Establishment and Characterization of Three New Continuous Cell Lines Derived from Human Breast Carcinomas

Linda W. Engel,1 Nathaniel A. Young, Tommie Sue Tralka, Marc E. Lippman, Stephen J. O'Brien, and Mary Jo Joyce

Laboratory of Pathology [L. W. E., N. A. Y., T. S. T., M. J. J.], Medicine Branch [M. E. L.], and Laboratory of Viral Carcinogenesis [S. J. O.], National Cancer Institute, NIH, Bethesda, Maryland 20014

ABSTRACT

Three continuous lines of mammary tumor cells (ZR-75-1, ZR-75-27, and ZR-75-30) have been established from malignant effusions of two women with breast cancer. Differentiated properties expressed by each cell line include: (a) epithelial morphology (by light and electron microscopy) resembling that of the parental tumors; (b) presence of receptors for estrogen and other steroid hormones; and (c) growth responsiveness to estrogen and/or progesterone. All three cell lines possess human karyotypes that differ from one another in modal chromosome number as well as in characteristic marker chromosomes. Two of the cultures (ZR-75-27 and ZR-75-30), although derived from the same patient, have stable differences in their karyotypes.

INTRODUCTION

Well-characterized cell lines derived from human mammary carcinomas are needed for in vitro model systems in multidisciplinary research. Since the first report in 1958 by Lasfargues and Ozzello (11), few such lines of undisputed pedigree have been reported. One well-studied line (MCF-7) has yielded an agent with the properties of an oncorna-virus (28), but characterization of this agent has been hindered by low, intermittent yields of virus. These findings prompted us to attempt to develop additional mammary tumor cell lines, cultured under conditions that minimize the possibility of inadvertent introduction of adventitious viral agents, as a prelude to our own virological investigations. In this report we describe the establishment and preliminary characterization of 3 differentiated epithelial cell cultures derived from malignant effusions of 2 women with metastatic breast cancer.

MATERIALS AND METHODS

Source of Cells

ZR-75-1 cultures were derived from a malignant ascitic effusion in a 63-year-old white female who had undergone modified radical mastectomy 34 months previously for infiltrating ductal carcinoma of the right breast. At the time of diagnosis, she was receiving postmenopausal estrogen therapy. Among the chemotherapeutic agents subsequently used without apparent benefit to the patient were tamoxifen (I.C.I. 46474, an antiestrogen) and fluoxymesterone (Halotestin). For several months before her death, weekly or biweekly abdominal paracentesis was required because of massive ascites. The effusion from which ZR-75-1 cells were derived was obtained 3 months after the beginning of antiestrogen therapy.

Two cell lines (ZR-75-27 and ZR-75-30) were derived from a 47-year-old premenopausal Negro woman who had undergone right radical mastectomy 1 year previously. The histological findings were infiltrating ductal carcinoma and axillary lymph node metastases. Estrogen-binding protein was detected in a cutaneous metastasis, and an oophorectomy was performed. Seventeen days before the first culture specimen was collected, treatment was begun with tamoxifen and fluoxymesterone. From this time until the death of the patient, repeated thoracenteses and abdominal paracenteses were required. ZR-75-27 originated from a pleural effusion, while ZR-75-30 was derived from ascitic fluid collected 32 days later, 2 days before the patient's death.

Culture Medium

Cell lines were established and are maintained in Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 25 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, penicillin (100 units/ml), streptomycin (100 μg/ml), neomycin (75 μg/ml), and fetal calf serum. Fetal calf serum (20%) was used for initiating cultures. When cultures had been established (5 to 10 passages), the serum concentration was reduced to 10%, and the cells were then fed 2 to 3 times/week by replacement of one-third to one-half of the conditioned medium.

Establishment of Cultures

ZR-75-1. Ascitic fluid (200 ml) was centrifuged at 250 × g for 10 min. The resultant cell pellets were resuspended in culture medium at 36°C to form a dense suspension and seeded in 75-cm² plastic flasks (Falcon Plastics, Oxnard, Calif.). Supernatants were serially decanted and reseded over 3 days. In this manner the newly seeded cultures were enriched with respect to slowly attaching tumor cells. In contrast, other more rapidly attaching cells, particularly mesothelial cells and fibroblasts, were selectively reduced. Cultures were maintained with minimal manipulation and twice-weekly feeding until resultant monolayers were passed 2-1, 47 days after initiation of the culture. Two subsequent 1:1 passages were made at weekly intervals, after which 1:2 passages weekly became routine.

ZR-75-27. Bloody pleural effusion (1000 ml) was left overnight at room temperature, following which 800 ml
were treated in a manner similar to that described for ZR-75-1, with the additional step of the lysing of RBC with 0.15 M NH₄Cl:0.01 M KHCO₃:10⁻⁴ M EDTA lysing buffer. Cultures of the heavily seeded material progressed poorly and were subsequently discarded. Multiple aliquots of 4 ml of the remaining 200 ml of fluid were carefully layered over 3 ml of Ficoll-sodium metrizoate solution (Lymphoprep R; Nyegaard & Co., Oslo, Norway) and spun at 240 x g for 30 min at room temperature. The interface and bottom pellet were collected separately and washed 3 times with Roswell Park Memorial Institute Tissue Culture Medium 1640, with the last wash containing 20% serum. Microscopy of filter preparations showed that the interface contained few RBC but increased, compared with that of the the pelleted cells (68 versus 33%). Cells collected at the interface were seeded heavily at 36° in two 75-sq cm plastic flasks. Twenty-four hr later the supernatants containing unattached cells were pooled, concentrated, and seeded in 1 flask. This culture did not grow to confluency as a monolayer. Rather, cells shed continually into the supernatant. After 2 months free-floating cells were repeatedly removed, packed by centrifugation, and reseeded cumulatively in one 25-sq cm flask. Within 3 weeks a 1:1 passage of the reseeded cells was made, and within several additional weeks it became possible to make 1:2 to 1:3 serial passages every 7 days.

**ZR-75-30.** More than 6 liters of bloody ascitic fluid were centrifuged at 1500 x g for 10 min. Packed cells were resuspended, pooled, and stored overnight at 4° (along with large clots that had formed in the collected fluid) for processing the next day. Pellets were seeded after treatment with 0.15 M NH₄Cl:0.01 M KHCO₃:10⁻⁴ M EDTA lysing buffer; these progressed poorly and were subsequently discarded. Clots were pooled, minced thoroughly with scissors, washed several times with phosphate-buffered saline (0.146 M NaCl:2.3 mM Na₂HPO₄:1.5 mM KH₂PO₄, pH 7.2), and dissociated with Dulbecco’s phosphate-buffered saline solution containing 0.25% trypsin:0.02% EDTA according to a modification of the method of DeOca (5). Cells from the first 4 serial trypsinizations were combined, pelleted, treated with lysing solution, and seeded heavily at 36° in one 75-sq cm flask. A culture of epithelial cells, admixed with fibroblasts, proliferated both in colonies and as free-floating cells. Within 24 hr supernatant cells were reseeded and concentrated in one 25-sq cm flask. Seven weeks later a 1:1 passage of the mixed culture again yielded a culture contaminated with fibroblasts. Since the fibroblasts detached more readily than did the epithelial cells, they were successfully eliminated by discarding an initial short trypsin rinse. The resultant cultures, all containing attached epithelial cells and floating lymphoblastoid cells, were maintained by serial passage at 1:2 to 1:4 split ratio every 7 to 14 days. The presence of Epstein-Barr virus nuclear antigen in the lymphoblastoid cells was demonstrated by indirect immunofluorescence. Lymphoblastoid cells were subsequently eliminated from the monolayer cultures by means of repeated washings with phosphate-buffered saline and frequent exchanges of medium over a 3- to 4-week period. They have been established in pure cultures separate from the epithelial cells.

At present, ZR-75-1 has been maintained in serial culture for over 2.5 years and through 68 passages. ZR-75-27 and ZR-75-30 have been in culture for 2 years and have been maintained through 25 and 30 passages, respectively, in a laboratory restricted to the handling of malignant effusions from breast cancer patients. Repeated cultures for Mycoplasma on all 3 lines, done by Dr. Leonard Hayflick, have been negative. Cells have been preserved in liquid nitrogen at passage levels 4 to 14.

**Electron Microscopy**

Monolayers were harvested with a rubber policeman, processed routinely for electron microscopy (29), and examined with a Siemens 1A electron microscope.

**Cell-doubling Time**

Cell-doubling times at 36° were measured by the counting of viable cells from freshly trypsinized monolayers by means of the trypan blue exclusion method. Counts were performed at 12- to 24-hr intervals for 9 to 11 days.

**Karyology**

Logarithmically growing cells were treated with Colcemid (0.1 µg/ml), swollen with hypotonic solution, and fixed with methanol:acetic acid (3:1). Following air drying metaphase plates were Giemsa stained for conventional morphological examination and counting of chromosomes. The trypsin-Giemsa method (30) was used for marker chromosome analysis. Chromosomes of ZR-75-1 cells were examined both in the original effusion and at the 38th passage level. Karyotypic analysis was made for ZR-75-27 at the third passage level and for ZR-75-30 at the sixth passage level.

**Phenotype Analysis**

To have a unique genetic signature for each cultured cell line, we determined allozyme (allelic isozyme) phenotypes for 8 polymorphic gene-enzyme systems as previously described (23). Crude extracts of packed cells were sonically dispersed; extracted in 0.05 M Tris-HCl:0.001 M EDTA, pH 7.1; and subjected to starch gel electrophoresis. Extracts from HeLa cells were used as a reference line.

**Hormone Receptors**

The detailed methodology used for steroid receptor analyses has been published previously (13, 15). Briefly, competition binding assays were performed on either cytoplasmic extracts or intact cells with the use of multiple concentrations of radiolabeled ligand. Binding was examined with the use of the Scatchard technique (26) to analyze data prepared by computer-assisted methods (1).

**Hormone Responsiveness**

Incorporation of [³H]thymidine into DNA was measured as previously reported (12), with minor modifications: (a)
the time allowed for cell attachment was variable (24 to 72 hr); (b) experiments were carried out in medium either supplemented with 5% charcoal:dextran-treated serum or under serum-free conditions; and (c) final concentrations of \(^{3}H\)thymidine ranged from 0.1 to 2 \(\mu\)Ci/ml. In individual experiments the effect of each hormone was assayed in 3 to 7 replicate flasks. Incorporation of \(^{3}H\) in the absence of added hormone was normalized to 1000 cpm so that results from individual experiments in which different concentrations of isotope were used could be pooled for statistical analysis. Student's t-test was used to determine the significance of differences between each of the hormonal treatments and the control.

RESULTS

Growth Characteristics and Light Microscopy

Cell-doubling times were 80 hr for ZR-75-1 (passage 40), 144 hr for ZR-75-27 (passage 14), and 110 hr for ZR-75-30 (passage 17). At present, ZR-75-1 cells may be split 1:6 each week, while ZR-75-27 and ZR-75-30 cells are divided 1:3 every 7 to 10 days.

ZR-75-1 cells in culture closely resemble malignant cells in the original breast biopsy (stained by hematoxylin and eosin) and exfoliated malignant cells in ascitic fluid (stained by the Papanicolaou technique) (Fig. 1). Each cell is polygonal, with abundant cytoplasm, and possesses a large nucleus with either a single prominent nucleolus or 2 to 4 prominent chromocenters. ZR-75-27 and ZR-75-30 cells in culture resemble each other as well as carcinoma cells in tissue obtained at autopsy (Fig. 2). Secretory vacuoles in the cytoplasm and prominent molding of the nuclei are additional features often seen both in vivo (Fig. 1B) and in vitro (Figs. 1C, 2B, and 2C). Although all 3 cell lines are typically epithelial, ZR-75-1 cells have more abundant cytoplasm and irregular contours, compared with ZR-75-27 and ZR-75-30, which tend to be rounded.

Ultrastructure

Characteristic features of the 3 cell lines are similar and have remained constant irrespective of passage history. Each closely resembles malignant cells in the original effusions from which they were derived. Cells connected by occasional desmosomes are often arranged in rosettes around duct-like lumens. Short microvilli typically extend from the apical borders of cells lining these "ducts" (Fig. 3). Large intracytoplasmic vacuoles, often displacing the nucleus and lined by microvilli, contain amorphous, membranous, secretory material (Fig. 4). Large, lobulated nuclei containing 1 or more prominent nucleoli are often located eccentrically. Chromatin is generally homogeneous but is seen occasionally in small aggregates. The cytoplasm contains abundant Golgi complexes. There are moderate numbers of vesicles of smooth endoplasmic reticulum and strands of rough endoplasmic reticulum; the latter contain variable amounts of lightly osmiophilic flocculent material. Free polysomes are numerous. Moderate numbers of pleomorphic mitochondria are present. Bundles of tonofibrils are frequent and are often located perinuclearly. Occasional desmosomes, glycogen rosettes and lipid granules, and rare multivesicular bodies are present. Pleomorphic secretory granules of variable density are abundant in all of the cell lines (Fig. 5). They are usually located at the apical portion of the cell, near the plasma membrane, or surrounding the membrane of the intracytoplasmic duct-like vacuoles, but they may also be seen lying within large intracellular spaces and duct-like lumens.

Karyology

Each of the 3 cell lines contains human chromosomes with characteristic markers distinct from each other and from those of HeLa cells (21).

ZR-75-1. The modal number of chromosomes is 74 to 75 (39% of metaphase plates) in the original effusion and 71 to 72 (52% of plates) at the 38th passage. However, the original population has a bimodal distribution, with a subpopulation (32% of plates) containing 69 to 72 chromosomes (Chart 1).

Three abnormally large chromosomes designated markers 1, 2, and 4 may be distinguished by conventional staining. Trypsin-Giemsa banding reveals an additional abnormal complement (markers 3 and 5 to 10) (Fig. 6). Seventy-nine % of cells from the original ascitic fluid contained markers by conventional staining, while after 17 months in culture and 38 passages these markers were found in 100% of the cells examined. In addition, minute chromosomes were found in 14% of the metaphase plates from the original effusion, while none were found at passage 38.

ZR-75-27 and ZR-75-30. The modal number for ZR-75-27 cells is 79 chromosomes (78 to 80 in 62% of the metaphase plates). The modal number for ZR-75-30 cells is 81, with 72% of the plates examined containing 80 to 82 chromosomes (Chart 2). These 2 lines, although derived from the same patient, therefore possess different modal numbers of chromosomes (p < 0.001; Kolmogorov-Smirnov 2-sample test). Trypsin-Giemsa-banded karyotypes are presented in Figs. 7 and 8. For ZR-75-27 cells markers 1 and 2 may be distinguished by means of morphology without resorting to banding techniques (Fig. 7). ZR-75-30 cells have 3 such markers, labeled 1, 2, and 4 (Fig. 8). An additional group of markers is revealed by banding techniques. Markers 1, 4, and 6 of ZR-75-27 cells are identical to markers 1, 3, and 6...
that the incorporation of nucleoside is increased significantly depicted in Chart 3. Although experiment-to-experiment variation is characteristic, analysis of pooled data shows that within individual experiments stimulation up to 81% is noted. In contrast, a consistent inhibitory effect on [3H]thymidine incorporation is apparent for dexamethasone (30%, p < 0.001). Maximal inhibition within individual experiments was 45%. Precursor incorporation into macromolecules is also below the control level for dihydrotestosterone (13%, p < 0.05). No effect of progesterone was observed. The specificity of estradiol stimulation is reinforced by the observed inhibitory effect of the antiestrogen tamoxifen (31%, p < 0.001). Furthermore, this inhibitory effect is blocked by addition of 10^-8 M estradiol along with tamoxifen, which restores stimulation to levels observed with estrogen alone (46%, p < 0.001).

The effects of steroid hormones on thymidine incorporation by ZR-75-27 and ZR-75-30 cells are shown in Chart 4. For ZR-75-27 cells significant increases above control are noted with estrogen (25%, p < 0.001), dexamethasone (26%, p < 0.001), and progesterone (24%, p < 0.001). Less dramatic stimulation is observed with dihydrotestosterone (16%, p < 0.05). Inhibition by tamoxifen and blocking of tamoxifen inhibition by 10^-8 M estradiol again confirm estrogen responsiveness, although in this case the responses are not statistically significant. For ZR-75-30 cells a significant increase above control is recorded for progesterone (19%, p < 0.05). Nonsignificant increases are recorded for dexamethasone (11%) and for estrogen (22%). The extent of stimulation in these experiments is apparently limited by intolerance of the cells to serum-free conditions.

DISCUSSION

Breast cancer cells have been established in long-term culture only with difficulty (7, 10). Attempts to culture cells have apparently been successful more often with malignant effusions than with solid tumors (3, 4). Effusions have the advantage of providing large numbers of dissociated, viable tumor cells with little or no contamination by fibroblasts. While the techniques used to establish each of our 3 cultures were different, 4 factors probably contributed to success. First, because we anticipated that the growth of the cells would be dependent on their concentration, we seeded the cells at extremely high densities. Second, supernatants were serially decanted over a period of several days to enrich the cultures for malignant cells, which attach more slowly than do other cells types, such as mesothelial cells and fibroblasts. Third, cultures were handled with minimal manipulation throughout a prolonged period of dormancy, during which little division occurred. Fourth, initial passages were made at low split ratios (2:1 and 1:1) and only after prolonged incubation.

The morphology of each of the 3 cell lines in vitro closely resembled that seen in biopsies or preparations of exfoliated cells from their respective donors. The cultured cells are clearly epithelial, and they exhibit ultrastructural features characteristic of breast carcinoma cells, such as desmosomes, tonofibrils, and intracytoplasmic vacuoles (2). All possess human chromosomes with markers readily distinguishable from those of HeLa cells by means of trypsin-Giemsa banding techniques. Moreover, analysis of enzyme phenotypes of these cells, when correlated with known population frequencies for the corresponding gen-
Gene-enzyme phenotypes of breast cancer cell lines and HeLa cells at 8 polymorphic loci

Crude extracts of packed cells were dispersed sonically; extracted in 0.05 M Tris-HCl:0.001 M EDTA, pH 7.1; and subjected to starch gel electrophoresis. The 8 loci tested were selected because they are polymorphic in human populations and can be measured in cultured cells. The numbers and letters are designations for the common alleles observed; e.g., ZR-75-30 is phenotypically PGM₁₋₂, which means that the patient was heterozygous for the PGM₁ and PGM₂ alleles.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Adenosine deaminase (ADA)</th>
<th>Phosphoglucomutase 1 (PGM₁)</th>
<th>Phosphoglucomutase 3 (PGM₃)</th>
<th>Glucose-6-phosphate dehydrogenase (G6PD)</th>
<th>Esterase D (ESD)</th>
<th>Peptidase D (PEP-D)</th>
<th>6-Phosphogluconate dehydrogenase (PGD)</th>
<th>Glyoxylase (GLO-1)</th>
<th>Frequency (signature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZR-75-1</td>
<td>1 (0.56)</td>
<td>1-2 (0.4)</td>
<td>2 (0.12)</td>
<td>B (0.85)</td>
<td>1 (0.63)</td>
<td>(0.6)</td>
<td>A (0.77)</td>
<td>2 (0.22)</td>
<td>0.0020</td>
</tr>
<tr>
<td>ZR-75-27</td>
<td>1 (0.56)</td>
<td>1 (0.52)</td>
<td>1-2 (0.45)</td>
<td>B (0.85)</td>
<td>1-2 (0.23)</td>
<td>1 (0.69)</td>
<td>AP (0.04)</td>
<td>1-2 (0.5)</td>
<td>0.0004</td>
</tr>
<tr>
<td>ZR-75-30</td>
<td>1 (0.56)</td>
<td>1 (0.52)</td>
<td>1-2 (0.45)</td>
<td>B (0.85)</td>
<td>1-2 (0.23)</td>
<td>1 (0.69)</td>
<td>AP (0.04)</td>
<td>1-2 (0.5)</td>
<td>0.0004</td>
</tr>
<tr>
<td>HeLa</td>
<td>1 (0.56)</td>
<td>1 (0.52)</td>
<td>1 (0.44)</td>
<td>A (0.15)</td>
<td>1 (0.76)</td>
<td>1 (0.69)</td>
<td>A (0.77)</td>
<td>2 (0.22)</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

Numbers in parentheses, frequencies of the indicated phenotype (genotype in the case of codominant allozyme alleles) in human populations. The probability that 2 independently derived lines have the same genotype by chance is estimated by multiplication of the individual genotypic frequencies and is presented for each cell line in the last column.

Table 2
Steroid hormone receptors in human breast cancer cell lines

<table>
<thead>
<tr>
<th>Receptor concentration (fmol/mg cytoplasmic protein)</th>
<th>Binding constant (nm)</th>
<th>Correlation coefficient</th>
<th>Receptor concentration</th>
<th>Binding constant</th>
<th>Correlation coefficient</th>
<th>Receptor concentration</th>
<th>Binding constant</th>
<th>Correlation coefficient</th>
<th>Receptor concentration</th>
<th>Binding constant</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines</td>
<td>ZR-75-1</td>
<td>29.0</td>
<td>1.3</td>
<td>0.982</td>
<td>34.0</td>
<td>0.53</td>
<td>0.911</td>
<td>74.0</td>
<td>43.0</td>
<td>3.7</td>
<td>0.964</td>
</tr>
<tr>
<td></td>
<td>ZR-75-27</td>
<td>18.0</td>
<td>6.4</td>
<td>0.994</td>
<td>60.0</td>
<td>0.31</td>
<td>0.999</td>
<td>51.0</td>
<td>14.0</td>
<td>0.57</td>
<td>0.942</td>
</tr>
<tr>
<td></td>
<td>ZR-75-30</td>
<td>7.3</td>
<td>1.07</td>
<td>0.999</td>
<td>89.7</td>
<td>3.70</td>
<td>0.981</td>
<td>100.6</td>
<td>20.1</td>
<td>0.94</td>
<td>0.994</td>
</tr>
</tbody>
</table>

Equilibrium dissociation constant.

Calculated according to the least squares method.

Evidence for functional differentiation of the cell lines is provided by studies of their responsiveness to steroid hormones. Each line possesses specific receptors for glucocorticoids, estrogen, and other sex steroids. Moreover, ZR-75-1 cells are specifically stimulated by estrogen, as well as by insulin (16, 18, 24), and are specifically inhibited by glucocorticoid and androgen. ZR-75-27 cells are stimulated by both estrogen and progesterone and, to a lesser degree, by dihydrotestosterone. For ZR-75-30 cells we have been able to demonstrate significant increases above control for progesterone only. However, the data suggest that the 2 sister cell lines may have similar if not identical responsiveness to other hormones. For example, estrogen stimulation...
of 25% above control was recorded for both ZR-75-27 and ZR-75-30 cells, but a statistically significant response by the ZR-75-30 cells may have been obscured by experimental variation.

Additional evidence for functional differentiation in our 3 breast cell lines was sought by means of assaying for casein and \( \alpha \)-lactalbumin, but these results were uniformly negative. Casein synthesis has not been detected by biochemical or immunological methods in any breast cancer cell in continuous culture (17). Furthermore, it appears that synthesis of \( \alpha \)-lactalbumin is not a sine qua non criterion of mammary origin. Kleinberg et al. (9) have reported that, while \( \alpha \)-lactalbumin was detected by radioimmunossay in 48.5% of histologically normal breast tissues, only 20% of breast cancer tissues were positive. These data suggest that some breast tumors may lose their capacity to synthesize \( \alpha \)-lactalbumin. Moreover, \( \alpha \)-lactalbumin has been detected in some nonbreast tissues, including amniotic fluid, cord blood, and uterus, suggesting the possibility of extra-mammary sources of this protein. Results of assays on breast cancer cells in continuous culture have been variable. Generally, only low levels of \( \alpha \)-lactalbumin have been detected, and even within a single laboratory repeated assays on a single cell line have not been consistently positive (9, 25, 27). Only for MCF-7 cells has production of this protein been confirmed independently in several laboratories.

In summary, the 3 malignant cell lines here reported are distinct from each other and are of human mammary origin according to the following criteria: (a) interspecies specificity, established by their human karyotype; (b) intraspecies specificity, demonstrated by genetic evidence supporting the origin of each cell line from different human donors, each having a genetic signature distinct from HeLa cells; (c) organ specificity, supported by morphological evidence of epithelial structure and secretory activity and especially by the expression of differentiated functions, in this case the presence of 4 classes of steroid hormone receptors and hormonal responsiveness.

Previous reports have described purported breast cell lines of human, non-HeLa origin and epithelial morphology as revealed by electron microscopy (2, 4, 6, 8, 11, 22, 28, 31). Differentiated function of these cells has generally been less convincingly demonstrated, and only MCF-7 cells have been shown to contain 4 classes of steroid hormone receptors and to be hormonally responsive (16). In some other breast cell lines, synthesis of \( \alpha \)-lactalbumin has been reported, but the significance of these results is at present unclear. Additional human breast cancer cell lines have been described, but their characterization in published reports is often insufficient for judging the legitimacy of their pedigrees. Many purported breast cell lines are in reality HeLa cells or are of nonhuman origin (19, 20).

Since no single characteristic affords conclusive evidence of human mammary origin, the most valuable breast cancer cell lines are those that have been the most thoroughly characterized. Until recently, MCF-7 cells provided the only \textit{in vitro} system for the study of hormone responsiveness in human breast cancer. The ZR-75-1 cell has already proven useful as a model system for the study of both estrogen and insulin responsiveness (14, 16, 18, 24). The availability now of 3 new \textit{bona fide} human breast cancer cell lines containing 4 classes of steroid hormone receptors, each of which is also hormonally responsive, should add to the value of these cells as research tools.

**ACKNOWLEDGMENTS**

We are grateful for the technical assistance of Thanda Wai, the expert assistance of Dr. Ann Cheng in arranging the trypsin-Giemsas-bandied karyotypes, and the unstinting help of Sue Hostler in preparing the manuscript.

**REFERENCES**

23. O'Brien, S. J., Kleiner, G., Olson, R., and Shannon, J. E. Enzyme...

Fig. 1. Photomicrographs of cultured ZR-75-1 cells and the parental tumor. A, infiltrating duct carcinoma cells in excised tumor. Hematoxylin & eosin. B, exfoliated carcinoma cells in pleural fluid. Papanicolaou stain. Cells are rounded and noncohesive and exhibit occasional nuclear molding. Nuclei are large and have 1 to 4 chromocenters. C, carcinoma cells in culture. Hematoxylin & eosin. Cells attached to the plastic surface show polygonal configurations that are typically epithelial. The characteristics of the nuclear chromatin resemble the carcinoma cells in the original tumor and in the exfoliated cells. Secretory vacuoles are seen in the cytoplasm of several cells. × 900.

Fig. 2. Photomicrographs of ZR-75-27 and ZR-75-30 cells in culture and the parental tumor. Hematoxylin & eosin. × 900. A, metastatic carcinoma cells in a lymphatic vessel at autopsy; B, ZR-75-27 cells in culture; C, ZR-75-30 cells in culture. The 2 cell lines resemble each other as well as the original tumor cells in their nuclear to cytoplasmic ratios and in the characteristics of their nuclear chromatin. Secretory vacuoles are present in the cytoplasm of occasional cultured cells and original tumor cells.
Figures 3 to 5. Electron micrographs of mammary carcinoma tissue culture cells. Uranyl acetate and lead citrate.

Fig. 3. ZR-75-1 cells showing typical duct-like lumen (L) formed by cells arranged in a rosette. An intracytoplasmic duct-like vacuole (IDV) is present in 1 cell. Bundles of tonofibrils (T) are prominent. Desmosomes (arrows) are connecting cells. × 6,900. Inset, desmosomes present in ZR-75-30 cells. × 23,000.
Breast Carcinoma Cells

Fig. 4. ZR-75-1 cell. Large intracytoplasmic duct-like vacuole (IDV) containing secretory material (arrows) and cross-sections of microvilli. The nucleus is large and irregular with a prominent eccentric nucleolus. × 4,600. Inset, secretory material in vacuole. × 13,800.

Fig. 5. ZR-75-1 cell. Apical border of cell displaying numerous secretory granules of varying densities. × 18,000.
Fig. 6. Karyotype of ZR-75-1 cell, passage 67, with 73 chromosomes, trypsin-Giemsa banding method. At least 1 complement of normal human chromosomes is present, but most chromosomes are in multiple copies. Rearranged "marker" chromosomes are labeled $m_1$ to $m_{10}$. 
Fig. 7. Karyotype of ZR-75-27 cell, passage 20, with 78 chromosomes. Trypsin-Giemsa banding method. Chromosomes 13 and 14 are not present. At least 1 complement of the remaining normal human chromosomes is present, but most are in multiple copies. Rearranged marker chromosomes are labeled m, to m14.
Fig. 8. Karyotype of ZR-75-30 cell, passage 21, with 80 chromosomes. Trypsin-Giemsa banding method. Chromosomes 13 and 21 are missing. At least 1 complement of all other normal human chromosomes is present, but most are present in multiple copies. Rearranged marker chromosomes are labeled m₁ to m₁₀. Identification of 2 chromosomes is uncertain.
Establishment and Characterization of Three New Continuous Cell Lines Derived from Human Breast Carcinomas

Linda W. Engel, Nathaniel A. Young, Tommie Sue Tralka, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/10/3352

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/38/10/3352.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.