Establishment and Characterization of SV40-transformed Human Breast Epithelial Cell Lines

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ABSTRACT

Human breast epithelial cells cultured from milk have been transformed with SV40. Indirect immunofluorescence tests using monoclonal antibodies show that cells from clones grown in soft agar have SV40 large T-antigen in their nuclei and epithelia-specific tonofilament antigens on their intermediate filaments. In primary cultures of milk epithelial cells, the tonofilaments form a characteristic delicate basketwork throughout the cytoplasm, but in the SV40-transformed epithelial cell strains, the filament network is grossly distorted. Insulin, hydrocortisone, and serum stimulate the growth of the cell strains. At passages 8 to 11, the cell strains become quiescent and usually die. One cell strain survived this crisis period and gave rise to the in vitro series of cell lines. Most cell lines have a cuboidal morphology and react with a monoclonal antibody that recognizes a differentiation antigen on the membranes of breast epithelia. Line fR2 expressed the highest level of this antigen whereas fR5, the only fR line isolated with fusiform morphology, had relatively little. The in vitro-transformed lines may be related to the two dominant epithelial cell types seen in primary milk cultures and could be useful for studying the relationship between transformation and differentiation in human mammary epithelial cells.

INTRODUCTION

While most epithelial cells in the adult organism show a fairly constant rate of growth and differentiation (e.g., skin, intestine), the epithelial cells of the female mammary gland grow and function only at specific stages. The question of cell lineage and its relation to possible stem cells in the breast and to malignant change is therefore of particular interest. The adult lactating mammary gland usually consists of a branching structure of ducts terminating in sac-like structures called alveoli; both structures are lined with epithelium which in the alveoli and smaller ducts has a secretory potential (1, 8, 12, 18). The alveoli and ductal epithelium are surrounded by myoepithelial cells, which by their ability to contract help to expel milk from the breast. Although the functioning mammary gland is very similar in all mammals, the physiology of its development during adult life varies considerably among species (5, 14, 36) so that other mammals may not provide completely appropriate models for the study of human mammary physiology and breast cancer.

One approach to investigating the relationship between cell lineage, differentiation, and cancer of the human breast is to study breast epithelial cells in culture, using immunological and other markers to classify them and to follow their interconversion or differentiation. Human milk contains a variety of cell types (13) including clumps of epithelial cells which can be readily grown in culture (3). In primary cultures, many adherent tissue macrophages are also present, but the cells that divide have been shown to be epithelial by morphological (35) and immunological (5) criteria. Although there is heterogeneity in both cell and colony morphology of the primary cultures, the dominant closed colony phenotypes can be identified containing either cuboidal or elongated cells (35). A minor but nevertheless distinct colony type that can also be identified is the open type, characterized by cuboidal cells separated from each other. In milk cultures growing in low serum, this open colony type proliferates more rapidly than either the closed cuboidal or elongated types (35). Experimental analysis of the relationship between the different colony phenotypes in vitro and specific cell types in the mammary gland (31) has been limited to primary cultures, since with passage the originally dominant morphological types disappear and a cell type emerges which by morphological and other criteria is different not only from the epithelial cells found in primary milk cultures but also from mammary fibroblasts (19). These cells may represent the glandular myoepithelial cells.

To obtain permanent cell lines of mammary epithelial origin which might retain some properties of the original cell types, we have transformed cells in primary milk cultures with SV40. Although it was relatively easy to obtain SV40-transformed epithelial cells with an extended in vitro life span, the cell strains eventually entered crisis (11) and died. One culture, however, survived crisis and has since given rise to several cloned cell lines with apparently unlimited growth potential. We have characterized these cell lines as being of human mammary epithelial origin by using 2 monoclonal antibodies, one of which (LE61; Ref. 16) recognizes a tonofilament component in nonkeratinizing epithelia and the other (1.10,F3; Refs. 2 and 32) is a marker for a differentiation antigen on the membranes of breast epithelia. Two cell lines which show several differences are of particular interest, fR2 with a cuboidal morphology and fR5 with an elongated morphology. We report their derivation and characterization and discuss the possibility that they might be related to the basic cell types found in primary milk cultures and how they and other related lines could be used in studying the relationship between transformation and differentiation in the human mammary gland.

MATERIALS AND METHODS

Primary Cultures of Milk Epithelial Cells. Epithelial cells and macrophages from pooled samples of early lactation milks were plated on

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1 S. Chang and E. B. Lane, unpublished results.
2 The abbreviations used are: RPMI 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640; FCS, fetal calf serum; TEGPED, 0.4% trypsin and 0.1% pancreatin in Hank’s buffer, 0.004% EDTA, 0.25% ethylene glycol bis(β-aminoethyl ether)-N,N,N',N’-tetraacetic acid in calcium- and magnesium-free Dulbecco’s phosphate-buffered saline (pH 7.0); PBS, phosphate-buffered saline.
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Radiolabeled Binding Assay for a Breast Epithelial Membrane Differentiation Antigen. freshly trypsinized cells were washed twice with assay medium (RPMI 1640, 0.2% bovine serum albumin, and 0.02% azide) and then aliquoted into microcups (Dynatech Laboratories) at 2 X 10^5 cells in 25 µl of assay medium. Next, 50 µl of monoclonal antibody 1.10.F3 used as neat culture supernatant were added to each well, in duplicate. The plates were shaken gently for 5 min and then incubated for 1 hr at 4°C. Control samples were incubated with RPMI 1640 containing 20% FCS. The cells were pelleted, washed 3 times with screening buffer, and then incubated for 1 hr at 4°C with 50 µl of fluorescein-conjugated rabbit anti-mouse IgG diluted 1:40 with PBS. After 4 washes with buffer, the cells were mounted in 15 to 20 µl of Gelvatol on slides and examined with a Zeiss photomicroscope.

RESULTS

Isolation of SV40-transformed Breast Epithelial Cell Strains. SV40-transformed breast epithelial cells were obtained by infecting suspensions of primary milk cultures with wild-type SV40. The infected cells were washed on plastic dishes for 3 to 4 weeks before they were cloned in soft agar, and this appeared to be necessary for the successful growth of clones in agar. In 1 of 10 experiments using different milk pools, 12 clones were obtained, and one-half of these came from the milk pool M566. Mock-infected milk cultures did not produce any clones in soft agar.

Clones were picked from soft agar 4 to 6 weeks after seeding, and the viable ones quickly attached to plastic dishes and proliferated (see Fig. 1A). These gave rise to cell strains which could be passaged 8 to 11 times, with splitting of the cultures 1:3 at each passage, before their growth slowed down and eventually stopped. The SV40 milk cell strains were passaged 6 to 8 weeks before crisis occurred, thus demonstrating at least a 2-fold increased life span compared to uninfected milk epithelial cells. All the strains showed a tendency to shed cells (up to 50% of the total cell number), and although these were viable, they neither attached to fresh plastic surfaces nor proliferated in suspension. Perhaps because of this, the strains never formed complete sheets but instead grew in a characteristic pattern consisting of ridges surrounding empty spaces.
(Fig. 1, B and C). This growth pattern was very different from that shown by the 2 dominant epithelial cell types seen in primary milk cultures (Fig. 1D; Ref. 35).

Crisis: Establishment of SV40-transformed Breast Epithelial Cell Lines. As indicated above, the growth of the SV40-transformed epithelial cell strains slowed down after 8 to 11 passages and then ceased. The quiescent cells slowly detached from the dishes, leaving only multinucleated giant cells. This phenomenon appears to be similar to the “crisis” originally described by Girardi et al. (11) for SV40-transformed human fibroblasts and also observed in SV40 transformation of other human cell types (10, 15, 23). Only one SV40 breast epithelial cell strain (clone M566.f at passage 9) survived crisis. During the quiescent period, these cells did not detach completely from the plastic dishes, and after 6 weeks, foci of viable cells appeared in the cultures. These foci were cultivated to form “intermediate” cell lines which readily formed confluent cultures with minimal shedding into the medium. Recloning of the intermediate cell lines in soft agar resulted in the isolation of the fR cell lines with different morphologies, as illustrated in Fig. 2. The derivation of the cell lines and their nomenclature are summarized in Chart 1.

Morphology of the SV40 Breast Epithelial Cell lines. Fig. 2 illustrates the morphology and growth pattern of an intermediate cell line and 4 of the recloned lines. The intermediate lines were morphologically heterogeneous, containing both cuboidal and elongated cells (Fig. 2, A and B). However, the dominant morphology shown by the recloned lines is cuboidal and is typified by fR9 and fR15 (Fig. 2, C and D). The 2 lines we have principally studied are fR2, which has a homogeneous cuboidal morphology, and fR5, the only cell line isolated which showed an exclusively elongated morphology. The growth pattern of fR2 is shown in Fig. 2E. As these cuboidal cells proliferate, they form compact flat colonies that merge together as the spaces between them become filled. In contrast, fR5 cells appear elongated and refractile under phase optics (Fig. 2F) and, unlike fR2 cells, tend to shed cells into the medium when they become confluent. A comparison of Fig. 2, E and F, with Fig. 1D shows that the fR2 and fR5 lines bear a resemblance to the closed cuboidal and elongated colony types, respectively, seen in primary milk cultures. The fR2 and fR5 lines have been passaged over 60 times (at least 150 doublings) and have retained their original phenotypes.

SV40 Transformation of Human Mammary Fibroblasts. For comparative studies with SV40-transformed milk cells, fibroblasts from reduction mammoplasty tissue (26, 34) were also transformed with SV40. SV40-infected fibroblasts were plated directly into soft agar, and 2 to 4 weeks later clones were visible macroscopically and were picked. About 20 viable SV40 fibroblast cell strains were isolated in this way from 2 transformation experiments. Mock-infected fibroblasts did not produce any clones. Fig. 3 illustrates the morphology of normal breast fibroblasts (HuMF) and a strain of SV40-transformed HuMF. All the SV40 HuMF strains formed confluent, often multilayered cultures like those illustrated in Fig. 3B. This is in marked contrast to the precrisis SV40-transformed breast epithelial cell strains which never formed confluent cell cultures and constantly shed cells into the medium. Like the SV40 milk epithelial cell strains, the SV40 mammary fibroblast strains had a longer life span in vitro than their uninfected counterparts. The SV40 fibroblast strains could be passaged 20 to 50 times (at least 60 divisions) over a 3- to 4-month period before crisis occurred. Normal mammary fibroblasts can usually be passaged 10 to 12 times over a 2-month period (about 30 divisions). No postcrisis SV40-transformed breast fibroblast cell line has yet been established.

T-Antigen Expression in SV40-transformed Breast Cells. Using the monoclonal antibody PAb 205 (formerl 3CS; Ref. 6), SV40 large T-antigen was detected by immunofluorescence in the nuclei of all the SV40 breast epithelial cell strains and lines. The presence of this specific antigen in the nuclei shows that SV40 DNA is present in these cells. Almost all the nuclei stained with a very high intensity (+ + + + ; Fig. 4A) in contrast to the nuclei of SV40 breast fibroblast strains which stained less brightly (+ to + + + ; Fig. 4B). The nuclei of uninfected milk cells and normal breast fibroblasts are not stained by PAb 205.

Expression of Tonofilament Antigen in Normal and SV40-transformed Milk Epithelial Cells. Tonofilaments or cytokeratin filaments are the intermediate filaments found in the cytoplasm of epithelial cells (9, 28); this type of filament is never found in connective tissue or cultured mesenchymal cells. Tonofilament-specific antigens can therefore be used to identify cultured cells as being of epithelial origin. The monoclonal antibody LE61 has been characterized extensively (16) and is a useful reagent for distinguishing simple epithelia from keratinocytes (stratified squamous epithelia) in vivo. By indirect immunofluorescence, LE61 shows tonofilaments in primary
cultures of milk cells to be arranged as a delicate basketwork throughout the cytoplasm with filaments anchored in desmosomes at the cell periphery (Fig. 5, A and B), a cytokeratin distribution typical of a variety of cultured epithelial cells. The fact that the primary milk cells stained with LE61 indicates that they are not epidermal cells that may have been sloughed from the skin or distal duct near the nipple during collection of the milk samples but rather that they are derived from the lining epithelium of the alveoli and smaller ducts of the mammary gland. The distribution of the cytokeratin filaments among the milk cells can be variable, a finding consistent with other observations of heterogeneous staining in primary epithelial cultures (7) and even within a cell line (17).

All the precrisis SV40-transformed milk strains we have looked at (M566. a, e, f, and h, in addition to 4 clones from 2 other SV40 milk transformation experiments) react positively with LE61, confirming their epithelial origin. However, the tonofilaments are not present here as a dispersed basketwork but are instead seen as a distorted, collapsed, and irregular filament system, often present only as a perinuclear ring or ‘cap’ (Fig. 5, C and D). The binding of LE61 to the postcrisis fR lines varied. Some lines showed a rudimentary filamentous network, e.g. fR17 (Fig. 5, E and F), while others like fR2 (Fig. 5, G and H) and fR9 (data not shown) had only a juxtanuclear aggregate of stained material. The cell line fR5 with the fusiform morphology was almost completely negative with LE61 as also were 3 fusiform cell lines (S1.3, S1.10, and S2.9) which were isolated as cloned variants from the positively staining cuboidal fR9 cell line. As no fusiform cell lines have been found which do have a tonofilament network, the transformed cell morphology and the absence of the tonofilament antigens could possibly be causally related. Normal and SV40-transformed breast fibroblast strains do not react with LE61.

Cytogenetic Analyses. Chromosome analyses of the SV40-transformed milk epithelial M566.f1 cells precrisis and postcrisis (intermediate and recloned cell lines including fR2 and fR5) showed them to be mainly hypotetraploid and characterized by several specific chromosomal markers. The identification of specific chromosomal markers in the precrisis M566.f cell strain and the postcrisis intermediate and recloned fR cell lines indicate that all these cells were derived from the same original clone. The SV40-transformed fibroblast strains N13F.20 and N13F.25 were near diploid.

Growth Requirements of SV40 Breast Epithelial Cells. The growth of primary milk cultures has been shown to be stimulated by insulin, hydrocortisone, and serum (34, 35). Chart 2, A and B, shows the growth curves for the precrisis SV40 milk strain M566.f1 at passage 7 (fp7) in the presence of 2 concentrations of serum, with and without insulin and hydrocortisone. Chart 2, C and D, shows similar data for the postcrisis intermediate cell line f10 at passage 9 (f10p9; see Chart 1 for nomenclature). The growth of SV40-transformed breast epithelial cell strains (represented by fp7) and cell lines (represented by f10p9), like primary milk cultures, are stimulated by insulin and hydrocortisone. Increasing the serum concentration from 1 to 10% also has an effect on growth, but this is more noticeable with the cell strains than the cell lines. Although the

SV40 breast fibroblast strains show relatively little growth response to insulin or hydrocortisone, they do respond to an increase in serum concentration from 1 to 10%.

Reaction of SV40-Transformed Cells with a Monoclonal Antibody to a Differentiation Antigen of Breast Epithelial Membranes. Antiserum to human milk fat globule membranes used to characterize cultured cells as being epithelial and of breast origin (4, 21). More recently, monoclonal antibodies against components of the milk fat globule membrane which

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2 S. Chang and J. Keen, unpublished results.
react specifically with human mammary epithelial cells in culture have been developed by Taylor-Papadimitriou et al. (32). Immunoperoxidase staining of tissue sections has shown that 2 of these antibodies react with antigens expressed on secretory epithelium in lactating mammary tissue and on primary breast carcinomas and to a much lower degree on epithelia in normal resting breast (2). The reaction of one of these antibodies (1.10.F3) with the SV40-transformed cell lines and strains was tested, using a radiolabeled binding assay. Chart 3 shows the radioactivity bound to precrisis strains, postcrisis lines, and cloned lines after incubation, first with antibody 1.10.F3 and then with 125I-labeled rabbit anti-mouse IgG Fab fragment. For comparison, primary milk cultures (HuME) and normal and SV40-transformed mammary fibroblasts were also tested. These data confirm the earlier observation that 1.10.F3 binds strongly to milk epithelial cells and not at all to mammary fibroblasts (32). The precrisis SV40 strains (epithelial and fibroblast) show a comparatively low level of expression of the differentiation antigen whereas most of the postcrisis SV40 epithelial lines express it strongly. It was of interest to note that 1.10.F3 is bound strongly to the cuboidal cell line fr2 but only weakly to the elongated cell line fr5. This difference was confirmed by indirect immunofluorescence staining of the cells; the fr2 cells had a spackled membrane staining (Fig. 6, A and B) whereas the fr5 cells had little (Fig. 6, C and D). The spackled fluorescence of fr2 was very similar to that observed with human breast cancer lines such as CAMA I (data not shown). The cell lines fr9, fr15, fr17, and fr18 also reacted with 1.10.F3 in a similar fashion to fr2 but to a lesser degree and consistent with the levels seen in the trace-binding assay (Chart 3).

A high proportion (55 to 60%) of primary milk cells react strongly with 1.10.F3 and the distribution of the target antigen on their cell surface appears to be uniform (Fig. 6, E and F). The observation that as much as 40% of the cells do not react with 1.10.F3 shows that in vitro we may have a population of cells at different stages of differentiation. That this is so is shown in Fig. 7 where it is evident that milk cells of either dominant morphological type can stain with 1.10.F3 to a greater or lesser extent or not at all. Since there is no obvious relationship between morphology and expression of the 1.10.F3 antigen in milk cultures, it is perhaps not surprising that a SV40-transformed milk epithelial cell line like fr5 which expresses a low level of 1.10.F3 has been isolated. Further evidence that morphology does not determine expression of the 1.10.F3 antigen is shown by the strong binding of 1.10.F3 to 3 fusiform cell lines (S1.3, S1.10, and S2.9) which were isolated as cloned variants from the cuboidal fr9 cell line.5

DISCUSSION

In this paper, we describe the first successful transformation of human mammary epithelial cells by SV40 and the subsequent isolation of cloned transformed cell lines. There are relatively few reports describing SV40 transformation of normal human epithelial cells, and in most cases lines with unlimited life span were not established. Instead, SV40 infection extended the in vitro life span of the cells (15, 23), but eventually they entered “crisis” and died (11, 24). On the other hand, there are reports of the establishment of SV40-transformed amnion epithelial cell lines (10) and of SV40-transformed skin keratinocytes (27) where “crisis” as described by Girardi et al. (11) did not appear to occur.5

The mammary epithelial cells which were transformed with SV40 were cultured from pooled samples of human milk. Transformed cells isolated as clones from soft agar were obtained from 4 of 10 SV40-infected milk pools. Twelve viable cell strains (6 from the milk pool M566) were established, and they all showed an extended in vitro life span compared to normal milk cultures. The absolute frequency of transformation of the milk cells was difficult to quantify because of the necessity of passaging the SV40-infected cells before cloning in agar. It was nevertheless obviously lower than for mammary fibroblasts which could be plated into soft agar directly after virus infection. The SV40-transformed mammary fibroblasts formed visible clones in soft agar within 2 to 4 weeks while the SV40 milk cells took 4 to 6 weeks. Like the SV40 milk epithelial cell strains, the SV40 fibroblasts proliferated in vitro for a longer time span than did their uninfected counterparts. The SV40 mammary fibroblast strains could usually be passaged 20 to 30 times (3 to 4 months) before crisis occurred, in contrast to 8 to 11 passages (6 to 8 weeks) for the most vigorous of the SV40 milk epithelial cell strains.

5 J. Taylor-Papadimitriou, unpublished results.
In our experience, the emergence from crisis of viable SV40-transformed mammary epithelial cells which can give rise to cell lines is a rare event. This may be due to the fact that the SV40 milk cell strains show a strong tendency to detach from the culture dishes, particularly during crisis. The one strain (M566.f) which did give rise to postcrisis cell lines detached much less during crisis than the other SV40 milk strains. Another explanation for the low recovery of breast epithelial cells from crisis is that the cell with the potential for emerging as an immortal cell line after SV40 transformation is a minority cell type in the culture. Conceivably, the milk pool designated M566 may have contained one milk sample which was enriched for the more susceptible cells, thus accounting for the relatively large number of SV40-transformed cell strains obtained from this milk pool. An enhanced susceptibility could be attributable to a variety of factors including stage of differentiation (25) and/or genetic defects (20, 22, 29, 30). In this context, it may be relevant that we have been unsuccessful in obtaining SV40-transformed cells, even as strains, from nonlactating breast tissue (reduction mammoplasty and nonmalignant fibroadenoma); possibly an epithelial cell present in the active mammary gland (and milk) and not in the resting breast could be the target for SV40 transformation.

The strongest evidence that the SV40-transformed milk cells are epithelial is the positive identification of tonofilaments (cytokeratins) in these cells, using the monoclonal antibody LE61 which specifically recognizes a tonofilament antigen in simple epithelia (16). Primary milk cultures stained with LE61 show a delicate network of cytokeratin filaments which is typical of the staining pattern shown by other nonkeratinizing epithelia in culture. While all the SV40 milk cell strains expressed the LE61 tonofilament antigen, thus confirming their epithelial origin, the arrangement of these filaments was different from that seen in primary milk cells. In all of the SV40 milk cell strains, the tonofilament network was distorted, with tonofilaments typically aggregated to form a perinuclear ring or “cap.” The function of intermediate filaments in epithelial cells is still incompletely understood, but it is possible that filament condensations such as we have observed are common responses to metabolic disturbances. All the cuboidal cell lines contained the tonofilament antigen, and only the fusiform cell line fR5 showed little or none of this marker. Interestingly, 3 other fusiform cell lines (S1.3, S1.10, and S2.9) which were isolated as cloned variants from fR9 also have very little cytokeratin filament material, suggesting that this fusiform transformed cell morphology and the absence of tonofilaments could be causally related. However, at the moment, we have no other evidence for such a relationship.

The positive staining of cells in primary milk cultures with LE61 shows that these cells did not originate from squamous epithelium from epidermis or the large ducts near the nipple but that they were derived from the lining epithelium of the alveoli or smaller ducts of the mammary gland. Another specific immunological marker which confirms the epithelial and secretory origin of cells is the monoclonal antibody 1.10.F3, which reacts with a determinant on lactating breast epithelial cells and some other epithelial cells involved in exocrine secretion (2, 32). The differentiation antigen recognized by this antibody is poorly expressed in the resting breast, but it is always found in primary breast carcinomas (2). The fact that all but one of the cloned fR cell lines express the 1.10.F3 determinant con-

firms that the cell from which they were derived came from the glandular epithelium of the breast.

About 60% of cultured milk epithelial cells react with 1.10.F3, and the cells that do react show a heterogeneity in the extent to which they express their antigen. This heterogeneity is clearly unrelated to the morphological phenotypes seen in the cultures. Modulation in the expression of the differentiation antigen can occur in culture as shown when milk cells are separated by a fluorescence-activated cell sorter on the basis of presence or absence of the 1.10.F3 determinant on their cell surface. Cells not expressing the 1.10.F3 antigen have greater in vitro growth potential than the positive ones and can subsequently give rise to cultures containing both 1.10.F3-positive and -negative cells. Thus, while a positive reaction with 1.10.F3 is evidence of the mammary gland origin, the converse is not necessarily true.

The low level of expression of the 1.10.F3 antigen detected by a trace-binding assay in the precrisis SV40 milk cell strain M566.f (and other SV40 milk strains) is interesting for it suggests that the transformation target cell(s) may not have been expressing the antigen, although clearly it must have had the potential to do so since the antigen is found on all but one of the postcrisis fR cell lines. The only fR cell line which did not express the differentiation antigen was fR5, the one fusiform line originally isolated. As with the primary milk cultures, however, there is no simple correlation among the transformed cell lines between cell morphology and expression of the 1.10.F3 antigen, since the 3 fusiform lines (S1.3, S1.10, and S2.9) which arose from the cuboidal fR9 cell line all express high levels of the antigen. As the 1.10.F3 differentiation antigen is expressed on all the established breast carcinoma cell lines that have been tested (32), it would seem that this antigen is generally conserved in stable cell lines isolated from in vivo- or in vitro-transformed breast epithelium.

The cell lines described in this paper came from a cloned cell strain and therefore almost certainly arose from the same parent SV40-transformed cell. This conclusion is supported by chromosomal analyses which show that the M566.f cell strain and the cell lines derived from it all share specific chromosomal markers. While the postcrisis intermediate cell lines show a mixed cuboidal and elongated morphology, 2 of the recloned lines (fR2 and fR5) are morphologically homogeneous and resemble the 2 major epithelial cell types seen in milk cultures; by analogy, these 2 milk culture phenotypes may derive from a single cell type. Whether in fact there is a stem cell in vivo which can give rise to the dominant closed cuboidal and elongated colony types seen in primary milk cultures (35) and at the same time represent a particularly susceptible target for SV40 transformation remains to be seen. However, it is interesting to speculate that, if such a stem cell does exist, it may be within the ubiquitous but minor open cuboidal colony type which is seen in primary milk cultures. In medium containing low serum, these open cells proliferate much more rapidly than the closed cuboidal or elongated colony types, and when the serum level is increased, open colonies often appear to develop into other colony types, particularly the closed cuboidal type (35).

Our results have shown that it is possible to isolate lines of SV40-transformed breast epithelial cells which retain some of

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6 S. Chang and J. Taylor-Papadimitriou, manuscript submitted for publication.
the properties of the original cells. These cell lines should be useful, not only for studying the effect of SV40 transformation on epithelial cells, but also for producing immunological markers for characterizing cells in the human breast. It will also be of interest to assess the relative sensitivity to experimental transformation of active mammary gland epithelial cells (from milk) against that of the inactive, nonfunctioning mammary gland. In this way, one may be able to define an experimental approach to the possibility of a relationship between differentiation and malignancy in the human mammary gland.

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Note Added in Proof

The monoclonal antibody 1.10.F3 is also referred to as HMFG-1.

REFERENCES

Fig. 1. Light micrographs of primary milk epithelial colonies and SV40-transformed milk epithelial cell strains. Living cells shown by phase-contrast optics. A, soft agar-derived SV40-transformed milk epithelial clone attached to a plastic surface; B and C, SV40 milk epithelial cell strains; B, strain M566.f at passage 4 (fp4; see Chart 1 for nomenclature); C, strain M566.e at passage 6 (ep6); D, 2 dominant closed epithelial colony types (cuboidal and elongated) found in primary milk cultures; bars, 100 μm.
Fig. 2. Light micrographs of SV40 breast epithelial cell lines. Living cells shown by phase-contrast optics. A and B, 2 colony types found in the intermediate cell line f4 (see Chart 1 for nomenclature); C, D, E, and F, cell lines obtained after recloning some intermediate cell lines in soft agar (see Chart 1); C, fr9; D, fr15; E, fr2 (cuboidal morphology); F, fr5 (elongated morphology); bars, 100 μm.
Fig. 3. Light micrographs of breast fibroblast cell strains. Living cells shown by phase-contrast optics. A, normal breast fibroblasts N13F; B, SV40-transformed breast fibroblast strain N13F.20; bars, 100 μm.
Fig. 4. Immunofluorescence staining for SV40 large T-antigen. See text for experimental procedure. Nuclei stained with PAb 205 (formerly 3C5), a monoclonal antibody against SV40 large T-antigen (6). A, SV40 breast epithelial cell line fR2; B, SV40-transformed breast fibroblast strain N13F.20; bars, 20 µm.

Fig. 5. Immunofluorescence staining for tonofilaments. Cytoplasmic tonofilaments were stained with monoclonal antibody LE61 as described in "Materials and Methods." A and B, primary culture of milk epithelial cells; C and D, SV40-transformed breast epithelial cell strain MS66.1 at passage 8 (tp8); E and F, SV40 breast epithelial cell line fR17; G and H, SV40 breast epithelial cell line fR2; bars, 20 µm.
Fig. 6. Immunofluorescence screening for a breast epithelial membrane differentiation antigen. Cells, in suspension, were stained with monoclonal antibody 1.10,F3 as described in "Materials and Methods." A and B, SV40 breast epithelial cell lines FR2; C and D, SV40 breast epithelial cell line FR5; E and F, milk epithelial cells, HuME; bars, 20 μm.
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Fig. 7. Immunofluorescence staining for a breast epithelial membrane differentiation antigen on milk epithelial cells. Primary milk cultures on coverslips were stained with monoclonal antibody 1.10.F3 as described in “Materials and Methods.” A and B, 2 dominant closed colony cell types (left, elongated; right, cuboidal) seen in milk cultures; C and D, a field of the closed colony cuboidal cells, bars, 20 μm.
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