Increased Progesterone Receptor Expression in Benign Epithelium of BRCA1-Related Breast Cancers

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Abstract

The study of pathologically normal breast epithelium of BRCA1 mutation carriers may yield insights into the early natural history of breast tumorigenesis. Hormone receptor expression was assessed in 24 cases of invasive breast cancer associated with a mutation in BRCA1 (n = 15) or BRCA2 (n = 9) and in 39 sporadic cases matched for patient age and tumor hormone receptor status. Expression of progesterone receptor was significantly (P = 0.0003) more common in normal breast epithelium adjacent to invasive breast carcinoma in BRCA1-linked cases compared with sporadic cases. The wild-type BRCA allele was retained in normal epithelium of all cases tested. We conclude that deregulation of progesterone receptor expression, as a result of BRCA1 haploinsufficiency, may represent an early event in BRCA1-linked breast tumorigenesis.

Introduction

An estimated 5–10% of breast cancers are associated with autosomal dominant genetic predisposition, and a substantial proportion of these are linked to BRCA1 or BRCA2 (reviewed in Ref. 1). The availability of genetic testing for BRCA1 mutation greatly increases the need for definition of the risks and benefits of various clinical interventions aimed at risk reduction. Options for persons determined to be at high risk for breast cancer as a result of BRCA1 mutation include surveillance, chemoprevention, and prophylactic mastectomy, but few prospective data exist to support the efficacy of these procedures. An increased understanding of the early natural history of breast tumorigenesis in BRCA heterozygotes with respect to hormone receptor expression is vital for a rational approach to the development of chemopreventive strategies for this population. Toward that end, an emerging body of data suggests that human cells heterozygous for a BRCA1 mutation display a phenotype consistent with BRCA haploinsufficiency, insofar as dermal fibroblasts and lymphocytes from BRCA1 heterozygotes display an enhanced sensitivity to ionizing radiation compared with cells from control individuals (2, 3), and in BRCA2 mutation carriers, biallelic inactivation of BRCA2 appears to be a late event in the development of pancreatic intraepithelial neoplasia (4). The purpose of this study was to search for evidence of BRCA haploinsufficiency in pathologically normal breast epithelium of BRCA1 heterozygotes with breast cancer, focusing on hormone receptors as clinically relevant molecular markers of this phenomenon.

Materials and Methods

Twenty-four patients with deleterious germ-line mutations in BRCA1 (n = 15) or BRCA2 (n = 9) and a diagnosis of invasive breast carcinoma were identified through the Clinical Genetics Service of the Memorial Sloan-Kettering Cancer Center. These patients had previously provided informed consent for BRCA genetic testing, and this particular study was approved by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center. For each BRCA-linked breast cancer case, two additional cases of presumed sporadic invasive breast carcinoma were selected from a Department of Surgery breast cancer database; these cases were matched for age at diagnosis (within 3 years) and tumor hormone receptor status with the BRCA-linked cancers. Premenopausal patients were not matched for menstrual cycle phase. Corresponding tissues were obtained as fixed and paraffin-embedded specimens from Department of Pathology archives, tissue sections were prepared as appropriate for the various analyses (see below), relevant clinicopathological annotation was attached to each case, and the tissue samples were then anonymized. Adequate tissue specimens were not available for 9 sporadic breast cancer cases, leaving 39 available for analysis.

All cases were reviewed by two study pathologists to confirm the diagnosis of invasive breast carcinoma, to delineate regions of normal epithelium, preinvasive lesions, and cancer, and to score tumors for hormone receptor expression status for the purpose of matching sporadic cases with BRCA-linked cases. Hormone receptor status was scored as positive if ≥10% of tumor cells were immunopositive for ER (estrogen receptor) or PgR (progesterone receptor) expression as assessed by immunohistochemical staining; these are the standard criteria used by the Department of Pathology at this institution and are considered to accurately predict for a positive clinical response to endocrine therapy.

For immunohistochemical analyses, several representative tissue sections of 6-μm thickness containing invasive carcinoma and/or normal epithelium were prepared from paraffin blocks associated with each case and placed on silanized glass slides. The DAKO LSAB2 System, Peroxidase, was used in conjunction with the DAKO Autostainer Universal Staining System according to instructions supplied by the manufacturer. Briefly, slides were deparaffinized in xylene and rehydrated in a graded series of ethanol/water rinses, and antigen retrieval was performed by heating slides in a steamer at 95°C–99°C in Target Retrieval Solution (DAKO) for 20 min. At room temperature, slides were then treated with 3% hydrogen peroxide for 10 min followed by primary antibody for 10 min. For ER, the monoclonal mouse antihuman clone 1D5 (DAKO) was used at a 1:50 dilution; for PgR, the monoclonal mouse antihuman monoclonal clone PgR 636 (DAKO) was used at a dilution of 1:75. Visualization using the LSAB2 System is accomplished using a biotinylated link antibody, peroxidase-streptavidin, and 3,3′-diaminobenzidine as chromogen. Slides were counterstained with methyl green.

Hormone receptor expression in normal breast epithelium, as a percentage of cells displaying positive nuclear staining, was scored as a continuous variable and potential differences between the various groups were assessed using the Student’s t test. All reported P values were corrected for multiple testing using the step-down Bonferroni procedure of Holm (5).

Microdissection of normal and tumor cells from tissue sections prepared from paraffin blocks was accomplished using a P.A.L.M. Laser Pressure Catapulting microdissection system (P.A.L.M. Mikrolaser Technologies). Quantitative allelotyping of DNA from microdissected cells was performed using a real-time multiplex allele-specific PCR procedure developed for the detection of specific framewise mutations in BRCA1 (18SdelAG) and BRCA2.
and probe sequences, are available upon request.

Results

The clinicopathological characteristics of BRCA-linked and sporadic breast cancer cases are summarized in Table 1. The sporadic cases were selected to closely match the BRCA-linked group for patient age at diagnosis and tumor hormone receptor expression status. In addition, the two groups were very similar with respect to histological cell type and grade, tumor size, presence of vascular invasion, presence of lymph node metastases, and stage.

For each case, hormone receptor expression in normal breast epithelium was assessed in two regions from the breast specimen from two separate paraffin blocks, one containing both normal epithelium and tumor and the second containing only normal epithelium. These two regions of normal epithelium were labeled as “adjacent to tumor” and “at an unknown distance from tumor,” respectively (Fig. 1). Expression of ER was not significantly different in normal breast epithelium from either region when comparing all BRCA-linked cases together to the sporadic cases (data not shown). When considering the BRCA1- and BRCA2-linked cases separately, there was a trend toward a higher proportion of cells expressing ER in both regions of normal epithelium in BRCA1-linked cases compared with either of the other two groups, but these differences did not reach statistical significance.

Although the proportion of cells expressing PgR was higher in all BRCA-linked cases compared with sporadic cases in both areas of normal epithelium assessed, these differences were not statistically significant (data not shown). However, when considered separately, BRCA1-linked cases showed significantly higher PgR expression than sporadic cases in normal epithelium adjacent to tumor (P = 0.0003) and higher PgR expression of marginal significance at an unknown distance from tumor (P = 0.06; Table 2). Expression of PgR was also marginally significantly higher in BRCA1-linked cases compared with BRCA2-linked cases in normal epithelium at an unknown distance from tumor (P = 0.06) but not adjacent to tumor. Expression of PgR in BRCA2-linked tumors was not significantly different from that in sporadic tumors.

Table 1  Clinicopathological characteristics of breast cancer cases

<table>
<thead>
<tr>
<th>Tumor category</th>
<th>All BRCA</th>
<th>BRCA1</th>
<th>BRCA2</th>
<th>Sporadic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td>45 (30–68)</td>
<td>43 (30–62)</td>
<td>47 (36–68)</td>
<td>45 (28–69)</td>
</tr>
<tr>
<td><strong>Histologic type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal</td>
<td>0.88a</td>
<td>0.93</td>
<td>0.78</td>
<td>0.85</td>
</tr>
<tr>
<td>Infiltrating lobular</td>
<td>0.08</td>
<td>0</td>
<td>0.22</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0.04</td>
<td>0.07</td>
<td>0</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Histologic grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.36</td>
<td>0.33</td>
<td>0.43</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.64</td>
<td>0.67</td>
<td>0.57</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
<td>1.7 (0.5–3.5)</td>
<td>1.9 (0.6–3.5)</td>
<td>1.3 (0.4–1.9)</td>
<td>2.1 (0.1–5.9)</td>
</tr>
<tr>
<td><strong>Multifocal disease</strong></td>
<td>0.04</td>
<td>0</td>
<td>0.22</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Vascular invasion</strong></td>
<td>0.32</td>
<td>0.21</td>
<td>0.50</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Nodal metastases</strong></td>
<td>0.68</td>
<td>0.57</td>
<td>0.88</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Hormone receptor status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER positive</td>
<td>0.33</td>
<td>0.14</td>
<td>0.55</td>
<td>0.28</td>
</tr>
<tr>
<td>PgR positive</td>
<td>0.12</td>
<td>0.07</td>
<td>0.33</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.23</td>
<td>0.29</td>
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<td>II</td>
<td>0.77</td>
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<td>0.84</td>
</tr>
<tr>
<td>III</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a Mean (range) in years.
b Proportion of tumors in category.
c Mean (range) in centimeters.

To search for evidence of loss of the wild-type BRCA allele in normal breast epithelium that may correlate with aberrant hormone receptor expression, a real-time PCR procedure was developed to quantitate the ratio of mutant to wild-type BRCA alleles in DNA from microdissected normal breast epithelium obtained from patients with the 185delAG BRCA1 mutation (n = 6) or the 6174delT BRCA2 mutation (n = 2). In these eight cases that were extensively analyzed, there was no evidence for allelic loss in normal epithelium either adjacent to or at an unknown distance from tumor, although loss of the wild-type allele was readily demonstrated in multiple cases of BRCA1-linked and BRCA2-linked invasive cancers (data not shown).

Discussion

These data indicate that PgR is aberrantly expressed in pathologically normal breast epithelium of BRCA1 mutation carriers with breast cancer. No evidence was found for statistically significant alterations in ER expression or cell proliferation in normal breast epithelium of BRCA mutation carriers compared with that of sporadic breast cancer patients. Retention of the wild-type BRCA1 allele in normal breast epithelium of these cases suggests that PgR deregulation is occurring in association with BRCA1 haploinsufficiency. These findings have several implications related to the early natural history of breast tumorigenesis in the BRCA1 population.

Although the literature on hormone receptor expression in normal breast epithelium is limited, available evidence indicates that ER expression is present in nearly all terminal duct lobular units but in only a small proportion of luminal epithelial cells in these units. In premenopausal women, the proportion of ER-positive cells ranges from 4 to 20%, depending on the phase of the menstrual cycle; these cells are distributed singly and are surrounded by ER-negative cells (6–9). With increasing age, the number of ER-positive cells increases, such that 30–40% of epithelial cells are ER positive in the postmenopausal breast (9, 10). Expression of PgR appears to coincide very closely with that of ER in the luminal epithelium (11, 12). Furthermore, there exists an inverse correlation between hormone receptor positivity and cell proliferation in the normal breast epithelium (8, 10–12), suggesting that hormone receptor-positive cells regulate mitogenesis in neighboring cells in a paracrine fashion (13).

In contrast, the great majority of epithelial cells in preinvasive breast lesions such as atypical hyperplasia and carcinoma in situ are ER positive, and there is a strong correlation between ER positivity and cell proliferation (9, 10, 14). These observations have led to the formulation of a mechanistic model in which ER-positive breast...
epithelial cells are at higher risk for progression to malignancy and that the development of autonomy from proliferative constraints by ER-expressing epithelial cells represents a critical early event in breast tumorigenesis. Consistent with this model are the observations that ER expression is increased in normal epithelial cells of cancer-bearing breasts (15) and that the proportion of ER-positive breast epithelial cells is lower in women at lower risk for breast cancer (16).

Data to suggest that hormone receptor expression may differ in BRCA-associated compared with sporadic breast cancers are beginning to emerge. It is well established that ~75% of primary breast cancers generally are classified as ER-positive, although this figure varies with age, race, nodal status, and other prognostic factors (17, 18), but the fraction of BRCA1-associated tumors with ER/PgR expression appears to be considerably lower (19–23). More notable perhaps is the observation that the majority of preinvasive ductal carcinoma in situ lesions from BRCA1 heterozygotes are ER/PgR negative, in marked contrast to the very high rate of receptor positivity in these lesions from the general population (24). Taken together, these data suggest that the general model of ER-positive breast epithelial cells developing proliferative autonomy followed by loss of ER expression may occur earlier or more rapidly during the process of breast tumorigenesis in BRCA1 heterozygotes.

In light of this discussion, we suggest that the most parsimonious explanation for the data presented here is that partial loss of BRCA1 function in breast epithelial cells of BRCA1 heterozygotes leads to derepression of ER activity, which results in increased expression of PgR. This is consistent with the established function of BRCA1 in repression of ligand-independent ER-α signaling activity (25, 26), possibly mediated by the cofactors p300 and cAMP-responsive element binding protein (27). Increased ER activity coupled with increased PgR expression and, presumably, activity, could thus impose a significantly increased risk of tumorigenic progression in normal breast epithelial cells of BRCA1 heterozygotes. In summary, the data presented here provide additional evidence for BRCA1 haplotypinsufficiency in breast epithelial cells of BRCA1 heterozygotes and have potential implications for chemoprevention in this population of women.

Acknowledgments

We thank Lee K. Tan, Department of Pathology, Memorial Sloan-Kettering Cancer Center, for her assistance with pathological review of the breast cancer cases studied.

References

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