Comparison of an Immunocytochemical Assay for Progesterone Receptor with a Biochemical Method of Measurement and Immunocytochemical Examination of the Relationship between Progesterone and Estrogen Receptors

Uta Berger, Pat Wilson, Suki Thethi, Richard A. McClelland, Geoffrey L. Greene, and R. Charles Coombes

ABSTRACT

Using a rat monoclonal antibody raised against human progesterone receptor (PR) we have developed an immunocytochemical technique to detect PR in human normal and malignant breast tissue and have compared the distribution of this with that obtained by the conventional dextran-coated charcoal steroid-binding assay.

Immunoreactive PR was detected exclusively in the nuclei of epithelial cells in 29/51 (56.9%) of breast cancers studied. There was an excellent correlation between the immunocytochemical and dextran-coated charcoal techniques, with concordance in 43/51 (84.3%) cases [regression coefficient (Spearman) = 0.78; P < 0.001].

The relationship between PR and estrogen receptor (ER) was also examined immunocytochemically using a monoclonal antiserum to ER. Twenty-eight out of 51 (54.9%) tumors were positive for both receptors and 13/51 (25.5%) negative for both. ER-positive, PR-negative tumors were found in 9/51 (17.6%) cases whereas only one case (2%) was PR-positive, ER-negative.

INTRODUCTION

Determination of the PR content of breast carcinomas has clinical utility since a high PR content predicts response to endocrine therapy and longer survival (1–5).

It has been suggested that, since the synthesis of PR results from estrogenic action, the presence of PR denotes a functional estrogen receptor (PR) and thus its measurement in association with ER can be of greater value than the measurement of ER alone (6–8).

Biochemical assessment of both ER and PR has certain limitations, principally that the amount of tissue obtained at biopsy is frequently insufficient to allow determination of both receptors. An immunocytochemical approach is able to overcome these limitations because receptor content can be assessed on fine needle aspirates and small tissue specimens (9) and in the case of ER, has been shown to agree with biochemical procedures (10–12). In needle aspirates immunocytochemically determined ER has been shown to correspond to clinical response data (13). Recently Flowers et al. have used similar response to therapy studies to demonstrate the significance of staining fine needle aspirates for both ER and PR (14).

In the following study we used a rat monoclonal antibody to PR to compare the immunocytochemical and biochemical approaches to determining PR status and to examine the relationship between PR and ER on tissue sections of 51 human breast cancers. A preliminary study of 27 human breast cancers has recently been published (15) utilizing two mouse monoclonal antibodies to PR, showing a good agreement with the biochemical assay.

MATERIALS AND METHODS

Material. Tissue samples were obtained from 51 patients with carcinoma of the breast and from seven patients with benign breast disease or normal breast tissue. All specimens were immediately snap frozen in liquid nitrogen and stored in the vapor phase of a liquid nitrogen bank for periods of time up to 1 year.

Patients. Patients were aged from 38 to 82 (mean age 59). Pathological tumor size ranged from 1 to 10 cm (mean size 2.75 cm). Thirty-seven patients had node dissections and 12 had evidence of node involvement.

Progesterone Receptor Assay. 100-μl aliquots of cytosol were incubated overnight at 4°C with six doubling dilutions of R5020 (New England Nuclear, 87.0 Ci/mmol) with an initial concentration of 2.5 × 10⁻¹² M in the presence or absence of 200-fold excess concentrations of unlabeled R5020 competitor. Unbound R5020 was absorbed by incubation with DCC (0.25% Norit A, 0.0025% dextran, 10 mM Tris, 1.5 mM EDTA, pH 8). Differences between uncompetited and competed tubes as measured by scintillation counting represent specifically bound radioligand. Free and specifically bound values for each case were obtained and manipulated to generate linearized transformations of the classic saturation curve to give reliable assessment of binding parameters (16, 17). Tumors containing greater than 15 fmol PR/mg cytosol protein are taken to be positive.

Protein estimations were by the Coomassie blue procedure (18) using Biorad protein dye reagent concentrate and BSA in PBS (<1 mg/ml) as standard.

Estrogen Receptor Assay. The procedure is as above for PR except [2,3,5,6-³H]estradiol is used as labeled steroid and diethylstilbestrol as competitor at similar concentrations as those described.

Immunocytochemistry. ER immunocytochemical determinations were carried out using the H222 monoclonal antibody ERICA kit (Abbott Laboratories, Chicago). H222 was raised in male Lewis rats immunized with estradiol receptor complex derived from MCF-7 human breast cancer cells (19).

PR determinations utilized the rat IgG, monoclonal antibody KD68. KD68 was raised to PR partially purified from T47D cells. It recognizes both A and B subunits of the PR molecule. It reacts with both complex and free receptor. Western blot analysis shows specificity to PR (20). The PR is localized in the nuclei of the target organs (20, 21).

Fixation. Three 5-μm cryostat sections were thaw mounted onto poly-L-lysine coated slides and immersed in cold acetone (−20°C) for 1 min and further washed in PBS. Then poly-L-lysine coated glasses were fixed 10 min in 3.6% formaldehyde in 0.1 M PBS (pH 7.2) immediately postsectioning. These were washed for 10 min in PBS, then immersed in cold methanol (−20°C) for 4 min and cold acetone (−20°C) for 1 min and further washed in PBS. Then
sections were incubated with 2% normal goat serum in PBS (to reduce nonspecific binding of antibodies). KD 68 antibody at the concentration of 5.11 μg/ml was added to one slide and a "control antibody" (normal rat IgG) to the other and incubated for 30 min. Then slides were washed three times in PBS. A second bridging antiserum (goat anti-rat IgG) was then added and incubated for 30 min. Following three PBS washes the peroxidase-antiperoxidase complex was added dropwise to sections for 30 min. This indirect peroxidase-antiperoxidase procedure is similar to that of Sternberger et al. (25). The receptors were visualized using the diaminobenzidine tetrahydrochloride: hydrogen peroxide chromagen-substrate reaction. Slides were counterstained in 1% (v/v) Harris’ hematoxylin in distilled water for 5 min and were then dehydrated and mounted in a xylene soluble mountant.

Staining was semiquantitatively assessed using an estimation of the number and intensity of stained tumor cells per section of tumor specimen. From this data the SII was calculated as previously reported. Briefly, four groups of reaction were reported: a, 0 negative; b, + weak; c, ++ moderate; and d, +++ strong.

\[
\text{SII} = \frac{\% \text{ of tumor cells stained at intensity a} \times 0 + \% \text{ of tumor cells stained at intensity b} \times 1 + \% \text{ of tumor cells stained at intensity c} \times 2 + \% \text{ of tumor cells stained at intensity d} \times 3}{100}
\]

This evaluation was done without knowledge of the simultaneously assessed ER and PR measurements performed by radioligand binding assays.

The entire slide was assayed at the proportion of positive cells estimated as previously reported (24).

**Statistical Methods.** Evaluation of the relationship between PR-ICA and the DCC-PR procedure was determined using the Spearman rank correlation test. Estimates of immunoreactive receptor content were obtained as described above, and biochemically assessed levels determined by standard Scatchard and Woolf procedures, with least squares and robust regression analyses.

**RESULTS**

The immunoreactive PR was exclusively localized in the nuclei of epithelial cells in normal breast tissue, benign breast disease and breast carcinoma. This reaction was consistent and did not occur in other cells or in the control slides incubated with rat IgG. (Fig. 1).

**Progesterone Receptor in Breast Carcinomas.** A good correlation was observed between the biochemical and immunocytochemical methods (Fig. 2; PS = 0.78; P < 0.001) with agreement in 43/51 cases (84.3%). Twenty-nine out of 51 (56.9%) carcinomas showed immunocytochemical staining for PR, the SII ranging from 0.1 to 2.95. The same number were positive using the biochemical method. Four samples were positive biochemically and negative immunocytochemically, and four were negative biochemically and positive immunocytochemically. Of the 43 which agreed 25 (58.1%) were PR-positive and 18 (41.9%) were negative.

**Relationship between Progesterone Receptor and Estrogen Receptor Status.** ER-ICA and PR-ICA positivity was observed in 28/51 (54.9%) cases and no staining observed by both procedures in 13/51 (25.5%) cases (Table 1). Nine out of 51 (17.6%) cases were ER-ICA positive but PR-ICA negative and only a single case (2%) was PR-ICA positive but ER-ICA negative.

**Immunocytochemistry of Normal and Benign Breast Tissue.** PR status was determined immunocytochemically on seven further cases in which only normal breast ducts and lobules or areas of benign hyperplasia were present in frozen sections. Nuclear staining was observed in epithelial cells in all of these specimens (Fig. 3). ER was localized immunocytochemically in five of these seven cases. These cases were examined biochemically and significant levels of PR were found in only four (range, 16–69 fmol/mg cytosol protein).

PR positivity was also observed in the nuclei of ductal and
Table 1 Distribution of ER and PR in breast cancer
Criteria for positivity: Biochemical assay = >15 fmol receptor/mg cytosol protein. Immunocytochemical assay = presence of any number of specifically stained tumor cell nuclei.

<table>
<thead>
<tr>
<th>Immunocytochemical assay</th>
<th>Biochemical assay</th>
<th>PR + ve</th>
<th>PR - ve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER+</td>
<td>ER-</td>
<td>ER+</td>
</tr>
<tr>
<td>1. PR+ and ER+</td>
<td>23</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2. PR+ and ER-</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. PR- and ER+</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>4. PR- and ER-</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Totals</td>
<td>26</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

Fig. 3. Immunocytochemical staining of normal breast tissue demonstrates PR-positivity in the nuclei of the secretory epithelia of the mammary gland. Magnification, x 300.

lobular epithelial cells in several cases of uninvolved tissue adjacent to areas of breast tumor.

DISCUSSION

Recently antibodies directed against human PR have been developed which are suitable for clinical immunodetection of PR in normal tissue and breast cancer (15, 20, 21, 26–28). This study demonstrates an immunocytochemical approach to determine PR status and its correlation to the biochemical assay. A nuclear localization of antigen has been observed consistent with that recently described for PR (20, 21). This localization is similar to that described for ER (13, 29) and the 1,25-dihydroxyvitamin D_3_ receptor (30). By this procedure PR was observed in 56.9% of cases and is in close agreement with the receptor distribution described by others using biochemical techniques (1, 5). Previous studies have shown that staining is abolished by coincubation with purified PR protein (21).

The overall agreement between the PR status assessed by both the DCC and immunocytochemical procedures was 84.3%, similar to that previously described using a similar antibody to estrogen receptor (13). For the KD 68 monoclonal antibody an overall agreement between immunocytochemically and biochemically determined PR of 77% with a sensitivity of 59% and specificity of 84% has been reported (31). Our results confirm these findings. We found immunologically reactive PR was present in four biochemically PR negative cases. A possible explanation for this discrepancy may be due to intratumoral steroid receptor heterogeneity resulting in selected areas of receptor positivity but with an overall amount below the limits of biochemical assay detection. Alternatively we may be detecting an immunologically reactive receptor which has no binding activity. This may be due to the epitope recognized by the antibodies being more resistant to degradation than the steroid binding site.

The biochemical distribution of both ER and PR in breast tumors has been well described (1, 4, 5). In these studies breast tumors have been shown to frequently possess significant levels of both steroid receptors (40–43%) and to be negative for both in 27–35% of cases. Our immunocytochemical results (Table 1) demonstrate a similar distribution of both receptors in breast cancer specimens.

An interesting subgroup of patients whose tumors are positive for one steroid receptor only has also been described. Biochemically ER-negative, PR-positive tumors occur rarely in 1–5% of cases and ER-positive, PR-negative tumors around 17–33% of cases. Again, these figures are similar to those obtained using the immunocytochemical technique. Subsequent follow up of our patients will determine whether these single receptor-positive patients respond to endocrine therapy.

We do not yet know the exact potential of this technique. Further study will show whether immunocytochemical expression of both receptors in breast carcinomas leads to a higher chance of predicting response to therapy. We also intend to examine the value of its determination in metastases such as bone marrow and pleural aspirates. Studies of its distribution in normal breast sections and aspirates may well help us understand how modulation of this receptor protein occurs and the way that it relates to proliferation of breast epithelial cells.

ADDENDUM

While this manuscript was in review, a further four papers have appeared describing a similar assay but using antibody J2B 39 (32) and a monoclonal antibody to rabbit PR (33), also Charpin et al. (34) and Elashry-Stowers et al. (35).

ACKNOWLEDGMENTS

We are very grateful to Ann Haydock and Doug Easton for their assistance. We would also like to thank Dr. Louise Przywara (Abbott Laboratories) for providing the ERICA kits.

REFERENCES


Comparison of an Immunocytochemical Assay for Progesterone Receptor with a Biochemical Method of Measurement and Immunocytochemical Examination of the Relationship between Progesterone and Estrogen Receptors

Uta Berger, Pat Wilson, Suki Thethi, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/18/5176