Estrogen Receptor Expression of Benign Breast Epithelium and Its Association with Breast Cancer

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ABSTRACT

We conducted a case-control study of estrogen receptor (ER) and progesterone receptor (PgR) expression in benign breast epithelium from 120 women (51 breast cancer cases and 69 benign disease controls) who underwent breast operations at University Hospital, Syracuse, New York. Benign samples were obtained and processed immunohistochemically for ER and PgR (Abbott, Chicago, IL). Receptor positivity was defined as any nuclear immunostaining. Proportionately more cases than controls were ER positive (84% versus 57%); PgR positivity was similar in cases and controls (86%). Logistic regression yielded an adjusted odds ratio of 6.5 for breast cancer among ER-positive women (95% confidence interval of 1.5 and 27.4); odds ratio of PgR positivity was 0.3 (95% confidence interval of 0.1 and 1.9). Adjustment for known risk factors for breast cancer did not change the odds ratio. ER-positive breast epithelium needs evaluation for breast cancer among ER-positive women (95% confidence interval of 1.5 and 27.4).

INTRODUCTION

Estrogen is known to be etiologically important in the development of breast cancer; estrogen effect on target organs is mediated through its receptor, and in the case of breast epithelium, it appears that estrogen effect includes the induction of proliferation, particularly of ductal tissue (1). Benign proliferative lesions of the breast indicate a higher risk for subsequent breast malignancy (2–4). It is possible, therefore, that the presence of ER+ in benign breast epithelium is of significance in the prediction of breast cancer risk. Earlier studies of the expression of ER in benign breast tissue were performed using ligand binding assays, and ER levels were found to be extremely low or undetectable (5, 6). This was attributed to the low epithelial cellularity of breast tissue. Interest in the receptor content of nonmalignant breast tissue lagged until the advent of highly specific monoclonal antibodies (7) which enabled immunohistochemical assays for ER and PgR. It is now possible to detect these receptors on small samples of breast epithelium and to control accurately for the cellularity of the specimen and for the presence of benign breast disease. Breast epithelium has since been studied by several investigators using immunohistochemistry (8–12), but there has been no systematic comparison of ER and PgR expression in the benign breast epithelium of women diagnosed to have breast cancer and those not known to have breast malignancy. We initiated a case-control study of ER and PgR expression in benign breast tissue of women undergoing breast surgery with the reasoning that the presence of ER in breast epithelium would render the tissue susceptible to the mitogenic stimulus of estrogen, and the presence of ER could thereby function as a risk factor for the development of breast cancer. PgR synthesis is a known consequence of estrogen-ER-DNA binding (13, 14); immunohistochemical PgR assays were therefore included in the study.

METHODS

Patient Population. All patients being scheduled for breast surgery through the Comprehensive Breast Care Program at State University of New York Health Science Center at Syracuse were invited to participate. They included women who had probably benign, palpable breast lumps as well as women who were known to have breast cancer and were undergoing definitive surgery for this problem. Patients undergoing biopsy for mammographically detected lesions were excluded to avoid problems with tissue availability for diagnosis. Self-administered questionnaires detailing menstrual and obstetrical history and history of known risk factors for breast cancer were completed preoperatively. The usual length of the menstrual cycle and the date of the last menstrual period were recorded for premenopausal women and were used to estimate the phase of the cycle at the time of surgery. Data on the use of oral contraceptives and hormone replacement therapy were also recorded. All patients gave informed consent.

All samples were snap frozen in liquid nitrogen after embedding in freezing medium and stored at −80°C. Five cryostat sections were prepared from each sample: one for routine hematoxylin and eosin staining, one each for ER and PgR histochemistry, and one each for negative controls. The assays were performed using commercially available ER-ICA and PgR-ICA kits (Abbott). Sections were processed according to directions included by the manufacturer, including the use of positive control slides for each batch. The sections were counterstained lightly with Harris hematoxylin and examined with light microscopy.

All sections were evaluated for adequacy of epithelial sampling and were excluded if less than five normal ducts or lobules were present. For the present analysis, all exclusions were made for the presence of benign disease. Epithelium was considered positive for ER or PgR if any brown epithelial nuclear staining was seen. All histochemical sections were compared to negative control sections to exclude nonspecific staining. In order to control for the multiple samples obtained from mastectomy specimens (since these came almost exclusively from breast cancer cases), we used a random numbers table to choose a single sample which was then scored qualitatively for receptor expression. Quantitation of hormone receptors was also performed using computer-assisted densitometry and area measurements (BIOQUANT Image Analysis System). Randomly chosen fields were analyzed (mean, 15/section) for the %PEN. The intensity of stain was measured for the positive nuclei and may be regarded as a summary quantitative value for receptor expression. If multiple samples from one breast were available, the section with highest technical quality and most intense staining was chosen for image analysis. This was not necessarily the same section as that chosen by a random digit table and qualitatively scored for the statistical analysis.

The use of the term “receptor positive” and “receptor negative” in this report refers to the qualitative assessment of histochemical sections. Data presented in tables reflect qualitative receptor status. Where quantitative image analysis data are used, this is specifically stated. Statistical analysis was conducted as described by Breslow and Day (15) using EGRET (Statistics and Epidemiology Research, Seattle).
RESULTS AND DISCUSSION

The characteristics of the study population are shown in Table 1. The subjects with breast cancer were older and more likely to be postmenopausal than the controls. The mean age of cases was 56 years (range, 23–80), and the mean age of controls was 37 (range, 17–80). There were proportionately more older women who were ER positive but this was not statistically significant (P = 0.2). There were no significant differences between cases and controls with regard to a family history of breast cancer, estrogen replacement therapy, age at menarche, age at first full-term pregnancy, history of oophorectomy, and body mass index. We were able to determine the phase of the menstrual cycle when the sample was taken for 45 (12 cases and 33 controls) of the 71 premenopausal women in the study (63%). Thirty-two samples were obtained in the luteal phase of the cycle and thirteen in the follicular phase. Breast cancer cases were more likely to have the sample taken during the luteal phase (10 of 12) than controls (22 of 33). Concurrent histochemical data on ER and PgR expression were available on 42 malignant lesions; the remaining cancer patients had undergone excision of their cancers before coming to mastectomy.

Qualitative ER Positivity and Presence of Cancer. The overall ER positivity rate for the study population was 68%; this did not vary significantly across menopausal strata (premenopausal, 66% ER positive; postmenopausal, 72% ER positive). However, proportionately more women who were ER positive had breast cancer than women who were ER negative. This was true for both pre- and postmenopausal women. Thus in premenopausal women, the ER positivity rate did not vary between cases and controls: 86.6% overall, 86% for premenopausal women. Thus in premenopausal women, the ER positivity rate was 94% (17 of 18) and for controls was 57% (32 of 56). Amongst postmenopausal women, 79% (26 of 33) of cases were positive as opposed to 54% (7 of 13) of controls.PgR positivity rates for cases was 94% (17 of 18) and for controls was 57% (32 of 56).

The crude and adjusted odds ratios for hormone receptor status and presence of cancer are shown in Table 2. Even after adjustment for other known risk factors of this disease, there was a strong relationship between ER positivity and breast cancer. The presence of any nuclear staining for ER rendered women 6.5 times as likely to have breast cancer as women with no ER staining.

Our finding of a strong association between ER positivity of benign breast epithelium and the presence of breast cancer is the first report of a difference in ER expression in benign breast tissue in women with breast cancer and those without. This persists after controlling for age and is independent of other known factors related to this disease (e.g., a family history of breast cancer, age at menarche, body mass index, oophorectomy, nulliparity, and late first term pregnancy). It is particularly marked in premenopausal women with menarche before age 12 and those with a positive family history. The calculated probability of breast cancer in the different receptor categories (Table 3) shows that, within each age stratum, risk is higher for ER-positive compared to ER-negative women. If these findings are confirmed in a larger study with longitudinal follow-up, there are important implications for breast cancer risk prediction and possibly for the selection of patients for chemoprophylactic intervention.

We have chosen to interpret ER positivity as the presence of any nuclear stain since the range of expression for nonmalignant breast tissue is not well established. In some subjects, this meant that as few as 10 cells showed faint but specific staining, not seen on the negative control sections. Other workers have used a cut-off level as high as 50% cells positive (9), whereas still others have reported the mean fraction of positive cells in their patient population without attempting to define a cut-off level for positivity (8, 11, 16). In comparison to these latter studies, we find a higher mean %PEN for ER (8, 11), suggesting that instances of ER-negative epithelium in our study are not related to receptor degradation due to poor tissue handling. In fact, benign tissue samples from breast cancer cases (84% ER positive) frequently came from mastectomy specimens, where warm ischemia time was longer on the average, whereas rapid freezing of tissue from...
benign disease controls (57% ER positive) was the rule. Our results are also in accordance with Ricketts et al. (9) who found a 29 to 40% ER negativity rate in breast epithelial sections from women without known breast disease. If the traditional cut-off for ER expression in breast cancer is used, 10 fmol/mg cytosol protein translates into 1% positive cells (17), which is close to what we have used.

**Comparison of Receptor Status of Benign Tissue and Cancer.** The hormone receptor status of benign epithelium and cancer within the same breast was compared in 42 women from whom malignant and benign samples were obtained at the same procedure. The data were analyzed as matched pairs of benign and malignant tissue, the results of which are presented in Table 4. We found that concordance of ER positivity was present in 23 of 36 (64%) of the matched samples. Among cancer patients with PgR-positive epithelium, tumors were concordantly positive in 27 of 39 (69%). Thus ER and PgR expression were significantly different between tumor and benign epithelium, although the difference in ER expression was of borderline significance. Interestingly in this data set, ER discordance between epithelium and carcinoma of the breast is bidirectional (13 cases with positive epithelium and negative cancer and four cases with negative epithelium and positive cancer). In contrast, the discordance in women with PgR-positive epithelium was unidirectional (twelve patients had PgR-positive epithelium and negative tumors, but none had PgR-negative epithelium and positive tumors). Thus tumors seem to be able to both gain and lose ER, whereas change of PgR status consists of loss of receptor by the tumor, with no patients in this series having PgR-negative epithelium with positive cancer. These results support the concept that “normal” hormone receptor expression in both pre- and postmenopausal breast malignancy consists of PgR positivity, with ER being expressed less frequently and at lower levels than PgR. ER positivity in breast cancer is more frequent than that of PgR (18), in contrast to the findings in benign epithelium, and suggest that this reversal in itself constitutes one step of dedifferentiation and loss of normal control of hormone receptor expression. If so, there is a great need for comparative studies of ER structure and function in benign epithelium and cancer.

The question of ER concordance between benign epithelium and cancer does not contradict our hypothesis that ER-positive breast epithelium would facilitate malignant transformation, since this is based on the concept that the role of ER is to render cells susceptible to the mitogenic stimulus of estrogen, thereby increasing mitotic events, which would provide opportunities for genetic mishaps and initiation of malignancy during cell division. Since there are many intermediate steps involved in the transformation to malignancy, the idea of ER positivity being a permissive state for the occurrence of other initiating events, during which ER expression may be lost, retained, or gained, is quite tenable. Conversely, an ER-negative state would protect cells from the mitogenic effects of estrogen and would endow cells with some resistance against transforming influences. The data are also compatible with the theory of the monoclonal origin of solid tumors since we believe we are measuring ER expression in benign epithelium which reflects the state of the tissue the initiated cell arose from, prior to initiation. ER-positive cancers are neoplasms which have retained, and in many cases increased, their ability to express the receptor; ER-negative tumors, even if they are associated with ER-positive epithelium, have ceased to express ER on their journey from benignity to initial malignancy.

**Table 2 Odds ratios for breast cancer and hormone receptor status**

<table>
<thead>
<tr>
<th>Cases n</th>
<th>Controls n</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ 43</td>
<td>39</td>
<td>4.1</td>
<td>1.7-10.1</td>
<td>6.5</td>
<td>1.5-27.4</td>
<td>6.5</td>
<td>1.4-28.9</td>
</tr>
<tr>
<td>ER- 8</td>
<td>30</td>
<td>0.9</td>
<td>0.3-2.7</td>
<td>0.3</td>
<td>0.1-1.9</td>
<td>0.3</td>
<td>0.1-2.2</td>
</tr>
</tbody>
</table>

**Table 3 Probability of having breast cancer in this study population**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>PgR status</th>
<th>ER+</th>
<th>ER-</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>PR+</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>PR-</td>
<td>0.40</td>
<td>0.09</td>
</tr>
<tr>
<td>50</td>
<td>PR+</td>
<td>0.60</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>PR-</td>
<td>0.81</td>
<td>0.40</td>
</tr>
<tr>
<td>70</td>
<td>PR+</td>
<td>0.91</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>PR-</td>
<td>0.97</td>
<td>0.82</td>
</tr>
</tbody>
</table>

**Table 4 Number of women by ER and PgR status of benign breast tissue and tumor tissue**

<table>
<thead>
<tr>
<th>Tumor tissue</th>
<th>ER status</th>
<th>Positive</th>
<th>Negative</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>ER</td>
<td>23</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>ER</td>
<td>4</td>
<td>2</td>
<td>0.049</td>
</tr>
<tr>
<td>PgR</td>
<td>Benign tissue</td>
<td>27</td>
<td>12</td>
<td>0.0005</td>
</tr>
<tr>
<td>Positive</td>
<td>PgR</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>PgR</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

* Exact test for matched pairs.
Quantitative Image Analysis Data. Possible trends in risk with increasing amounts of ER as determined by image analysis were investigated (Table 5). Three categories of %PEN and MIOD were created; as the percentage of ER positive cells increased, there was no graded increase in breast cancer risk. Similarly, there was no uniform rise in odds ratios with increasing values for ER MIOD and %PEN. Therefore, it appears, at least in this data set, that any ER positivity increases breast cancer risk, regardless of amount. This was true even though, in the event of multiple samples being available from the same patient, a single sample was chosen for image analysis based on the highest degree of positivity.

These results are not surprising given the evidence for estrogen down-regulation of ER in the breast; expression tends to be lowest in the luteal phase of the menstrual cycle, when estradiol levels are high, (10, 11) and the presence of estradiol down-regulates ER expression in the breast cancer cell line MCF-7 (19). Although exposure of MCF-7 cells to estradiol results in a decrease of ER expression, the effect is temporary, and receptor levels do not go down to zero; our finding of a strong association between breast malignancy and any presence of ER suggests the concomitant presence of estrogen and ER and is compatible with present knowledge of ER mediating estrogen action on breast epithelium, estrogen regulation of ER quantities, and estrogen exposure increasing risk of breast cancer.

Although the presence of ER in breast carcinoma has a modest survival advantage, the presence of ER in benign breast tissue (at least in quantities which are histochemically detectable) may be disadvantageous from the viewpoint of breast cancer risk. This is in agreement with evidence pointing to differences in the hormonal control of normal breast tissue where progesterone appears dominant, as opposed to the more important effect of estrogen in the growth stimulation of breast cancer (20). Thus ER positivity in cancer is more frequent than PgR positivity (21), and the quantitative expression of ER is greater in cancer than in nonneoplastic breast tissue (12). In contrast, PgR staining is stronger than ER staining in benign breast tissue (9, 16); and in the present study, PgR positivity was also more frequent than ER positivity. Finally, the ER-positive/PgR-negative phenotype constitutes 25–30% of ER-positive cancers (22) but was seen in benign epithelium from only three patients (2.5%) in the current study, all from breast cancer cases.

Relationship between ER and PgR. There was a statistically significant association between ER status and PgR status (P < 0.001). Women who were ER positive were 22 times more likely to be PgR positive than PgR negative. Inclusion of PgR status in the model changed the OR for ER positivity and breast cancer; therefore, PgR was included in the final model. Although there was an inverse association between PgR status and breast cancer (adjusted OR = 0.3), it was not statistically significant.

Progesterone receptor positivity was not only more frequent than ER when we considered any staining versus no stain, but the intensity of staining using image analysis data was uniformly greater than that for ER (PgR-MIOD, 0.67; ER-MIOD, 0.45). Similarly, the %PEN for PgR was 24, and for ER was 15.6. The PgR:ER ratio for both %PEN and MIOD were calculated. In premenopausal women, these equaled two; whereas in postmenopausal women, the ratio for mean %PEN was 1.2 and that for MIOD was 1.4. The normal pattern of receptor expression in breast tissue, therefore, consists of very high rates of PgR positivity, with PgR being expressed by a greater proportion of epithelial cells and at higher levels than ER.

The probability of having breast cancer for women of different ages (Table 4) shows a trend within each age group, with the highest probabilities occurring in patients who are ER positive and PgR negative. This is again explainable on the basis that progesterone appears to be the dominant hormone controlling the normal breast, and the normal pattern of receptor expression in benign breast tissue appears to be a predominance of PgR expression, with ER being expressed both less frequently and to a lesser degree (16). An aberrant ER/PgR receptor pattern (i.e., loss of PgR dominance) might indicate that normal control of receptor expression has been lost and that epithelium displaying this pattern is particularly susceptible to neoplastic transformation.

ER Relationship with Menstrual Cycle. For the women in whom menstrual cycle phase at the time of tissue sampling could be determined, the relationship between ER and PgR status and phase was investigated. There was a trend for a larger proportion of women in the follicular phase of the cycle to be ER positive (11 of 15; 73%) than in the luteal phase (20 of 32; 62.5%), but this was not statistically significant. The less marked menstrual cycle variation seen in this group of patients when compared to other reports (10, 11) may be due to the fact that almost half our patient population were breast cancer cases, and it is possible that receptor periodicity may not be as marked in this group. Eight of 46 postmenopausal women were on hormone replacement therapy, and 5 of them (63%) were ER positive, opposed to 28 of 38 (74%) of those not on replacement hormones.

Analysis of Multiple Samples from Mastectomy Specimens. Forty-one patients underwent mastectomy, five of these being bilateral. Four separate tissue samples were obtained from each mastectomy specimen, but some of these contained an inadequate amount of epithelium. Data on only one quadrant was available in three mastectomy patients, and those are excluded for the purposes of assessing within-breast concordance. A mean of three evaluable samples/breast were obtained from the remaining 33 unilateral mastectomy patients. Of these, 18 (55%) showed complete concordance for ER expression, 27 (82%) were positive in more than one quadrant, and there were 6 breasts (18%) with only 1 positive sample. We also assessed the between-breast concordance in women who had undergone bilateral mastectomy, either prophylactic or therapeutic. In no patient was one breast ER positive and the other ER negative. Two patients had 1 of 2 positive samples in one breast, but the other breast had 3 of 3 and 2 of 3 positive samples, respectively. Higher degrees of concordance between breasts (18%) with only 1 positive sample. We also assessed the between-breast concordance in women who had undergone bilateral mastectomy, either prophylactic or therapeutic. In no patient was one breast ER positive and the other ER negative. Two patients had 1 of 2 positive samples in one breast, but the other breast had 3 of 3 and 2 of 3 positive samples, respectively. Higher degrees of concordance between breasts (18%) with only 1 positive sample. We also assessed the between-breast concordance in women who had undergone bilateral mastectomy, either prophylactic or therapeutic. In no patient was one breast ER positive and the other ER negative. Two patients had 1 of 2 positive samples in one breast, but the other breast had 3 of 3 and 2 of 3 positive samples, respectively. Higher degrees of concordance

### Table 5

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of cells ER positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>34</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>0.01–14.99</td>
<td>42</td>
<td>15.0</td>
<td>2.2–102.3</td>
<td>20.0</td>
<td>1.9–210.8</td>
</tr>
<tr>
<td>15.00–84</td>
<td>42</td>
<td>5.9</td>
<td>0.9–36.8</td>
<td>6.2</td>
<td>0.7–54.1</td>
</tr>
<tr>
<td>ER MIOD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>34</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>0.01–0.49</td>
<td>36</td>
<td>11.2</td>
<td>1.9–66.9</td>
<td>9.8</td>
<td>1.3–73.4</td>
</tr>
<tr>
<td>0.50–1.10</td>
<td>48</td>
<td>6.8</td>
<td>1.2–37.1</td>
<td>7.7</td>
<td>1.1–54.0</td>
</tr>
</tbody>
</table>

* The logistic model included ER status, PR status, and age.
* CI, confidence interval.
* The logistic model included ER status, PR status, age, menopausal status, family history of breast cancer, age at menarche, body mass index, estrogen replacement therapy, and oophorectomy.
were observed in the remaining patients (6 of 6, 6 of 8, and 7 of 8 positive samples).

The degree of concordance within breasts in terms of ER status is an important issue in the design and analysis of future studies as well as in the interpretation of this study. All 38 patients from whom we obtained multiple evaluable samples (33 unilateral and 5 bilateral mastectomies) were included in the process of using a random numbers table to identify a single sample, the receptor status of which was unknown until the study was finished. The hormone receptor status of epithelial proliferation and breast cancer site of relapse in primary breast cancer. Cancer (Phila.), 61: 758-768, 1988.


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