Original contribution

Expression of BRCA1 protein in breast cancer and its prognostic significance

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Received 17 August 2007; revised 24 September 2007; accepted 10 October 2007

Keywords: Breast cancer; BRCA1; Tissue microarray; Immunohistochemistry; Prognosis

Summary BRCA1 is a tumor suppressor gene which, when mutated, is associated with the development of hereditary breast cancers. In sporadic tumors, although inherent gene mutations are rare, loss of BRCA1, resulting from reduced expression or incorrect subcellular localization, is postulated to be important. The purpose of the current study was to examine the expression and localization of BRCA1 protein and to assess its prognostic value, in a well-characterized series of unselected breast carcinomas.

We have examined BRCA1 in a series of invasive breast carcinoma (1940 cases) using tissue microarray and immunohistochemistry, to evaluate its expression pattern and to correlate this with clinicopathologic variables and patient outcome. In breast cancer, complete loss of nuclear expression was observed in 223 cases (15%) and cytoplasmic expression was found in 541 breast cancers (36.6%). Absent or reduced nuclear BRCA1 expression was observed more frequently in ductal carcinoma of no special type and medullary-like carcinoma and less frequently in lobular and tubular mixed carcinomas. It was also associated with high-grade, advanced lymph node stage, larger size, vascular invasion, negative estrogen receptor, progesterone receptor and androgen receptor expression, and positive p53 and P-cadherin expression, and with the basal-like class of breast cancer. Altered BRCA1 was associated with shorter disease-free interval. Cytoplasmic expression was also associated with development of recurrence and positive EGFR and HER2 expression. It showed an inverse association with survival particularly in low-grade, small-size, and estrogen receptor–positive subgroups. In the grade 1 subgroup, multivariate analysis with adjustment for other prognostic factors showed that cytoplasmic expression of BRCA1 was an independent predictor of disease-free interval. BRCA1 alteration may play a significant role in the development and progression of breast cancer. Immunohistochemical assessment of BRCA1 expression could provide additional clinically relevant information in routine classification of breast cancer.

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1. Introduction

Breast cancer is the most common cancer and second leading cause of cancer death among women in Western countries [1]. It is recognized to be biologically and clinically heterogeneous. Thus, understanding its biology, the ability to detect its growth potential, and assessment
of certain prognostic factors are of great importance in predicting disease outcome and planning treatment strategies. Moreover, additional molecular markers are being sought to further refine classification of breast cancer, especially in patient subgroups whose outcome cannot be predicted accurately using conventional parameters. One of the biomarkers that has received a great deal of attention in breast cancer is the breast cancer susceptibility gene-1 (BRCA1).

BRCA1 has been mapped to chromosome 17q21 [2]. It encodes a nuclear protein of 1863 amino acids [2] that regulates, at least in part, transcriptional activation, DNA repair, apoptosis, cell-cycle checkpoint control, and chromosomal remodeling [3]. In familial breast cancer, BRCA1 is a classical tumor suppressor gene [4]. The presence of inherited mutations in BRCA1 continues to be one of the best-defined overall risk factors for the development of breast cancer; however, these familial mutations, together with familial BRCA2 mutations, occur in less than 10% of all diagnosed cases [5,6]. By definition, germ-line BRCA1 gene mutations are virtually undetectable in sporadic breast cancers [7], where some authors postulate that loss of BRCA1 protein function, resulting from reduced expression or incorrect subcellular localization, is important in these tumors and provides evidence for the tumor suppressor function [7-11]. They suggest that it is involved in carcinogenesis of these sporadic tumors via mechanisms other than gene mutation. For example, there is some evidence that epigenetic loss of BRCA1 function may occur at the level of transcription or a subsequent step affecting RNA accumulation [12] and promoter hypermethylation [13].

Although the role of BRCA1 in hereditary breast cancers and the characteristics of tumors with germline mutation have been extensively studied, its role in sporadic tumors is still not well defined and there remains controversy regarding the significance of BRCA1 expression and its subcellular localization among different studies. Some studies have demonstrated a relationship between BRCA1-associated breast cancer and development of distant metastasis [14,15], and shorter disease-free survival [10], whereas others did not find any association with survival [15] or other variables [12,16]. The subcellular localization of BRCA1 has been reported to range from nuclear, to cytoplasmic invaginations into the nucleus of the normal cells, and to the abnormal cytoplasmic location [7-9,17,18]. These contradictory reports about the pattern of expression may reflect differences in the specificity of antibodies used, tissue fixation and immunostaining methods, and the presence of different splice variant isofoms of BRCA1.

In this study, we have assessed, using immunohistochemistry (IHC), the expression of BRCA1 protein in a large and well-characterized series of unselected breast cancer with a long-term follow-up, prepared as tissue microarrays (TMA), to evaluate the pattern of expression and the prognostic significance of BRCA1 in this common cancer.

2. Materials and methods

2.1. Patients and tumors

A consecutive series of 1940 cases of invasive breast carcinoma entered into the Nottingham Tenovus Primary Breast Carcinoma Series between 1986 and 1998 were used. This is a well-characterized series of primary operable invasive breast cancer that has been previously used to study a wide range of proteins [19-21]. Patients' clinical history and cancer characteristics including tumor type [22], histologic grade [23], tumor size, lymph node (LN) status, vascular invasion (VI), Nottingham Prognostic Index (NPI) [24,25], and survival data including survival time (overall survival [OS]), disease-free interval (DFI), recurrence, and development of distant metastasis were available for all patients. These patients were not selected on the basis of a family history (eg, a known family history of breast and/or ovarian cancer) or age at diagnosis.

In addition, data on postoperative chemotherapy and hormonal therapy as well as other biomarkers including estrogen receptor (ER), progesterone (PR) and androgen (AR) receptors, p53, ERBB2 (HER2), EGFR (HER1), ERBB3 (HER3), ERBB4 (HER4), P-cadherin, Bcl2, basal cytokeratins (CKs) (CK5/6 and CK14), luminal CKs (CK7/8 and CK19), FHIT protein, MUC-1, and p21 expression [21,26,27] were also available.

2.2. Tissue microarrays and IHC

TMAs were prepared as previously described [28]. In brief, tissue 0.6-mm-diameter cores were punched from representative tumor regions of each donor block and arrayed into a new recipient paraffin blocks using a tissue microarrayer (Beecher Instruments, Sun Prairie, WI). TMA blocks were constructed in duplicate, each containing one sample from a different region of the tumor. TMA sections, 4 μm, were stained immunohistochemically using the standard streptavidin-biotin complex method as previously described [19,29,30]. Briefly, tissue slides were deparaffinized with xylene and then rehydrated through 3 changes of alcohol. Endogenous peroxidase activity was blocked by incubation in a 0.3% hydrogen peroxide/methanol buffer. Antigen retrieval was carried out by microwave treatment of the slides in sodium citrate buffer (pH 6.0) for 20 minutes. The slides were rinsed in Tris-buffered saline (TBS) (pH 7.6) and incubated with normal swine serum in TBS (1:5) to block nonspecific staining. The slides were incubated for 1 hour with the BRCA1 Ab-1, a mouse monoclonal antibody directed against the amino terminal 304 amino acid residues (clone, MS110, Oncogene Research Products, Cambridge, MA, diluted at 1:150) [31]. Several studies have confirmed the reliability of this antibody in formalin-fixed tumors and in microwave antigen retrieval [32]. Several blocking agents were tested including normal swine serum, Marvel, and
bovine serum albumin. The preferred blocker was found to be bovine serum albumin 1%. After washing with TBS, sections were incubated with the secondary antibody (biotinylated goat antimouse/rabbit immunoglobulin; Duet K 0492, DakoCytomation, Glostrup, Denmark) (1:100) for 30 minutes followed by the avidin-biotin complex (1:100) for a further 45 minutes. 3-3′Diaminobenzidine tetrahydrochloride (Dako liquid DAB plus, K3468, Dako, Glostrup, Denmark) was used as a chromogen with the addition of copper sulfate to enhance staining. All sections were counterstained with Mayer’s hematoxylin. Positive (normal breast sections) and negative control slides (according to manufacturer’s data sheet) were included in every experiment.

2.3. Evaluation of immunohistochemical staining

Of the 1940 breast cancer cases, sufficient tissue was available to perform scoring in 1482 cases and these form the basis of this study analysis. As controls, a whole tissue section containing normal breast tissue was included, as well as any normal glandular tissue entrapped in the cores. Assessment of staining was based on a semiquantitative approach. A modified histochemical score (H-score) [33] was used which includes an assessment of both the intensity of staining and the percentage of stained cells. For the intensity, a score index of 0, 1, 2, and 3 corresponding to negative, weak, moderate, and strong staining intensity was used and the percentage of positive cells at each intensity was estimated subjectively. A final score of 0 to 300 is the product of both the intensity and the percentage. In addition, assessment of the subcellular localization and stromal expression was conducted. The pattern of expression was nuclear, cytoplasmic, or combined nuclear and cytoplasmic staining. Two cores were evaluated from each tumor. Each core was scored individually, then the mean of the 2 readings was calculated. If one core was uninformative, either lost or contained no tumor tissues, the overall score applied was that of the remaining core [34]. The cases were scored without knowledge of the patient outcome by one observer on 2 separate occasions and a good correlation between the results was found. For the purpose of this study, positive expression of BRCA1 was identified by positive staining in 5% or more of the neoplastic cells. Positive nuclear expression was further stratified into 2 groups below and above the median of expression (H-score = 100) corresponding to reduced and strong expression. Cutoff values for the other biomarkers included in this study were chosen before statistical analysis and were the same as for previously published patient series [26,35] (Table 1).

2.4. Statistical analysis

Statistical analysis was performed using SPSS 15.0 statistical software (SPSS Inc, Chicago, IL). Associations between BRCA1 expression and clinicopathologic variables were analyzed using the χ² and Mann-Whitney U tests. The association of altered BRCA1 expression upon survival was analyzed initially by Kaplan-Meier plot and log rank test and also with Cox regression to adjust for other prognostic indicators in breast cancers. A P value less than .05 was considered significant.

3. Results

Of the informative breast carcinoma cases (n = 1482), 260 cases were grade 1, 485 cases were grade 2, and 737 were grade 3. At the time of the primary diagnosis, 564 (38%) patients had LN-positive disease (445 cases with one to 3 positive nodes, 119 cases with 4 or more positive). Recurrence occurred in 256 cases (17.6%), distant metastases in 158 cases (10.9%), and 149 (9.7%) patients died from breast cancer. The patients had a median age of 54 years (range, 18–70 years). Median follow-up time was 62 months (range, 1–192 months).

<table>
<thead>
<tr>
<th>Antibody, clone</th>
<th>Dilution</th>
<th>Source</th>
<th>Pretreatment</th>
<th>Cutoff values</th>
</tr>
</thead>
<tbody>
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<td>ER, clone 1D5</td>
<td>1:80</td>
<td>DakoCytomation</td>
<td>Microwave</td>
<td>&lt;1% (negative)</td>
</tr>
<tr>
<td>PR, clone PR 636</td>
<td>1:100</td>
<td>DakoCytomation</td>
<td>Microwave</td>
<td>&lt;1% (negative)</td>
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<td>EGFR, clone EGFR.113</td>
<td>1:10</td>
<td>Novocastra, Newcastle, UK</td>
<td>Microwave</td>
<td>&lt;10% (negative)</td>
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<tr>
<td>HER2, cerbB-2</td>
<td>1:250</td>
<td>DakoCytomation</td>
<td>No</td>
<td>&lt;10% (negative)</td>
</tr>
<tr>
<td>c-erbB3, clone RTJ1</td>
<td>1:20</td>
<td>Novocastra</td>
<td>Microwave</td>
<td>&lt;5% (negative)</td>
</tr>
<tr>
<td>AR, clone F39.4.1</td>
<td>1:30</td>
<td>Biogenex, San Ramon, CA</td>
<td>Microwave</td>
<td>&lt;1% (negative)</td>
</tr>
<tr>
<td>p53, clone DO7</td>
<td>1:50</td>
<td>Novocastra</td>
<td>Microwave</td>
<td>&gt;5% (positive)</td>
</tr>
<tr>
<td>CK5/6, loneD5/16134</td>
<td>1:100</td>
<td>Boehringer Mannheim Biochemica, Mannheim, Germany</td>
<td>Microwave</td>
<td>≥10% (positive)</td>
</tr>
<tr>
<td>CK14, clone LL002</td>
<td>1:100</td>
<td>Biochemica,</td>
<td>Microwave</td>
<td>≥100 (H-score; median)</td>
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<tr>
<td>Anti–E-cadherin (clone HECD-1)</td>
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<td>Zymed Laboratories, South San Francisco, CA</td>
<td>Microwave</td>
<td>≥5% (positive)</td>
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<tr>
<td>Anti–P-cadherin (clone 56)</td>
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<td>BD Biosciences, San Jose, CA</td>
<td>Microwave</td>
<td>≥5% (positive)</td>
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<tr>
<td>Bcl2, clone 124</td>
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<td>Microwave</td>
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<tr>
<td>Anti-FHIT (clone ZR44)</td>
<td>1:600</td>
<td>Zymed Laboratories</td>
<td>Microwave</td>
<td>&gt;5% (positive)</td>
</tr>
</tbody>
</table>
3.1. BRCA1 expression in breast cancer

In the breast, staining was localized to the nuclei of the parenchymal cells with no cytoplasmic or membranous staining. In the malignant tissues, unlike normal breast, the expression intensity was heterogeneous and frequently was less intense than in normal breast and was detected in both nuclei and, in some cases, cytoplasm of the malignant cells. Complete loss of nuclear expression was observed in 223 breast cancer cases (15%). Reduced expression was detected in 578 (39%), whereas strong immunostaining (H-score >100) was seen in 681 (46%) breast cancers. Staining was mainly localized to the nuclei, but unlike normal breast tissue, the expression intensity was heterogeneous and was frequently less intense than in normal breast (Figs. 1 and 2). Cytoplasmic expression was detected in 541 breast cancers (36.6%), which was associated with nuclear expression except in 67 cases (5.1%) that showed cytoplasmic expression only. The average percentage of BRCA1-positive cells was 70% (81% in grades 1 and 2, and 58% in grade 3). BRCA1 stromal staining was weak and insignificant, and, therefore, it was not considered in the analysis. This may reflect the size of cores (TMA), their high proportion of tumor cells or due to genuine weak or absence of expression of BRCA1 protein in the stroma of the malignant breast tissues.

3.2. Nuclear BRCA1 expression in relation to clinicopathologic features

Altered BRCA1 expression, either absent or reduced nuclear expression, was more frequent in ductal carcinomas of no special type (duct/NST) (66%) and medullary-like carcinomas (77%) and less frequent in lobular (27%), mucinous (12%), and tubular mixed carcinomas ($\chi^2 = 132.9$, $df = 9$, $P < .001$). Complete loss or reduced BRCA1 nuclear expression was associated with high tumor grade, advanced LN stage, large tumor size, definite VI, negative hormone receptors (ER [$\chi^2 = 133.4$, $P < .001$], PR [$\chi^2 = 112.8$, $P < .001$], and AR [$\chi^2 = 98.6$, $P < .001$]), and positive p53 expression (Table 2). Loss or reduced expression also showed positive associations with expression of P-cadherin ($\chi^2 = 6.3$, $P = .04$), HER3 ($\chi^2 = 44.5$, $P < .001$), and HER4 ($\chi^2 = 127.8$, $P < .001$), with negative expression of FHIT protein ($\chi^2 = 47.8$, $P < .001$), luminal CKs (7/8 and 19) ($\chi^2 = 21.7$, $P < .001$), and MUC-1 protein ($\chi^2 = 17.2$, $P = .007$), and with cytoplasmic expression of MUC-1 ($\chi^2 = 36.3$, $P < .001$).

Although not significant, loss or reduced BRCA1 nuclear expression showed a trend for development of recurrence ($\chi^2 = 4.7$, $P = .09$), distant metastasis ($\chi^2 = 5.1$, $P = .07$), and negative Bcl-2 expression ($\chi^2 = 3.1$, $P = .08$). No association was found with breast cancer death rate, expression of HER1, HER2, neuroendocrine markers (chromogranin-A and synaptophysin), or p21 expression.

To evaluate the relation between BRCA1 IHC expression and basal-like class of breast cancer, we assessed its expression in the different categories used to define basal-like cancer [36-38]. An association was found between altered nuclear BRCA1 expression and basal-like cancer as defined by (i) Nielsen criteria (ER-negative, HER2-negative, CK5/6-positive, and/or EGFR-positive [36]), in which 123 cases (76% of basal-like cancer) showed altered BRCA1 nuclear expression ($\chi^2 = 31.9$, $P < .001$); (ii) positive basal CK expression (CK5/6-positive and/or CK14-positive [37]) ($\chi^2 = 14.2$, $P = .001$); (iii) positive CK5/6 and/or EGFR ($\chi^2 = 8.9$, $P = .009$). An association was also found between altered nuclear BRCA1 expression and triple-negative phenotype (ER-negative, PR-negative, and HER-negative) [38], in which 185 cases (80% of triple-negative
phenotype) showed altered BRCA1 nuclear expression ($\chi^2 = 68.3, P < .001$).

### 3.3. Cytoplasmic BRCA1 expression

In contrast to nuclear expression, positive rather than absence of cytoplasmic staining was detected more frequently in duct/NST and medullary-like carcinomas and was rarely identified in lobular tumors ($\chi^2 = 56, P < .001$). Cytoplasmic expression was also associated with high grade, large size, development of local recurrence ($\chi^2 = 4.4, P = .036$), p53 expression, P-cadherin, EGFR ($\chi^2 = 33.3, P < .001$), HER2 ($\chi^2 = 14, P < .001$), and basal CKs ($\chi^2 = 11.6, P = .001$) but not associated with PR, AR, or the triple-negative phenotype ($\chi^2 = 0.2, P = .94$). An association of borderline significance was found between positive cytoplasmic BRCA1 expression and ER negativity ($\chi^2 = 3.8, P = .05$).

Interestingly, when we stratified cases into different subgroups, we found that in grade 1 tumors (260 cases), cytoplasmic BRCA1 was associated with larger size ($\chi^2 = 4.3, P = .03$), development of recurrence ($\chi^2 = 4, P = .04$) but no such association was found in grades 2 and 3 tumors.

### 3.4. Patients’ outcome

Survival analyses revealed an association between nuclear BRCA1 and DFI (LR = 7, $P = .03$). The difference was mainly found between the negative group (which showed absence of expression) and the other 2 groups (reduced expression and strong expression) (LR = 8.2, $P = .004$) with obvious overlap in the 2 curves presenting the last 2 groups (LR = 0.5, $P = .8$) (Fig. 3A). However, no significant association has been identified in relation to OS in the whole series (LR = 2.7, $P = 0.3$) or in the different subgroups (Fig. 3B).

Cytoplasmic BRCA1 expression was inversely associated with DFI in the whole series (LR = 5.9, $P = 0.015$) (Fig. 4A). Cytoplasmic expression also showed an inverse association with DFI in grade 1 subgroup (LR = 8.2, $P = .02$), in the smaller size subgroup ($\leq 1.5$ cm) (LR = 7.6, $P = .02$), in the LN-negative (LR = 10.3, $P = .006$), and in the ER-positive subgroups (LR = 11.9, $P = .002$), but not in the advanced more aggressive tumors: high-grade, larger size, LN-positive, and ER-negative subgroups. Moreover, in the ER-positive subgroup (1012 cases), cytoplasmic BRCA1 was also inversely associated with OS (LR = 6.3, $P = .043$) (Fig. 4B).
Multivariate analyses showed that neither nuclear nor cytoplasmic expression of BRCA1 has an independent prognostic significance in the whole tumor series with regard to DFI or OS. However, in grade 1 subgroup, multivariate analysis with adjustment for other prognostic factors including tumor size, LN status, VI, and ER expression showed that cytoplasmic expression of BRCA1 was an independent predictor of DFI (Table 3).

4. Discussion

The complex genomic organization of the BRCA1 gene, the wide array of mutations, the presence of multiple polymorphisms, and the genetic heterogeneity of breast cancer imply that rapid screening in a routine diagnostic setting will be a major technical challenge [39]. Previous studies have demonstrated that IHC detection of loss of BRCA1 protein can identify cases associated with BRCA1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazards Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic BRCA1 expression</td>
<td>3.25 (1.38-7.6)</td>
<td>.007</td>
</tr>
<tr>
<td>LN stage</td>
<td>0.326</td>
<td></td>
</tr>
<tr>
<td>1 vs 3</td>
<td>0.295 (0.06-1.51)</td>
<td>.143</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>0.275 (0.04-1.89)</td>
<td>.189</td>
</tr>
<tr>
<td>Tumor size (≥1.5 cm)*</td>
<td>0.971 (0.41-2.32)</td>
<td>.948</td>
</tr>
<tr>
<td>ER expression</td>
<td>0.25 (0.1-0.75)</td>
<td>.014</td>
</tr>
</tbody>
</table>

* Compared with tumor size <1.5 cm.
Expression of BRCA1 protein in breast cancer

Gene mutations with high sensitivity and specificity [11,40]. This study has investigated the expression profile, prognostic value, and clinical implication of BRCA1 protein expression in breast carcinomas using IHC. To minimize confounding variables on the significance of its expression, we have studied a large and well-characterized series of breast cancer using TMA.

In breast, strong uniform nuclear expression of BRCA1 was observed in the normal parenchymal tissues. Altered expression (absent or reduced nuclear expression or positive cytoplasmic BRCA1 expression) was only found in the malignant tissues. This observed altered expression of BRCA1 was associated with other parameters of poor prognostic and with shorter DFI. In breast cancer, altered expression was seen more frequently in duct/NST and medullary-like carcinomas, tumors with poor NPI, development of recurrence and distant metastasis, negative hormone receptor, and positive p53 and P-cadherin expression. Our results are consistent with previous IHC and mutation analyses of BRCA1 in breast cancer, which demonstrated that BRCA1 mutation–positive tumors or altered expression of BRCA1 was associated with similar characteristics and with parameters of poor prognosis and shortened survival [10,14,15,41-45]. However, it is important to mention that some studies evaluating BRCA1 protein expression and prognosis have shown different results. For example, Taylor et al [11] noted both nuclear and cytoplasmic staining in the majority of normal breast epithelium and lack of correlation between cytoplasmic staining and clinical features; Fraser et al [46] showed no relation to outcome or tumor pathology, however, in their study; MS110 (the antibody used in the current study) showed cross reactivity by Western blotting suggesting antibody nonspecificity.

There is still an ongoing debate concerning the cellular localization of BRCA1 protein in breast cancer and the significance of its abnormal cellular localization. Cytoplasmic expression as detected by IHC was documented in sporadic breast carcinomas and was suggested to play a role in these tumors [46-48]. Interestingly, we have observed that cytoplasmic expression of BRCA1 protein in breast cancer, as detected by the methodology used in this study, is related to behavior of less aggressive tumor types (low-grade, small-size, and ER-positive tumors). In these tumors, positive cytoplasmic expression was associated with other variables of poor prognosis and showed an inverse association with survival. For example, in grade 1 tumors, cytoplasmic BRCA1 expression was an independent predictor of DFI whereas size and LN stage were not. These results indicate that BRCA1 dysregulation may also play a role in early well-differentiated tumors as well as those poorly differentiated, hormone receptor–negative tumors that are known to be associated with BRCA1 gene mutation. Our findings are also supported by (1) the study of Wilson et al [49] who showed that among the different splice variants of BRCA1, the BRCA1-delta 11b, which lacks most of exon 11, was localized in the cytoplasm instead of the nucleus. They suggest that this variant and the full-length BRCA1 may have distinct roles in cell growth regulation and tumorigenesis; (2) previous demonstration that different breast tumor grades have distinct molecular origins, pathogenesis, and behavior with the presence of distinct genetic differences between grade I and grade III tumors [50]. Therefore, an alternative explanation of the presence of cytoplasmic expression could be production of abnormal BRCA1 protein through specific epigenetic changes or nontruncation mutations that are different from those identified in familial cancers. This abnormal protein may play a role in the development of sporadic breast cancer which is different from that produced by germline mutation of BRCA1 gene in the familial cases. However, this point needs further study to assess the protein and gene status in the tumors which showed cytoplasmic expression. Moreover, taking into account that this series is an unselected population of breast cancer patients with subsequent heterogeneity of systemic treatments given to individual cases, the result of correlation with variables of outcome needs to be interpreted with caution.

There are several lines of evidence to suggest a link between BRCA1 deficiency and basal-like breast cancer [51]. Previous studies have demonstrated that many phenotypical, immunohistochemical, clinical characteristics, and molecular features are shared by basal-like breast cancers, as defined by cDNA expression microarrays and tumors that arise in carriers of BRCA1 germline mutations. The majority of BRCA1-associated tumors are “triple negative,” express basal CKs, in addition to other markers commonly seen in basal-like tumors such as p53 and P-cadherin [43,52-58], and, in most studies, both patient groups have a poor outcome [43,59]. To emphasize the relationship between basal-like tumors and BRCA1, some authors have demonstrated that the use of basal CK staining in combination with ER and morphology provides a more accurate predictor of BRCA1 mutation status than previously available and may be useful in selecting patients for BRCA1 mutation testing [51,60,61]. Interestingly, in the current study, an association between IHC expression of BRCA1 and basal-like tumors, using different definitions, was found. In addition, 80% of cases with triple-negative phenotype showed altered nuclear BRCA1 expression. Similarly, an association between altered BRCA1 nuclear expression and positive basal CKs (CK5/6 and/or CK14 [37]) expression was found. These findings also support the relationship between basal-like class of breast cancer and BRCA1 abnormalities and may emphasize the role of IHC detection of BRCA1 to identify basal-like cancer in routine practice.

In conclusion, we have confirmed in a large series of breast cancer cases that aberrant expression in terms of level of expression and subcellular localization of BRCA1 protein is related to biological and pathologic prognostic characteristics as well as clinical outcome. The features of tumors with altered BRCA1 in our study were similar to those described...
in previous studies of familial cancers with BRCA1 germline mutation and showed the same association with other variables indicating that BRCA1 alteration in sporadic tumors are more important than previously thought and confirmed the value of IHC in detection of BRCA1 alteration in routine practice. Its clinical role in prognostic evaluation of these tumors merits further investigation.

References


Expression of BRCA1 protein in breast cancer


