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

<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 Aminoglutethimide</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>MPA</td>
<td>0</td>
<td>6</td>
<td>6</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>

where Intensity A is no specific nuclear staining visible; B, weak; C, moderate; and D, strong. This index has a range of 0–3, where 3 represents 100% of tumor cells staining at Intensity D.

Results

Correlation of ER-ICA with DCC Method. Our previous manuscript described a good correlation between these two procedures, demonstrating qualitative comparability between them in 80 of 90 (88.9%) cases and iER to be present in 66.7% of tumor samples. Furthermore, it showed correlations between the DCC content and the SII on the percentage of tumor cells stained (P < 0.001).

In the present study specific staining of iER was observed in the nuclei of a proportion of cells in 39 of 56 (69.6%) cases. Thirty-five of 56 (62.5%) patients were classified as DCC positive, their tumors containing > 15 fmol of ER per mg of cytosol protein. The >15-fmol/mg of cytosol protein cut off level for ER positivity used here was found to give optimum prediction of response to therapy (73.2%), while cut offs above and below this level predicted less accurately (e.g., > 10 fmol/mg of cytosol protein, 66.1%; > 20 fmol/mg of cytosol protein, 71.4%). Using this optimum cut-off point and a similarly optimized SII cut off of ≥0.5, we observed that of the 56 patients only 5 of 17 (29%) of the premenopausal group had ER-ICA positive cancers while 24 of 39 (62%) of postmenopausal patients had positive cancers. Similar results were obtained using an optimal ≥50% proportion of tumor cells stained as cut off. This compares with 41% and 74%, respectively, using the DCC assay.

Overall the DCC results for premenopausal women were lower than for postmenopausal patients (means of 23.6 fmol/mg of cytosol protein and 82.7 fmol/mg of protein, respectively), and the ER-ICA results showed a similar trend (0.42 and 0.83, respectively). No relationship was seen with primary procedures, demonstrating qualitative comparability between them.

Response to Therapy. A total of 70 courses of various forms of endocrine therapy was given to the 56 patients (Table 1). All patients had at least one endocrine maneuver, and 22 of 56 (39%) patients responded to therapy on at least one occasion.

Twenty-one of 22 responders had an ER-ICA SII score ≥0.5; 8 of 34 nonresponders had an ERICA score ≥0.5; when nonresponders and responders with a positive ER-ICA test were examined for the actual percentage of tumor cells stained in the sections, similar results were seen. No difference was observed between intensity score and outcome of therapy in those with positive ER-ICA tests. The single patient who had a negative ER-ICA and who responded had a strongly positive tumor by 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 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. 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>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 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>DCC +</th>
<th>DCC -</th>
<th>ER-ICA +</th>
<th>ER-ICA -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>20</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>8*</td>
<td>6*</td>
<td>0</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%).

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 tumors 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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References

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