Steroid Receptor Analyses of Nine Human Breast Cancer Cell Lines

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ABSTRACT

Nine human breast cancer cell lines in permanent tissue culture and currently available to researchers have been assayed for their content of cytoplasmic estrogen receptors, progesterone receptors, androgen receptors, and glucocorticoid receptors, as well as for the presence of unfilled or hormone-filled nuclear estrogen receptors.

Receptor distribution varied considerably among the nine lines and differed from the expected distribution predicted from solid tumors. We find that estrogen receptor, when present, is usually localized in the nucleus as unfilled nuclear estrogen receptor. Progestosterone receptor is correlated with presence of unfilled nuclear estrogen receptor. Glucocorticoid receptors are ubiquitous; they were found in all cell lines tested. The distribution of androgen receptor and progesterone receptor differed, suggesting that these proteins are dissimilar.

INTRODUCTION

It has been known for many years that breast tumors may be either hormone dependent or autonomous. However, it has only recently been demonstrated that these 2 groups can be distinguished, at least in part, on the basis of the presence or absence of cellular hormone receptor proteins. The ER has been the most widely studied and is most widely applied as a marker of hormone dependence (14).

Recently, we suggested that the presence or absence of PGR might improve the accuracy of the ER assay in predicting hormone-responsive tumors (9). This follows from the observation that PGR is synthesized under control of estradiol (4) so that presence of PGR in a tumor indicates that a part, at least, of the estrogen response system is operating normally in that cell.

Since breast tumors occasionally regress in response to other steroid hormones, namely, androgens and glucocorticoids, proposals have surfaced suggesting use of receptor proteins for these steroids as markers (5, 16, 20).

Steroid receptors are not restricted to the cytoplasm alone. If the cell is exposed to a steroid hormone, the appropriate cytoplasmic receptor complexed to the hormone translocates to the nucleus, followed presumably by gene activation (10). In our search for estrogen receptors in the nuclei of human breast tumor cells in culture and in solid tumors, we have shown that both RNE and RN sites are present (6, 21). Clearly, this opens a new area in studies of the relationship between hormone receptors and hormone-dependent tumors. A tumor might be classified appropriately as negative on the basis of cytoplasmic assays if nuclear receptors, both filled and unfilled, are not considered as well.

Breast cancer-derived cell lines are ideal model systems for studying the mechanisms of steroid action in tumors. In this paper we report the distribution of the 4 common cytoplasmic steroid receptors, as well as both RNE and RN in 9 human tumor cell lines, which are available to researchers. Our studies show that each of these lines is distinctly different with respect to receptor content. Because of the type of receptors that they do or do not contain, we suggest that certain lines might be more useful than others to answer very specific questions about the roles of steroid hormones in breast cancer.

MATERIALS AND METHODS

Cells. The cells used in this study are described in Table 1. Cells plated in Falcon plastic T-150 flasks (150 sq cm) were shipped to San Antonio from the human cell culture bank of the EG and G/Mason Research Institute, Md., in their routine growth medium (Table 1). Before shipment, routine sera were removed and replaced by 5% calf serum stripped of endogenous steroids by 30 min incubation at 45° with a dextran-coated charcoal pellet (0.25% Norit A and 0.0025% dextran in 0.01 M Tris-HCl, pH 8.0, at 4°, 1 ml/ml serum).

In our laboratory cells were maintained approximately 7 to 10 days on the same medium in 5% CO₂ in air at 37° until they were harvested when approximately 90% confluent. Each cell line was assayed once.

Cell Harvest. Cells were removed by a 10-min incubation at 37° with 1 mm EDTA in Ca²⁺, Mg²⁺-free Hanks’ balanced salt solution, and washed once with Hanks’ balanced salt solution at 4° and once in phosphate buffer (5 mM sodium phosphate, pH 7.4, at 4°, 10 mm thiglycerol, and 10% glycerol).

Cytosols and Nuclear Extracts. Cells were resuspended in phosphate buffer (1 ml/ml packed cells) and homogenized in a Teflon-glass homogenizer (Kontes Glass Co., Vineland, N. J.) until they were more than 90% disrupted as determined by trypan blue exclusion and phase-contrast microscopy.

The homogenate was centrifuged 800 x g for 10 min; the supernatant plus 2 nuclear pellet washes, each with 1 ml of phosphate buffer, were combined. This crude cytosol was centrifuged at 105,000 x g for 30 min in a Beckman 75 Ti rotor, and the supernatant (cytosol) was used immediately.

The washed, crude nuclear pellet was resuspended in 1
Steroid Receptors in Breast Cancer Cell Lines

Table 1

<table>
<thead>
<tr>
<th>Designation and refs.</th>
<th>Tissue of origin</th>
<th>Investigator and laboratory of origin</th>
<th>Culture medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. SW 613</td>
<td>Breast tumor tissue 1° adenocarcinoma</td>
<td>A. Leibovitz Scott and White Clinic Temple, Texas</td>
<td>L-15, 10% FBS; 10% bovine amniotic fluid, insulin, 10 μg; glutathione, 16 μg/ml; 2 mm glutamine; penicillin, 50 units/ml; and streptomycin, 50 μg/ml</td>
</tr>
<tr>
<td>2. MDA MB 175 (3)</td>
<td>Breast tumor pleural effusion, infiltrating duct mammary cancer</td>
<td>Reïda Cailleau M. D. Anderson Hospital Houston, Texas</td>
<td>L-15, 10% FBS; 10 μg insulin; glutathione, 16 μg/ml; 2 mm glutamine; penicillin, 50 units/ml; and streptomycin, 50 μg/ml</td>
</tr>
<tr>
<td>3. T47D</td>
<td>Pleural effusion, infiltrating ductal cancer</td>
<td>Iafa Keydar Tel Aviv University, Tel Aviv, Israel</td>
<td>RPMI 1640; 10% FBS; insulin, 0.2 IU/ml; 2 mm glutamine; penicillin, 50 units/ml; and streptomycin, 50 μg/ml</td>
</tr>
<tr>
<td>4. HBL-100</td>
<td>Normal human breast lactating milk</td>
<td>Edwin V. Gaffney Pennsylvania State University, State College, Pa.</td>
<td>McCoy's 5A modified plus 10% FBS; 2 mm glutamine; penicillin, 50 units/ml; and streptomycin, 50 μg/ml</td>
</tr>
<tr>
<td>5. MDA MB 361 (3)</td>
<td>Brain tumor, metastatic from breast</td>
<td>Reïda Cailleau M. D. Anderson Hospital Houston, Texas</td>
<td>L-15; 10 to 15% FBS; insulin, 10 μg/ml; glutathione, 15 μg/ml; 10% bovine amniotic fluid, 2 mm glutamine; penicillin, 50 units/ml; and streptomycin, 50 μg/ml</td>
</tr>
<tr>
<td>6. MDA MB 231 (3)</td>
<td>Pleural effusion, breast cancer</td>
<td>Reïda Cailleau M. D. Anderson Hospital Houston, Texas</td>
<td>Same as MDA MB 175</td>
</tr>
<tr>
<td>7. MCF-7 (19)</td>
<td>Pleural effusion, scirrhous cancer of the breast</td>
<td>H. D. Soule Michigan Cancer Foundation Detroit, Mich.</td>
<td>Same as HBL-100 plus insulin, 10 μg/ml</td>
</tr>
<tr>
<td>8. MCF-7 (7, 19)</td>
<td>Pleural effusion, scirrhous cancer of the breast</td>
<td>H. D. Soule Michigan Cancer Foundation Detroit, Mich.</td>
<td>Eagle's minimal essential medium with Earle's balanced salt solution, nonessential amino acids (1%); 2 mm glutamine, insulin, 0.006 μg/ml; 10% calf serum; gentamycin, 25 μg/ml</td>
</tr>
<tr>
<td>9. BT 20 (11)</td>
<td>Breast cancer, infiltrating ductal</td>
<td>E. Y. Lasfargues Institute for Medical Research, Camden, N. J.</td>
<td>RPMI 1640; 10 to 20% FBS; insulin, 10 μg/ml; penicillin, 50 units/ml; and streptomycin, 50 μg/ml</td>
</tr>
</tbody>
</table>

* FBS, fetal bovine serum.

Steroid receptors in breast cancer cell lines were assayed by sucrose density gradient as previously described (7).

R-5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione[6,7-3H], 51.4 Ci/mmol; Roussel-Uclaf), dexamethasone (9-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione[1,2,4-3H], 10 Ci/mmol; Schwartz/Mann, Orangeburg, N. Y.), 17β[2,4,6,7-3H]estradiol (114 Ci/mmol; New England Nuclear, Boston, Mass.) or 5α[1,2,4,5,6,7-3H]dihydrotestosterone (175 Ci/mmol; Amersham/Searle, Arlington Heights, Ill.) were added in 2 μl of ethanol to 250 μl of cytosol and incubated for 4 hr at 4°. Final concentrations were: estradiol, 4 nM; R5020, 20 nM; dihydrotestosterone, 20 nM; dexamethasone, 50 nM. Parallel samples were preincubated for 15 min with 100-fold excess unlabeled hormone [R5020, diethylstilbestrol (4,4'-dihydroxy-α,β-diethylstilbene), dexamethasone, or 5α-dihydrotestosterone] added in 1 μl of ethanol. Pellets were prepared from a 1-ml suspension of dextran-coated charcoal by a 10-min centrifugation at 2000 × g. The charged cytosol was transferred onto the pellet, mixed, incubated for 10 min to adsorb unbound radioactivity, and pelleted. A 200-μl aliquot of the supernatant was layered onto a 5 to 20% sucrose gradient prepared in the homogenization buffer in polyallomer tubes. 14C-Labeled bovine serum albumin (17), 1500 cpm/10 μl buffer, was added to each gradient as an internal marker. Gradients were centrifuged in a Beckman SW 60 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) at 53,000 rpm for 16.3 hr. Four-drop fractions were collected and counted in 5 ml of modified Bray's solution (1). Cytosol protein concentration was measured by the method of Lowry et al. (13). Nuclear Estrogen Receptors. Nuclear extracts were di-
luted 8-fold with phosphate buffer, 500-μl aliquots were precipitated with 250 μl of a 1 mg/ml concentration of protamine sulfate (USP injection without phenol preservative; Eli Lilly, Co., Indianapolis, Ind.), and then incubated with [3H]estradiol with doses ranging from $4 \times 10^{-11}$ M to $10^{-8}$ M. Parallel samples contained [3H]estradiol plus diethylstilbestrol (10^{-6} M) to assess nonspecific binding. Total (estradiol-filled plus unfilled sites) were determined by incubation at 30°, 5 hr; unfilled sites (RN) were measured by binding at 4°. The difference yields the values for estradiol-filled sites (RNE).

Following incubation, tubes were washed twice with phosphate buffer, extracted, and counted directly in 5 ml of toluene scintillation fluor (4.0 g of POPOP and 1 liter of toluene) in a Beckman LS 233 counter with a counting efficiency of 50%. The data were analyzed by the method of Scatchard (18) following subtraction of nonspecific binding.

### RESULTS

The RC, PGR, androgen receptor, and GLUCR content of each cell line, as well as the free and bound nuclear estrogen receptor levels, are shown in Table 2. Cytoplasmic receptor values are considered negative with 8S peaks less than 3 fmol/mg protein, equivocal if 8S is between 3 and 10, and positive if 8S is greater than 10. Our interpretation of the data is summarized in Table 3.

There are several salient features to be noted. (a) All but one of these cell lines (MCF-7, obtained from Dr. Herbert Soule and maintained in our laboratory for 3 years) have little or no cytoplasmic ER. However, in addition to our MCF-7 cells, 3 of these RC negative lines (T47D, MDA MB 361, and MCF-7, Mason) contain appreciable levels of RN. Curiously, the same 4 lines are the only ones that contain PGR. (b) One line (MDA MB 175) has only bound RNE. The nature of the ligand is unknown since free steroids were presumably removed from the growth medium, but it must have a relatively high affinity for the receptor since no exchange is observed during incubation at 4°. This rules out the possibility that the ligand is one of the weaker estrogens (estriol or estrone) or a known antiestrogen or androgen. All these compounds, although they bind nuclear ER, have an affinity sufficiently low (at least 5- to 10-fold lower than estradiol) that some exchange is observed at 4°. No PGR was demonstrable in this cell line. (c) Every line assayed contains GLUCR. (d) Six of the 9 cell lines have androgen receptor; 2 of these do not have PGR.

### DISCUSSION

The absence of cytoplasmic ER from all but one of these cell lines is surprising. Judging from the distribution of RC in solid human tumors (14), we would have expected 50 to 60% to be positive. If nuclear receptors are also considered, then the number of ER-positive lines (5 of 9) equal the predicted value. It is tempting to speculate that the nuclear localization of the receptor is a result of tissue culture conditions. Nothing is known of the effects of different sera, for instance, on receptor distribution. Were these receptors in the cytoplasm in the tissues of origin? Conversely, it is possible that culture conditions stabilize the receptor in situ, that its usual cytoplasmic position is somehow artifactual. Most attempts to establish tissue culture cell lines from human breast cancer specimens

### Table 3

<table>
<thead>
<tr>
<th>Cell line</th>
<th>ER</th>
<th>PGR</th>
<th>GLUCR</th>
<th>Androgen receptor</th>
<th>Nuclear ER (fmol/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8S</td>
<td>4S</td>
<td>Total</td>
<td>8S</td>
<td>4S</td>
</tr>
<tr>
<td>1. SW 613</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>2. T47D</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1221</td>
<td>1221</td>
</tr>
<tr>
<td>3. MDA MB 175</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>4. HBL-100</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>5. MB 361</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>97</td>
<td>78</td>
</tr>
<tr>
<td>6. MDA MB-231</td>
<td>0</td>
<td>56</td>
<td>56</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7. MCF-7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>19</td>
<td>52</td>
</tr>
<tr>
<td>8. MCF-7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93</td>
<td>0</td>
<td>93</td>
<td>240</td>
<td>60</td>
</tr>
<tr>
<td>9. BT-20</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> The 2S form.
<sup>b</sup> MCF-7 EG and G/Mason Research Institute stocks.
<sup>c</sup> MCF-7 San Antonio stock Passage 117 to 124.
have been unsuccessful. It is thus possible that the presence of RC is somehow inimical to successful adaptation and that the established lines represent selection of a unique cell type from an original heterogeneous cell population.

Garola and McGuire (6) have recently demonstrated free nuclear ER in solid human breast tumors. In that series RN is always associated with measurable levels of cytoplasmic receptors. Since the present data show that in cell lines RN can exist without RC, then extrapolation to solid tumors would suggest that measurement of ER in one or another subcellular compartment alone may result in tumor misclassification. Although some tumors may have both cytoplasmic and nuclear receptors, others may have only one or the other. Moreover, measurement of RN alone may fail to detect receptor in a tumor that contains only RNE (MDA MB 175).

The correlation between the presence of PGR and RN is interesting. We have recently shown that our MCF-7 cells, which have only low PGR levels in the absence of estradiol, respond to estradiol or antiestrogen treatment with marked induction of PGR. Is RN involved in the maintenance of basal PGR? Can estradiol induce higher levels in the absence of RC (i.e., MDA MB 361) or is RC required? Our data with MCF-7 suggest that both RC and RN may be involved in PGR induction by estradiol, while PGR induction by an antiestrogen may involve only RC (8).

Similar questions can be raised about the mitogenic effects of estrogens. It is possible that estradiol enhances growth in cells that contain RN but fails to do so in those that contain no ER or only RC. Clearly, judicious selection of cell lines for these and similar studies would greatly enhance our understanding of the mechanisms involved in estrogen control of growth and specific protein synthesis.

Little is known about the role of androgen receptors in human breast tumors. MCF-7 cells have been shown to be androgen responsive (12). However, this effect of androgens appears to be mediated through the ER system (22). That 2 of the cell lines in this series (SW 613 and MDA MB 175) have androgen but no PGR supports the conclusion that these 2 proteins are distinct entities.

The ubiquitous presence of GLUCR in these cell lines requires further comment. When solid human breast tumors are assayed for GLUCR, only 15 to 30% contain the receptor (15, 20). Yet in culture all the cells in our series, and 7 of 10 cells in a series assayed by Lippman et al. (C. K. Osborne, personal communication) are positive. Again, this extraordinary frequency of GLUCR may reflect adaptation or selection of cells in culture. The discrepancy between data obtained directly from human tumor biopsy specimens and those obtained from cells in culture suggests that, despite the obvious advantages that an in vitro model system provides, definitive conclusions about the use of steroid receptors as markers of hormone dependence or their role in steroid hormone action require parallel studies in intact systems.

ACKNOWLEDGMENTS

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REFERENCES

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