were ultimately developed from polycyclic aromatic hydrocarbon-induced mammary tumors in the Wistar-Furth rat strain. However, the present findings show that conversion in vitro to the elongated, myoepithelial-like phenotype does not occur to a detectable extent in Rama 800 cell populations. Occasional Rama 800 cells assume a relatively elongated morphology in culture, but this state appears to be temporary, as judged by the failure of attempts to select or clone elongated variants. The temporary elongation of the cells may be associated with cell locomotion, since lamellipodia-like formations (18) are observed at the cell apices. The lack of myoepithelial-like cells in Rama 800 tumors and collagen gel cultures is apparent from the ultrastructural studies and from the absence of staining of the cells with antisera to myoepithelial-specific markers such as keratin, actin, and laminin. Our findings suggest that the absence of myoepithelial-like cells in metastasizing rat mammary tumors may be a result of the inability of more malignant epithelial cells to undergo phenotypic conversion to the myoepithelial state. The reason for this lack of conversion of the metastatic cells is not known, but it may be associated with the pronounced genetic instability of Rama 800 cells.

The lack of cellular differentiation markers in Rama 800 cells also extends to a number of epithelial features such as lumen formation, desmosomes, and the presence of MFGM antigens, and the absence of myoepithelial antigens, although some tumor cells do stain with anti-MFGM serum and abundant microvilli are seen on the surfaces of the Rama 800 cells. The absence of MFGM-positive cells in collagen gel cultures of Rama 800 cells relative to their tumors may have been due to the comparatively small number of cells examined in the cultures, since the proportion of cells in the tumors staining strongly with anti-MFGM serum is less than 1%. This finding suggests that MFGM-positive cells may arise as variants during growth of the initially nonstaining Rama 800 cells in tumors, as reported for human breast cancers (15).

In conclusion, we have shown that the Rama 800 metastasizing cell line is unable to generate variants with a myoepithelial-like phenotype in contrast to the behavior of nonmetastasizing rat mammary tumor cell lines described previously. This lack of conversion may account for the absence of myoepithelial-like cells and their products such as basement membrane proteins in metastasizing rat (4) and human (7) mammary cancers.

D. Dunnington, manuscript in preparation.
D. J. Dunnington et al.

ACKNOWLEDGMENTS

We thank Dr. M. J. Warburton for supplying the rabbit antisera used in this study and Linda Lovell for animal care.

REFERENCES

Fig. 1. Scanning electron micrographs. A, rounded Rama 800 cells showing surface microvilli and membrane blebs; B, elongated Rama 800 cells showing blebs and apical lamellipodia-like formations (arrows). A, × 2,040; B, × 2,080.
Fig. 2. A, Rama 800-induced tumor showing cuboidal cells with lack of glandular structuring; B, nodular Rama 800 lung metastasis similar in appearance to the primary tumor. C, immunocytochemical treatment with antiserum to laminin of Rama 800 tumor showing staining of blood vessels (arrows) but not of tumor cells; D, collagen gel culture of Rama 800 cells with no cell-associated staining; E, treatment with antiserum to MFGM of Rama 800 tumor, some cells stain intensely (arrows), others weakly and others fail to stain. F, collagen gel culture of Rama 800 cells; no staining is observed. H & E, × 475.
Fig. 3. Immunocytochemical staining of normal rat mammary gland with antiserum to MFGM showing staining of adluminal epithelial cells (A); keratin showing staining of myoepithelial cell layer (B); actin showing staining of myoepithelial cells (C); and laminin showing staining of basement membrane (D). A, B, and D, ×475; C, ×1,125.
Fig. 4. Electron micrograph. A, cell in Rama 800 tumor showing indented nucleus, abundant rough endoplasmic reticulum, and cytoplasmic thin filaments (arrow); B, Rama 800 cell in collagen gel showing membrane bleb devoid of organelles. A, x 15,600; B, x 13,860.
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