ABSTRACT

The qualitative and quantitative relationships between cytoplasmic estrogen receptors (ERC), total nuclear estrogen receptors (ERN), and cytoplasmic progesterone receptors (PGR) in 74 primary and 23 metastatic human breast cancer tissues were studied. A positive correlation between the age of the patients and the receptor concentration was found only for ERC. Although ERN and PGR were more frequent in tumors with a higher level of ERC, there was no significant correlation between concentrations of either ERN or PGR and ERC. However, PGR were more frequent in ERN-positive than in ERN-negative tumors, irrespective of the presence of ERC. There was also a highly significant correlation between PGR and ERC concentrations. These findings support the assumption that induction of PGR by estrogen in human breast cancer is mediated by a mechanism involving nuclear receptors. Therefore, the ERN assay might increase the validity of steroid receptor determination for prediction of hormonal sensitivity of human breast cancer.

INTRODUCTION

Although the ERC\(^3\) is not an unequivocal indicator of hormonal sensitivity of a tumor tissue, it is generally accepted that the ERC assay is useful for prediction of the response of breast cancer to hormonal therapy. Only some (50 to 60%) of the ERC-positive and a few (less than 10%) of the ERC-negative tumors respond to hormonal therapy (18), and several hypotheses were offered to explain this. It seems that the tumor cells are heterogeneous with respect to the ERC content, and it was shown that the response to hormonal therapy increases with the increase of the ERC level (21). It is possible that the mechanism of the estrogen action is disturbed in malignant cells. The defect may be at the level of translocation of the estrogen-receptor complex to the nucleus, or subsequent biochemical events might be blocked (20). Detection of the nuclear estrogen receptors (6), or of the nuclear estrogen content (13), was proposed as an additional test for assessment of hormonal sensitivity of breast cancer. After a failure to find ERN, it would be possible to reveal the hypothetical ERC-positive tumors, which are resistant to hormones due to impaired estrogen receptor translocation. A certain relation between the endogenous estrogen level and the tumor ERC content may result in depletion of cytosol of ERC due to translocation to the nucleus.

If the ERC assay detects such tumors as ERC-negative ones, ERN determination should show estrogen receptor in the nuclear compartment (19).

The apparent product of estrogen action is PGR. It was thus proposed to measure PGR as a marker for the integrity of the mechanism of estrogen action (11), and a high rate of response to hormonal therapy in breast cancer with both ERC and PGR was found (2, 4, 9, 16).

In this paper, we describe the results of the studies on qualitative and quantitative relationships of ERC, ERN, and PGR in human breast cancer.

MATERIALS AND METHODS

Tissue samples from 74 primary and 23 metastatic breast cancers were placed on ice immediately after excision during mastectomy or biopsy. Within 30 min, they were frozen in liquid nitrogen and stored in it for no longer than 1 week. The methods for measuring ERC, ERN, and PGR were based on those of McGuire (17), Gorlita and McGuire (6), and Horwitz and McGuire (8), respectively.

\[2,4,6,7-^3H\]Estradiol (specific activity, 85 to 110 Ci/mmol) and \[^3H\]R5020 (specific activity, 87 Ci/mmol) were supplied by the Radiochemical Centre, Amersham, United Kingdom, and New England Nuclear, Boston, Mass., respectively.

Frozen tissue was pulverized in a liquid nitrogen-cooled metal mortar and then homogenized with 4 volumes of phosphate buffer [5 mM sodium phosphate (pH 7.4)-1 mM monothioglycerol-10% (v/v) glycerol], using a Polytron PT10 homogenizer with Setting 2 and 3 \(\times\) 20-sec bursts. The homogenization and all subsequent procedures were performed at 4\(^\circ\)C if not stated otherwise. The homogenate was centrifuged at 800 \(\times\) g for 10 min to obtain the nuclear pellet. The pellet was washed twice by resuspension in phosphate buffer and by centrifugation at 800 \(\times\) g for 10 min. All supernatants were pooled and centrifuged at 260,000 \(\times\) g for 1 hr to isolate the cytosol fraction.

To measure the ERC, the cytosol was incubated in duplicate for 18 hr with \[^3H\]estradiol in final concentrations of 50 to 800 pm. The number of binding sites was calculated by Scatchard analysis of bound hormones, after adsorption of the free hormone on DCC. The results were corrected for nonspecific binding, calculated from the binding of \[^3H\]estradiol (final concentration, 800 pm) in the presence of a 200-fold excess of diethylstilbestrol (5).

To measure ERN, the nuclear pellet was extracted with a buffer containing 0.5 M KCl. The unoccupied and the total binding sites were measured by incubation in triplicate of receptors adsorbed on hydroxylapatite with \[^3H\]estradiol (final concentration, 1 nm) at 4\(^\circ\)C and 30\(^\circ\)C, respectively, during 3 hr, with or without a 100-fold excess of diethylstilbestrol. PGR were measured by the one-point assay, incubating...
cytosol in triplicate with [3H]R5020 (final concentration, 8 nM) with or without a 200-fold excess of cold R5020. The free [3H]R5020 was separated by adsorption on DCC. When a sufficient quantity of tumor tissue was available, Scatchard analysis was performed using the same method as for ERC, except that the concentrations of ligands ([3H]R5020 and cold R5020) were 10-fold higher. In some instances, the sucrose density gradient analysis (8) was performed using polycarbonate tubes and a 6- x 4.2-ml MSE titanium swing-out rotor. The gradients were centrifuged at 4° and 350,000 x g for 12 hr. Recently, in addition to the DCC single-point assay, the hydroxylapatite assay for PGR was used (24).

The sensitivity of the methods was such that the tumors with a binding capacity lower than 3 fmol/mg of cytosol or nuclear extract protein for ERC or ERN, respectively, and lower than 10 fmol/mg of cytosol protein for PGR were considered negative.

RESULTS

Among the 97 samples of primary and metastatic breast cancer tissues, ERC were found in 55%, ERN in 38%, and PGR in 49% (Table 1). The receptor content varied from 3 to 3440 fmol/mg of the cytosol proteins for ERC, from 3 to 50 fmol/mg of the nuclear extract proteins for ERN, and from 10 to 310 fmol/mg of the cytosol proteins for PGR. The results for PGR, obtained by the single-point assay, agree well with those of the Scatchard and the hydroxylapatite analyses and with the 8S fraction of the sucrose density gradient.

ERN refers to total nuclear estradiol-binding sites. Unoccupied nuclear estradiol receptors were found in 19% of the ERN-positive tumor tissue (Chart 1), always parallel with the occupied binding sites.

Both ERN and PGR were present more frequently in the ERC-positive than in the ERC-negative group of tumors. Also, the percentage of both ERN and PGR increases with the increase of ERC content in the tumors (Table 2). The relationship of ERC content, presence of ERN and PGR, and the age of patients are shown on Chart 2. There is a positive correlation between the age of patients and the ERC content. The proportion of ERC-positive tumors also containing ERN or PGR does not vary with the age of the patients. There are similar percentages of ERC-positive tumors with ERN positivity and PGR positivity in groups of patients under and over the age of 50 (55 and 66% under versus 58 and 64% over). In our patients, we did not find a statistically significant association between either ERN or PGR contents and the age of patients.

The frequency of various combinations of ERC, ERN, and PGR are presented in Table 3. A great majority of the ERN-positive tumors contain also PGR, irrespective of the presence of ERC, but only one-third of the ERC-positive tumors without PGR have PGR. PGR is also present in some tumors negative for ERC and ERN.

A more detailed insight into the relationship between ERC, ERN, and PGR was obtained with the quantitative correlation analysis. In Chart 3, the concentration of ERN is plotted against that of ERC, and the concentrations of ERN in ERC-negative and ERN in ERN-negative tumors are presented. The correlation analysis did not show a statistically significant association between the ERN and ERC levels. In tumors containing only ERC or ERN, the receptors were present in a concentration range similar to that of tumors positive for both ERC and ERN.

The quantitative relation between PGR and ERC and levels of PGR in ERC-negative and ERN in PGR-negative tumors is shown in Chart 4. There was no statistically significant correlation between concentrations of PGR and ERC. Although the range of the PGR concentrations was similar in both ERC-positive and ERC-negative tumors, the PGR content tended to be higher in the former than in the latter (54% in ERC-positive and 25% in ERC-negative values for PGR were higher than 50 fmol/mg protein). The range of ERC concentrations was lower in PGR-negative than in PGR-positive tumors.

However, analyzing the relationship between the concentrations of ERN and PGR (Chart 5), we found a highly significant positive correlation between levels of these receptors. The quantitative relationship between PGR and ERN is linear with the regression equation: \( y = 16.7 + 5.2x \).

DISCUSSION

Although several groups of authors have emphasized the importance of analyzing the functional ability of the estrogen receptor system for the assessment of hormonal sensitivity of human breast cancer (11, 13), the relationship, particularly quantitative, between ERC, ERN, and PGR has not been studied in detail. Many authors found that breast cancer tissue from menopausal women contains on an average more ERC than that from premenopausal patients (7, 12, 22). For PGR, some
Table 2

Relation of ERN and progesterone receptors to ERC in human breast cancer
ERC-positive > 3 fmol/mg cytosol protein; ERN-positive > 3 fmol/mg nuclear protein; PGR-positive > 10 fmol/mg cytosol protein.

<table>
<thead>
<tr>
<th>ERC-positive total</th>
<th>ERC-negative 3–10</th>
<th>ERC-negative 11–100</th>
<th>ERC-negative &gt;100</th>
<th>ERC-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERN-positive</td>
<td>6/44 (14)*</td>
<td>10/22 (45)</td>
<td>14/23 (61)</td>
<td>7/8 (88)</td>
</tr>
<tr>
<td>PGR-positive</td>
<td>12/44 (27)</td>
<td>16/22 (55)</td>
<td>15/23 (65)</td>
<td>8/8 (100)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Table 3

Relation of progesterone receptors to ERC and ERN in human breast cancer
ERC-positive > 3 fmol/mg cytosol protein; ERN-positive > 3 fmol/mg nuclear protein; PGR-positive > 10 fmol/mg cytosol protein.

<table>
<thead>
<tr>
<th>PGR-positive</th>
<th>ERC-positive</th>
<th>ERC-negative</th>
<th>ERC-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGR-negative</td>
<td>28/31 (90)*</td>
<td>5/6 (83)</td>
<td>7/22 (32)</td>
</tr>
<tr>
<td>PGR-positive</td>
<td>7/38 (18)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Chart 2. Age distribution of ERC in human breast cancer. Correlation coefficient, 0.27; p < 0.05. Presence of ERN and PGR is shown.

Chart 3. Quantitative relationship between ERC and total ERN in human breast cancer. Correlation coefficient, 0.089; p < 0.53. Concentrations of ERC in ERN-negative and ERN in ERC-negative tumors are shown along x and y axes, respectively.

Chart 4. Quantitative relationship between ERC and PGR in human breast cancer. Correlation coefficient, 0.084; p < 0.96. Concentrations of ERC in PGR-negative and PGR in ERC-negative tumors are shown along x and y axes, respectively.

Authors found a higher level in premenopausal women (14, 25), whereas others found no difference between the two groups (1). A positive correlation between the patient’s age and the ERC content (1) and a lack of such correlation for PGR have been reported (1, 3). Similar to this was the relationship between the age and the ERC and PGR content in our patients. In addition, we found no correlation between age and ERN content. Apparently, levels of circulating estrogens are sufficient for receptor translocation and PGR induction in both premenopausal and postmenopausal women (26).

Although we found ERN more frequently in the groups of tumors with a higher level of ERC, there was no quantitative correlation between these receptors. This lack of correlation probably reflects many factors involved in the estrogen action, including the hormone level and its effect on receptor synthesis, translocation and processing, and the functional ability of the receptors, which could be impaired in tumor tissue. Like others, we found ERN in some ERC-negative tumors (6, 15, 27). This is probably due to translocation of measurable receptors to the nucleus.

Considering the relationship between ERC and PGR, we found only a qualitative association but no quantitative corre-
In some tumors, we found PGR in absence of either ERN or both ERN and PGR. It is possible that the PGR synthesis in some tumors is independent of the estradiol receptor mechanism (2).

For understanding the relationship between various combinations of the receptor content and hormonal sensitivity of the human breast cancer, it is necessary to accumulate more clinical data on the response to hormonal therapy of tumors with various combinations of the receptors. In addition to ERC and PGR assays, determination of the nuclear estrogen receptor, demonstrating the integrity of the estrogen receptor system, could improve the predictive value of the steroid receptor assays for the assessment of hormonal sensitivity of the human breast cancer.

REFERENCES


Steroid Receptors in Human Breast Cancer


Relationship of Cytoplasmic and Nuclear Estrogen Receptors and Progesterone Receptors in Human Breast Cancer

Ranka Romic-Stojkovic and Stjepan Gamulin


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