Immunocytochemical Assay for Estrogen Receptor: Relationship to Outcome of Therapy in Patients with Advanced Breast Cancer

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Abstract

We have used an immunoperoxidase technique utilizing a monoclonal antibody to the estradiol receptor to identify immunoreactive estradiol receptor in breast carcinomas and have examined the relationship between the immunoreactive estradiol receptor and response to therapy in patients with advanced breast cancer.

Fifty-six patients were found to be assessable for response to endocrine therapy. Twenty-two showed an objective response to some form of endocrine manipulation, and all these had positively stained carcinomas. None of the 17 patients with negatively stained carcinomas responded to endocrine therapy.

We conclude that the monoclonal antibody to estradiol receptor can help identify breast cancer patients who may respond to endocrine therapy.

Introduction

Quantitative binding of estrogen and progesterone to tumor tissue is an inaccurate predictor of response to endocrine therapy in breast cancer. Thus, in the case of ER, nearly twice as many patients' tumor tissue will show significant binding as will show an objective response to endocrine therapy. The possibility exists, therefore, that the ER-ICA (1-3) may more accurately predict responding patients.

We have therefore used this assay to visualize the ER in tumor tissue obtained from patients who have subsequently received endocrine therapy for advanced breast cancer. This report describes the correlation between response to endocrine therapy and the receptor content visualized by ER-ICA and estradiol binding to tumor tissue ER as measured by a conventional DCC assay.

Patients, Materials, and Methods

Tumor Samples

Biopsy tissue was obtained from 56 patients, immediately snap frozen in liquid nitrogen, and stored in vapor phase liquid nitrogen for up to 6 yr prior to assay. Primary tumor tissue was obtained from 41 patients and metastatic skin nodule or lymph node tissue from the remaining 15 patients. All were histologically proven to be of breast cancer origin.

Seventeen patients were premenopausal, and 39 were postmenopausal. All have received various forms of endocrine therapy for treatment for advanced breast cancer (Table 1).

Patients were included in this study only if a biopsy specimen was taken at the time of primary surgery or, in the case of a biopsy from a metastatic lesion, prior to endocrine or cytotoxic chemotherapy. Furthermore, patients must have subsequently received some form of endocrine manipulation, drug or surgical, following a relapse at any site. An additional requirement was that the response to such treatment after relapse was assessable according to standard International Union against Cancer criteria (4).

Receptor Assay Methodology

DCC Steroid Binding Assay. ER assessment by the DCC technique is described in detail elsewhere (5). Briefly, 6 paired tissue cytosol aliquots are incubated with 6 known concentrations of tritium-labeled estradiol in the presence or absence of diethylstilbestrol, an unlabeled competitor for the receptor. The difference in radiolabel between noncompeted and competed tubes following the removal of unbound steroid with charcoal represents specifically bound radioligand. Multipoint assays such as this allow the calculation through Scatchard and Woolf analyses of specific receptor content, which is expressed in terms of fmol of receptor per mg of cytosol protein.

Protein estimations of appropriately diluted cytosol samples were performed using the Bio-Rad protein dye reagent method (6) with bovine serum albumin in PBS (<1 mg/ml) as standard.

ER-ICA. The monoclonal antibody H222 used in this study was raised in a male Lewis rat immunized with estradiol-receptor complex derived from the MCF-7 human breast cancer cell line (7). Similar antibodies have been shown to react with both complexed and free receptor (8) and to localize in the nuclei of various mammalian reproductive tissues (1), a phenomenon also observed in this study.

The localization of ER was performed using a PAP technique similar to the procedure described by Sternberger et al. (9), and it is described in some detail in other publications (Ref. 3; Footnote 4).

Two cryostat sections of each specimen (10 μm) on poly-L-lysine-coated glass slides were fixed for 10 min in 3.6% formaldehyde in 0.1 M PBS (pH 7.2) immediately after sectioning and were transferred to a PBS bath for at least 10 min. Sections were then placed in cold methanol (−20°C) for 4 min and then in cold acetone (−20°C) for 1 min before being returned to PBS. Slides were then incubated for 15 min with 2% normal goat serum in PBS to reduce nonspecific binding of primary antibody. H222 or a control antibody (normal rat immunoglobulin) with 2% normal goat serum in PBS was added dropwise to sections at a concentration of 20 μg/ml and incubated for 30 min. Sections were then washed twice with PBS for 5 min.

A bridging antibody, goat anti-rat IgG, in PBS was then added dropwise over the sections at a dilution of 1:100, and they were incubated for 30 min. Sections were then washed twice with PBS for 5 min each.

PAP complex at a dilution of 1:100 in 2% normal goat serum-PBS was added dropwise to cover sections which were further incubated for 30 min. Sections were again washed twice with PBS for 5 min, and the chromogen, diaminobenzidine tetrahydrochloride, and substrate solution (0.06% hydrogen peroxide in PBS) were mixed together and immediately added dropwise to the sections for 6 min.

Sections were subsequently washed in running tap water for 5 min and then counterstained for 5 s in 1:10 dilution of Harris hematoxylin in distilled water. After being washed for 5 min in running tap water, sections were dehydrated and mounted in xylene-soluble mountant.

The SII was calculated as follows:

\[
\text{SII} = \frac{\% \text{ of tumor cells stained at Intensity } A \times 0}{\% \text{ of tumor cells stained at Intensity } B \times 1} + \frac{\% \text{ of tumor cells stained at Intensity } C \times 2}{\% \text{ of tumor cells stained at Intensity } D \times 3}
\]

1 Presented at the Symposium on "Estrogen Receptor Determination with Monoclonal Antibodies," December 14, 1984, Monte Carlo.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: ER, estrogen receptor; ER-ICA, estrogen receptor immunocytochemical assay; DCC, dextran-coated charcoal; PBS, phosphate-buffered saline; PAP, peroxidase anti-peroxidase; SII, staining intensity index; iER, immunoreactive estrogen receptor.

of endocrine therapy was given to the 56 patients (Table 1). All staging at the time of presentation.

This compares with 41% and 74%, respectively, using the DCC method (182.5 fmol/mg of cytosol protein). It was not possible to reassess this tumor by ER-ICA since all remaining tissue was used for the DCC assay. A possible explanation for the discrepancy in this result may be drawn from the observations of Poulsen (10) who demonstrated intratumoral ER variations. ER-ICA may be subject to such variation, results being based on the staining of a single section. Statistical manipulation of results showed that combining the ER-ICA test with the DCC assay did not improve predictive value (Table 2). Furthermore, we were unable to show any clear predictive capacity for one method over the other regarding any one form of therapy, although ER-ICA appeared to predict slightly more accurately. Thus, for tamoxifen therapy 25 of 31 (81%) correct responses were predicted by ER-ICA, 22 of 31 (71%) by DCC, and for aminoglutethimide therapy 12 of 14 (86%) correct responses were predicted by ER-ICA and 11 of 14 (79%) by DCC, i.e., an improved prediction of 9.6% by ER-ICA for tamoxifen-treated patients and 7.1% for aminoglutethimide-treated patients. Table 3 illustrates the relative overall predictive abilities of the two techniques and shows that ER-ICA correctly classified (i.e., responding ER positives, or nonresponding ER negatives) 47 of 56 (83.9%) and the DCC 41 of 56 (73.2%).

Discussion

The results from this study indicate that the ER-ICA technique offers an excellent alternative to the DCC procedure and can be used to select patients with hormone-sensitive breast cancer. The techniques have similar predictive ability and correlate well in their determination of ER presence in tumors. The monoclonal antibody used here has been shown to be specific for ER by nitrocellulose blotting, distribution within frozen sections of normal tissue, and inhibition of staining by excess ER (1, 3). The present technique offers substantial technical advantages over the DCC technique and appears to have similar accuracy. However, the results indicate that, as with the DCC method, some patients whose tumors are shown to possess iER fail to respond to hormone therapy. Furthermore, when we examined this group of ERICA positive tumors specifically to compare the intensity of tissue staining and the percentage of positively stained cancer cells, no difference could be found between responders and nonresponders whose tumors contained iER. This confirms other studies which have shown that, for the DCC assay, there is no difference in the kinetics, affinity, or inhibition of binding of labeled estradiol in patients

Table 2 Immunocytochemical stain for estrogen receptor: relationship to menopausal status and value of combining with DCC results

This table illustrates the distribution of ER-ICA and DCC ER-rich tumors by menopausal status against response to endocrine therapy and demonstrates that ER-ICA can predict outcome of therapy slightly better than DCC in both premenopausal (15 of 17 versus 11 of 17) and postmenopausal (32 of 39 versus 30 of 39) groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>DCC ER ≥ 15 fmol/mg</th>
<th>ER-ICA ≥ 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-menopausal</td>
<td>Post-menopausal</td>
<td>Pre-menopausal</td>
</tr>
<tr>
<td>Responders</td>
<td>22</td>
<td>2/3</td>
<td>19/19</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>34</td>
<td>5/14</td>
<td>9/20</td>
</tr>
</tbody>
</table>

Table 1 Endocrine therapy given for treatment of recurrent breast cancer (70 trials in 56 patients)

This table details different therapies given to the patients whose tumors were studied. The majority of patients received tamoxifen, but some received medroxyprogesterone (MPA) or aminoglutethimide only.

<table>
<thead>
<tr>
<th>Endocrine treatment</th>
<th>Pre-menopausal</th>
<th>Post-menopausal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen</td>
<td>8</td>
<td>23</td>
<td>31</td>
</tr>
<tr>
<td>Aminoglutethimide</td>
<td>0</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Tamoxifen and MPA</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Various</td>
<td>11</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Totals</td>
<td>22</td>
<td>48</td>
<td>70</td>
</tr>
</tbody>
</table>
Table 3  Comparison of predictive abilities for outcome of endocrine therapy of
the DCC and ER-ICA techniques

This table shows the misclassification rates of ER-ICA compared with DCC,
i.e., the number of patients who were seen to respond to therapy but were identified
as ER poor or failed to respond to therapy but whose tumors were ER rich.

<table>
<thead>
<tr>
<th></th>
<th>DCC +</th>
<th>DCC -</th>
<th>ER-ICA +</th>
<th>ER-ICA -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>6</td>
<td>6^</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

\* DCC —, 14 positive nonresponders and 1 negative responder (15 of 56,
26.8%); ER-ICA —, 8 positive nonresponders and 1 negative responder (9 of 56,
16.1%).
^ Misclassification for ER-ICA.
6 Misclassification for DCC.

who respond to therapy when compared to those whose tumors
contain receptor and who fail to respond. The most likely
explanation is that this monoclonal antibody recognizes both
biologically active and inactive ER, as has been inferred by
Horwitz and McGuire (11) for DCC-ER assessments. Their
hypothesis suggests a defect in the receptor mechanism distal
to the steroid binding step may be associated with a lack of
hormone responsiveness by the tumor, and this may also be
similarly applied to ER-ICA.

In spite of our findings that 8 of 24 breast carcinomas from
nonresponding patients were ER-ICA positive, the technique
has many potential clinical applications. These include
measurement of ER in cytological preparations from primary tu-
ors or in samples from metastases in different sites, reducing
the need for surgical biopsy. This may also enable us to improve
our understanding of the mechanism of response to endocrine
therapy in breast cancer, since repeat sampling and analysis
will be possible in individual patients.

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