BRCA1 Immunohistochemistry in a Molecularly Characterized Cohort of Ovarian Carcinomas

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Abstract

BRCA1 and BRCA2 dysfunction, frequently seen in high-grade serous ovarian carcinomas, often results from germline mutations, somatic mutations, and promoter methylation. Identification of tumors with BRCA defects has therapeutic and prognostic implications. Identifying germline BRCA mutations is also important given the increased risk for hereditary breast and ovarian carcinoma. Our goal was to assess if immunohistochemistry for BRCA1 is an effective method for detection of BRCA1 dysfunction in molecularly characterized high-grade ovarian serous carcinoma. We identified 43 high-grade ovarian serous carcinomas with known events in BRCA1 and BRCA2 included in The Cancer Genome Atlas Project (TCGA). BRCA1 stain was first assessed without knowledge of *BRCA* status, and a semiquantitative assessment for intensity and amount of staining was performed. The stains were re-evaluated and divided into 3 categories (retained, loss, and equivocal) based on correlation with genotyping data. Presence of retained BRCA staining was considered normal, while the other patterns, including equivocal staining or loss of staining, were considered abnormal. Two pathologists, blinded to *BRCA* status, then scored 2 sets of validation cases selected based on available molecular data—one with only germline mutation status available (n=31) and one with comprehensive genomic data (n=39). The pathologists agreed 88% of the time in the training set and 91% in the validation sets. In the training set, abnormal BRCA staining was seen in 24 cases, of which 21 (87%) showed *BRCA1* genetic abnormalities, one showed *BRCA2* mutations, and 2 showed no *BRCA* abnormalities. Abnormal BRCA1 staining was noted in all 5 cases with *BRCA1* germline mutations, in 3 (60%) of 5 with *BRCA1* somatic mutations, and in 13 (93%) of 14 with *BRCA1* promoter methylation. The 2 validation sets included 70 additional patients, and all cases with germline *BRCA1* mutations (n=11) showed abnormal BRCA1 staining. Tumors with *BRCA1* promoter methylation also showed abnormal staining in 6 (86%) of 7 cases. In the entire study, no cases with *BRCA1* germline mutation showed intact immunostaining (negative predictive value= 100%). This study shows BRCA1 immunohistochemistry is well correlated with molecular events in ovarian

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carcinoma. Considering the high negative predictive value for germline mutations, BRCA1 immunohistochemistry appears to be an effective approach to stratify patients for germline genetic testing and to detect other mechanisms of BRCA1 dysfunction in high-grade serous ovarian carcinomas.

Keywords

BRCA1; BRCA1 immunohistochemistry; ovarian carcinoma; protein expression; serous carcinoma

Introduction

Patients with ovarian cancer usually present at advanced stages and have high rates of morbidity and mortality. High-grade serous carcinoma accounts for the majority of these cases (1). There are many risk factors for ovarian high-grade serous carcinoma, including BRCA1 and BRCA2 dysfunction. BRCA dysfunction frequently results from germline mutations, as seen in hereditary breast and ovarian cancer syndrome. Germline mutations in *BRCA1* and *BRCA2* are detected in 14.5% of serous ovarian carcinomas (2). Other mechanisms of BRCA dysfunction include somatic mutations and promoter methylation. Methylation of the *BRCA1* promoter has been found in 11% of ovarian cancers (2), and somatic mutations of *BRCA1/BRCA2* have been found in 6% of high-grade ovarian cancers (2). BRCA dysfunction appears to be mostly confined to high-grade serous carcinomas of the ovary and does not appear to be associated with increased risk for other histologic subtypes of ovarian cancer, including clear cell, mucinous, and endometrioid carcinomas.

The presence of *BRCA* germline mutations in ovarian carcinomas has been shown to have prognostic and therapeutic significance, including increased sensitivity to platinum-based chemotherapy and susceptibility to PARP (polyADP-ribose polymerase) inhibition. *BRCA1* and *BRCA2* are critical components of double-strand DNA break repair by the conservative mechanism of homologous recombination (HR). Loss of BRCA function results in DNA repair by alternative non-conservative mechanisms, which are prone to error, leading to genomic instability and increased cancer susceptibility. BRCA defective cancers are therefore highly susceptible to DNA damaging chemotherapeutic agents such as platinum analogs (3, 4).

PARP is important for the repair of DNA single-strand breaks. Inhibition of PARP leads to accumulation of single-strand breaks that are converted to double-strand breaks during replication; these are normally repaired by the HR mechanism. In the absence of intact HR (as with BRCA dysfunction), there is accumulation of irreparable double-strand DNA breaks, resulting in increased sensitivity to PARP inhibitors (3).

In addition to implications for therapy, defects in BRCA function also have prognostic significance in ovarian carcinomas. The presence of *BRCA* germline mutations has been shown to correlate with improved clinical outcomes; this may be attributable to younger patient age, ability to achieve optimal debulking, and/or increased sensitivity to platinum agents (3–5). Detection of *BRCA* germline mutations has important implications for patients and their family members; they may benefit from genetic counseling regarding their increased risk for breast and ovarian carcinoma. It also appears that somatic mutations in *BRCA1* and *BRCA2* may confer improved clinical outcomes, similar to those seen with *BRCA* germline mutations (5).

For the reasons outlined above, the identification of ovarian cancers with defective BRCA is clinically relevant and therapeutically important. However, *BRCA* mutation analysis is cumbersome, expensive, and may be impractical as a screening method for detection in all patients. Moreover, patients with *BRCA* promoter methylation and other mechanisms of loss are not identified using current approaches.

Immunohistochemistry (IHC) is an easy and inexpensive technique that is available to most pathologists, and has been incorporated as a routine test for screening for other hereditary syndromes, like Lynch syndrome in colorectal cancer, and more recently, endometrial cancer (6–9). A large study showed that screening with IHC for DNA mismatch repair proteins, followed by confirmatory tests such as mutation analysis or hypermethylation studies, is the most cost-effective method for detection of Lynch syndrome patients (10).

We similarly hypothesize that IHC for BRCA1 could serve as a screening test for detection of BRCA1 dysfunction. This would provide an efficient and low-cost initial screen to select patients for targeted therapies, specifically PARP inhibition. This methodology will also narrow the pool of patients who may benefit from screening for hereditary cancer syndromes and should provide prognostically relevant information.

Materials and Methods

After Institutional Review Board (IRB) approval, we identified a training set of 43 high-grade ovarian serous carcinomas included in The Cancer Genome Atlas (TCGA), selected for known events in *BRCA1* and *BRCA2*.

Genetic subgroups represented included the following:

1. *BRCA1* germline mutation (n=5)

2. *BRCA1* somatic mutation (n=5)

3. *BRCA1* promoter methylation (n=14)

4. *BRCA2* germline mutation (n=5)

5. *BRCA2* somatic mutation (n=3)

6. *BRCA* unaffected (n=11)

The IHC staining method for BRCA1 was optimized using the antibody in a variety of different conditions until one was chosen based on its ability to discriminate different staining patterns in a small group of ovarian cancers with known *BRCA* genotype. One whole section from each of these 43 high-grade serous ovarian carcinomas was evaluated with a commercially available monoclonal antibody against BRCA1 (Ab-1) clone MS110 (mAb) from Calbiochem (Catalogue number OP92). Whole sections (and not TMAs) were used. Heat retrieval was performed with steaming with EDTA pH 8 for 30 minutes. This was followed by incubation of primary antibody for 30 minutes at room temperature (dilution 1:100), followed by incubation with labeled polymer from Envision TM+ System HRP (Dako) for 30 minutes at room temperature. DAB (3,3'-diaminobenzidine) was used as counterstain.

BRCA1 staining was first assessed by a single pathologist (RAS) without knowledge of BRCA status. On initial review, a semiquantitative assessment for intensity and amount of staining was performed. The intensity of staining was recorded relative to that seen in internal positive control. The stains were then re-evaluated with knowledge of the BRCA status, and a cutoff separating tumors into distinct groups based on genotype was selected.

In other words, semiquantitative assessment was used to develop categorical criteria for scoring cases as BRCA1 loss, BRCA1 retained, or BRCA1 equivocal.

The staining pattern was recorded as BRCA1 loss, BRCA1 retained, or BRCA1 equivocal as follows:

1. BRCA1 loss:

   - Complete absence of staining with presence of positive internal control (Figure 1A), or

   - Staining in less than 5% of tumor cells (Figure 1B).

2. BRCA1 retained:

   - Staining in more than 10% of tumor cell nuclei (Figure 2A), or

   - Moderate intensity staining in 5–10% of tumor nuclei with a moderately intense internal control (Figure 2B).

3. BRCA1 equivocal staining:

   - Weak staining in 5–10% of tumor cell nuclei, in presence of moderate to strong internal positive control (Figure 3A).

   - Complete absence of staining without positive internal control (Figure 3B).

The same set of slides was then reviewed by a second pathologist (KG) using the above defined scoring criteria, but without knowledge of the BRCA genotype status. The 2 pathologists, using the above criteria while blinded to BRCA status, then scored 2 sets of validation cases selected based on available molecular data (BRCA1 and BRCA2 germline mutation status only or comprehensive BRCA1/2 genomic status).

Simple associations were performed and tested for statistical significance using Fisher's Exact Test, with a two-sided P < 0.05 considered significant. Cases with loss of staining and equivocal staining were finally combined into an "abnormal BRCA staining" set when correlation between staining and BRCA1 molecular abnormalities was further evaluated in the validation sets.

TCGA genomic data for the training set were obtained from various sources, but all data are available on the MSKCC Computational Biology Center Cancer Genomics Portals at http://www.cbioportal.org/public-portal/. DNA and RNA were co-isolated from ovarian tumors using standard methods. RNA was hybridized to one or more gene expression platforms, and expression values were normalized as previously described (2). Median-centered gene expression values for BRCA1 were extracted from microarray data. DNA was bisulfite modified and hybridized to the Illumina Infinium HumanMethylation27 arrays to identify regions of promoter methylation. DNA methylation beta values reflect the proportion of methylated to unmethylated probes. Four BRCA1 probes were averaged to obtain a mean value for BRCA1 methylation. To identify which samples had BRCA1 promoter methylation, K-means clustering (assuming K=2) on the two-dimensional space of DNA methylation and expression data was used to separate the epigenetically silenced group and non-epigenetically silenced group of samples (2). This process allowed for the selection of cases that had evidence of BRCA1 promoter methylation based on both DNA methylation and RNA expression.

Results

Training set

Interobserver concordance in interpretation of stains—In the initial training set of 43 TCGA cases, interpretation of the BRCA1 IHC stain was concordant between both pathologists in 88% of cases. There were no cases interpreted as positive (loss of BRCA1 immunostaining) vs negative (no loss) by the 2 pathologists. There was disagreement in 5 (12%) of 43 cases, all of which involved an equivocal call by one of the pathologists; the interpretation was equivocal versus positive (n=3) or equivocal versus negative (n=2) (Figure 3C). For the 5 discrepant cases, the slides were re-reviewed by both pathologists and a consensus agreement was reached.

Staining characteristics—Twenty (47%) of 43 cases had loss of BRCA1 expression by IHC, 19 (44%) had intact BRCA1 expression, and 4 (9%) showed equivocal staining for BRCA1. Cases scored as BRCA1 loss (n=20) showed either complete absence of staining (n=6) or weak staining in <5% of tumor cells (n=14). Cases scored as intact BRCA1 (n=19) showed moderate to strong staining in >10% of tumor nuclei (n=17) or moderate intensity staining in 5% of tumor nuclei with a moderately intense internal control (n=2). In most cases, positive staining was noted in approximately 20–30% of tumor cells only. Diffuse strong staining of all tumor cells was not observed in any tumor. Cases scored as equivocal (n=4) showed weak staining in 5–10% of tumor cell nuclei in the presence of moderate to strong internal positive control (n=2), intense staining in 1% tumor nuclei (n=1), or lack of staining in tumor and surrounding normal tissue (n=1). Correlation between BRCA1 IHC and *BRCA* genotype is shown in Table 1.

Results stratified by BRCA1 IHC staining pattern—Among the 20 cases with BRCA1 IHC loss, 19 showed *BRCA1* genetic abnormalities (promoter methylation=12, germline mutation=4, somatic mutation=3), and one had a *BRCA2* germline mutation. Of the 4 cases that showed equivocal BRCA1 staining, one showed *BRCA1* promoter methylation, one had a *BRCA1* germline mutation, and 2 were *BRCA* unaffected. Of the 19 cases with retained BRCA1 IHC staining, 9 were *BRCA* unaffected, 7 showed *BRCA2* abnormalities (3 somatic and 4 germline mutations), 2 showed *BRCA1* somatic mutations, and one showed *BRCA1* promoter methylation.

Results stratified by genotype—The test results were correlated with genomic results by considering all *BRCA1* events together: promoter methylation, germline mutation, and somatic mutation. Loss of BRCA1 immunostaining is abnormal and was considered a positive test result. Only loss of BRCA1 immunostaining and retention of BRCA1 staining were considered in the metrics presented. Equivocal cases were excluded for this component of the analysis, as it is unclear to which group they belong. Considering all genetic and epigenetic abnormalities, the positive predictive value (PPV) of BRCA1 IHC was 95% (19 of 20), and the negative predictive value (NPV) was 84% (16 of 19). Sensitivity and specificity for cases in the training set were 86% and 94%, respectively. If only cases with retained BRCA1 staining were considered "normal" and cases with loss of BRCA1 staining and equivocal staining were considered abnormal, then the PPV, NPV, sensitivity, and specificity were 87%, 87%, 87%, and 84%, respectively. The NPV for BRCA1 germline mutation, specifically, was 100%.

Interestingly, most of the *BRCA1* somatic mutations are insertions or deletions. However, one of the 2 *BRCA1* somatic mutations with retained immunostaining involved a missense mutation of uncertain functional significance at C47W that does not appear in the BIC database (Breast Cancer Information Core Database, http://research.nhgri.nih.gov/projects/

bic/). The other case contained a nonsense mutation at the very end of the protein, R1835*, which may result in a sufficiently long protein that is potentially protected from nonsense mediated decay. The one case of *BRCA1* methylation with retained immunostaining had the lowest level of promoter methylation of all the designated methylated cases. It is therefore possible that low level methylation, in contrast to higher levels of methylation, results in detectable BRCA1 IHC staining. This pattern is, apparently, uncommon (Figure 4).

Validation sets

We used 2 sets of high-grade serous ovarian carcinomas for validation purposes. One set was composed of 31 high-grade serous ovarian carcinomas with known *BRCA1* and *BRCA2* germline mutation status from patients who had clinical genetic testing at MSKCC. The status of somatic mutation or promoter methylation was not known for these cases. The second set consisted of 39 separate TCGA high-grade serous ovarian carcinomas for which comprehensive genomic status, including *BRCA1* methylation, somatic and germline mutations, was known.

Interobserver concordance in interpretation of stains—The 2 pathologists, without knowledge of each other's interpretation or the molecular data, independently assessed BRCA1 IHC on these cases. In the first validation set, agreement between pathologists was reached in almost all cases (29 of 31, 93.5%). In the 2 cases with differences in interpretations, one was scored as equivocal versus negative, while the second was interpreted as equivocal versus positive. In the second validation set, agreement between pathologists was reached in 35 (90%) of 39 cases. All 4 cases with discrepancies were interpreted as retained versus equivocal by the 2 pathologists.

Results stratified by genotype—In the first validation set (Table 2), there were 11 cases with germline *BRCA1* mutations; 7 showed loss of BRCA1 staining (64%) and 4 showed equivocal staining (36%). There were no cases with retained BRCA1 staining in this group. There was one case with a *BRCA2* germline mutation that showed retained BRCA1 staining. There were 19 cases lacking *BRCA1/2* germline mutations for which somatic mutation and methylation status were not known, and all showed intact BRCA1 staining. Whether equivocal cases are excluded or included as abnormal, the sensitivity, specificity, PPV, and NPV for BRCA1 IHC are all 100% when considering germline *BRCA1* mutations. In the second validation set (Table 3), there were 7 cases with *BRCA1* promoter methylation; of these, the staining was interpreted as lost in 4 cases, equivocal in 2 cases, and retained in one case. Two additional cases showed loss of BRCA1 staining: one with a *BRCA1* somatic mutation and one without a known mutation. Seven showed equivocal staining, including the 2 methylated cases, one *BRCA1* somatic mutation, and 4 without known mutation. Twenty-six cases showed retained staining, including one case with a BRCA1 somatic mutation and one with *BRCA1* methylation; the remaining 24 cases included 5 cases with *BRCA2* mutations and 19 cases with no known *BRCA* genomic events. If the equivocal cases are again excluded, and considering all genetic and epigenetic abnormalities, the sensitivity is 71%, the PPV is 83%, the specificity is 96%, and the NPV is 92% for BRCA1 IHC. If only cases with retained BRCA1 staining were considered "normal" and cases with loss of BRCA1 staining and equivocal staining were considered abnormal, then the PPV, NPV, sensitivity, and specificity are 62%, 92%, 80%, and 83%, respectively. The NPV for BRCA1 germline mutation, specifically, was 100%.

Interestingly, of the 3 cases with *BRCA1* somatic mutation, the one case with retained immunostaining had a nonsense mutation, whereas the other 2 cases had a frameshift mutation. The one case of *BRCA1* methylation with retained immunostaining had much

lower levels of DNA promoter methylation than all of the other designated methylated cases (Figure 5).

Combined training and validation sets—When data were combined from the training and both validation sets, 40 (89%) of 45 cases with known *BRCA1* events showed abnormal BRCA1 staining (Table 4). If the equivocal cases are again excluded, the sensitivity is 86%, the PPV is 94%, the specificity is 97%, and the NPV is 92% for BRCA1 IHC. If only cases with retained BRCA1 staining were considered "normal" and cases with loss of BRCA1 staining and equivocal staining were considered abnormal, then the PPV, NPV, sensitivity, and specificity are 83%, 92%, 89%, and 88%, respectively. Overall, of 16 cases with BRCA1 germline mutation, no cases showed intact BRCA1 IHC staining, indicating a 100% NPV for this molecular defect compared to all other results. A summary of all performance metrics is provided in Table 5.

Discussion

Detection of BRCA dysfunction in ovarian carcinomas has prognostic and therapeutic significance. Immunohistochemistry for BRCA1 is an inexpensive and easy initial screen to detect these patients. Our study shows that BRCA1 IHC is an effective method to detect BRCA1 dysfunction in ovarian carcinoma. Based on our data, BRCA1 immunostaining has a negative predictive value of 100% for *BRCA1* germline mutations (all staining intact cases lack *BRCA1* germline mutations). This result is clinically relevant since at present only BRCA germline genetic testing is routinely offered to patients considered at high risk for hereditary breast and ovarian cancer syndromes. BRCA1 immunostaining could also potentially be used to screen for *BRCA1* genetic defects other than germline mutation if the clinical significance of these defects becomes better understood and new agents are developed for targeted therapy (Table 4). Thus, BRCA1 IHC provides a robust initial screen for triaging patients for *BRCA1* germline mutation testing and will also detect other relevant genetic defects in *BRCA1*.

Nearly all cases with intact staining lacked a *BRCA* abnormality, while almost all cases with staining loss were found in cases with *BRCA1* germline mutation, somatic mutation or promoter methylation, but there were exceptions. Of 33 cases with BRCA1 IHC loss, one was found to have a *BRCA2* mutation and one lacked a detectable abnormality in the *BRCA1* gene. Of 66 cases with intact BRCA1 staining, there were 3 with a *BRCA1* somatic mutation and 2 with *BRCA1* promoter methylation. BRCA1 IHC appeared to be the least sensitive for tumors with *BRCA1* somatic mutations, although the data are limited by a small sample size. The reason for this is not clear, but since it appears that haplo-insufficiency of *BRCA* may be sufficient to initiate breast tumorigenesis, it is plausible that it is not essential for tumors to demonstrate complete loss of BRCA1 staining in the presence of somatic *BRCA1* mutation (11). Furthermore, the type of mutation type may also be responsible for the observed staining pattern. Work by Kashima et al (12) indicates that antibodies against the N-terminus of *BRCA1* fail to detect tumors with BRCA1 mutations upstream of exon 11. The antibody used for the current work, likewise, recognizes only the N-terminus of *BRCA1* and one case with a somatic *BRCA1* mutation indeed had intact BRCA1 staining. Both cases with *BRCA1* promoter methylation and retained immunostaining had lower levels of promoter methylation, and each had equal or higher mRNA expression compared to other methylated cases, indicating that BRCA1 IHC does not recognize all cases with *BRCA1* promoter methylation, especially when methylation levels are low. The reason for loss of BRCA1 IHC staining in one case of *BRCA2* mutation is not clear, although it is possible that an undetected *BRCA1* alteration may exist or other unknown molecular function could be relevant.

The prevalence of somatic mutations and *BRCA1* promoter methylation could not be determined in the first validation set. This set was selected from a cohort that had undergone genetic testing and was therefore preselected—all cases were known to be either mutated or wild type in the germline. Somatic mutations in *BRCA1* occur at a relatively low frequency, and in fact, the 8 *BRCA1* somatically mutated cases included in the entire study set of 113 (7%) over-represents this subgroup based on an expected prevalence of approximately 3%. It was nevertheless unexpected that there were no IHC abnormal cases lacking germline mutations in this set.

The BRCA1 antibody has some limitations and can be difficult to interpret. The stain cannot be assessed at scanning or low magnification, but instead it requires assessment of the entire tumor at relatively high magnification. The internal control may show only patchy and weak staining, although it is often moderately intense and diffuse. Comparison of the staining intensity between tumor and non-neoplastic elements must be taken into consideration for the purposes of interpretation. It is also important to note that even tumors with retained staining do not typically show strong and diffuse staining; rather, staining is often focal and patchy with moderate to strong intensity and limited in distribution to less than 30% of tumor cells. Interpretation does become easier and more consistent with experience, however. In our study, the 2 pathologists reached agreement in 38 of 43 cases in the training set and in 64 of 70 cases in the validations sets. In all discrepant cases, one reviewer had diagnosed an equivocal staining pattern, such that none of the discrepancies involved a negative versus positive result. We found that many of the cases with equivocal staining showed *BRCA1* genetic abnormalities. As we gain more experience with the antibody, we may be able to further segregate these cases. Currently, we advocate that these cases should be treated like cases with BRCA1 IHC loss so as to not miss cases of potential clinical significance.

Several groups have previously published the results of BRCA1 IHC in ovarian cancer. As long ago as 2000, Byrne and colleagues reported on the sensitivity and specificity of BRCA1 IHC in a small group of ovarian carcinomas and matched controls (13). The sensitivity, specificity, positive and negative predictive values were 100%. Recently, investigators have described tight associations between the loss of BRCA1 expression and relatively improved survival, although genetic analyses were not performed (14–16). There is also a number of papers describing loss of BRCA1 expression in the setting of *BRCA1* gene mutation (12, 17, 18). Kashima described loss of expression in 8 cases with *BRCA1* germline mutation (12), while Skytte (17) reported a sensitivity of 80%, a specificity of 93%, and an estimated PPV of 73% based on having studied 15 "BRCA1 cancers." In comparison, our study included significantly larger numbers of carcinomas lacking germline mutations and a detailed analysis of genetic abnormalities other than germline mutations. Furthermore, the methodology we employed provided robust results based on having tested the validity of the IHC scoring system derived in the test set using 2 additional validation cohorts. In addition, we have presented information regarding interobserver variability in the interpretation of the stains and recognized an equivocally stained group of cases, all of which may be useful for adaptation of the methodology for clinical practice.

This is therefore a comprehensive study of a BRCA1 IHC antibody in ovarian serous carcinomas. Going forward, the utility of this antibody would be primarily as a screening test to select patients for genetic counseling and testing, although it is acknowledged that we cannot, at present, use morphologic (19) or IHC algorithms to detect patients at highest risk of having a *BRCA2* germline mutation. BRCA1 IHC may also be useful to detect ovarian cancer patients with BRCA dysfunction who might benefit from PARP inhibitors. PARP inhibitors have been shown to be effective in serous carcinomas with germline *BRCA* mutations, and it has been suggested that they may also be beneficial to patients with

sporadic ovarian cancers that have defective HR due to other mechanisms, including promoter methylation and somatic mutations (20–22). Studies correlating BRCA1 staining patterns and response to PARP inhibitors would therefore be helpful to determine utility of IHC for this purpose. Considering the mutual exclusivity between *BRCA1* methylation events and mutation of *BRCA1* or *BRCA2* in the germline or tumor (2), a dedicated *BRCA1* methylation assay may help to further stratify patients without intact BRCA1 IHC for genetic counseling or testing.

Conclusions

These data suggest that BRCA1 IHC is an effective method to screen for *BRCA1* alterations in patients with high-grade serous ovarian cancer. The overwhelming majority of cases with BRCA dysfunction and only a few of the *BRCA* unaffected cases showed loss of staining with a high NPV. Immunohistochemistry for BRCA1 may be a useful initial screening test to select patients for targeted therapy and to detect patients at risk for hereditary breast and ovarian cancer syndromes. These preliminary data indicate that BRCA1 IHC is a clinically useful test.

Acknowledgments

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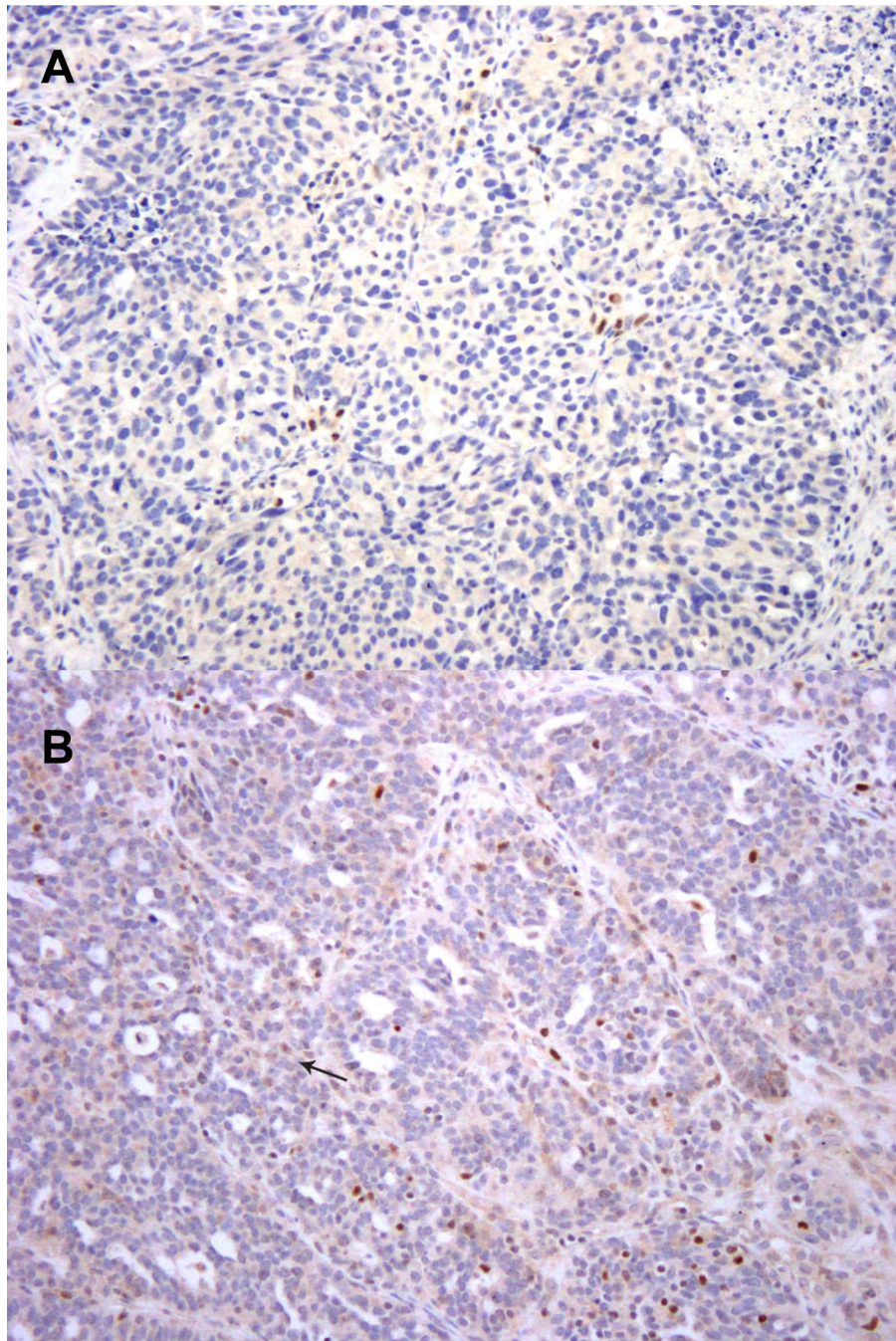**Figure 1.**
A. High-grade serous carcinoma lacking nuclear expression of BRCA1. There is a positive internal control. This is interpreted as "BRCA1 loss."
B. High-grade serous carcinoma with very rare BRCA1 positive tumor cell nuclei (example indicated by arrow). The weak staining intensity of positive tumor cell nuclei contrasts with the strong staining seen in tumor infiltrating lymphocytes. This is interpreted as "BRCA1 loss."
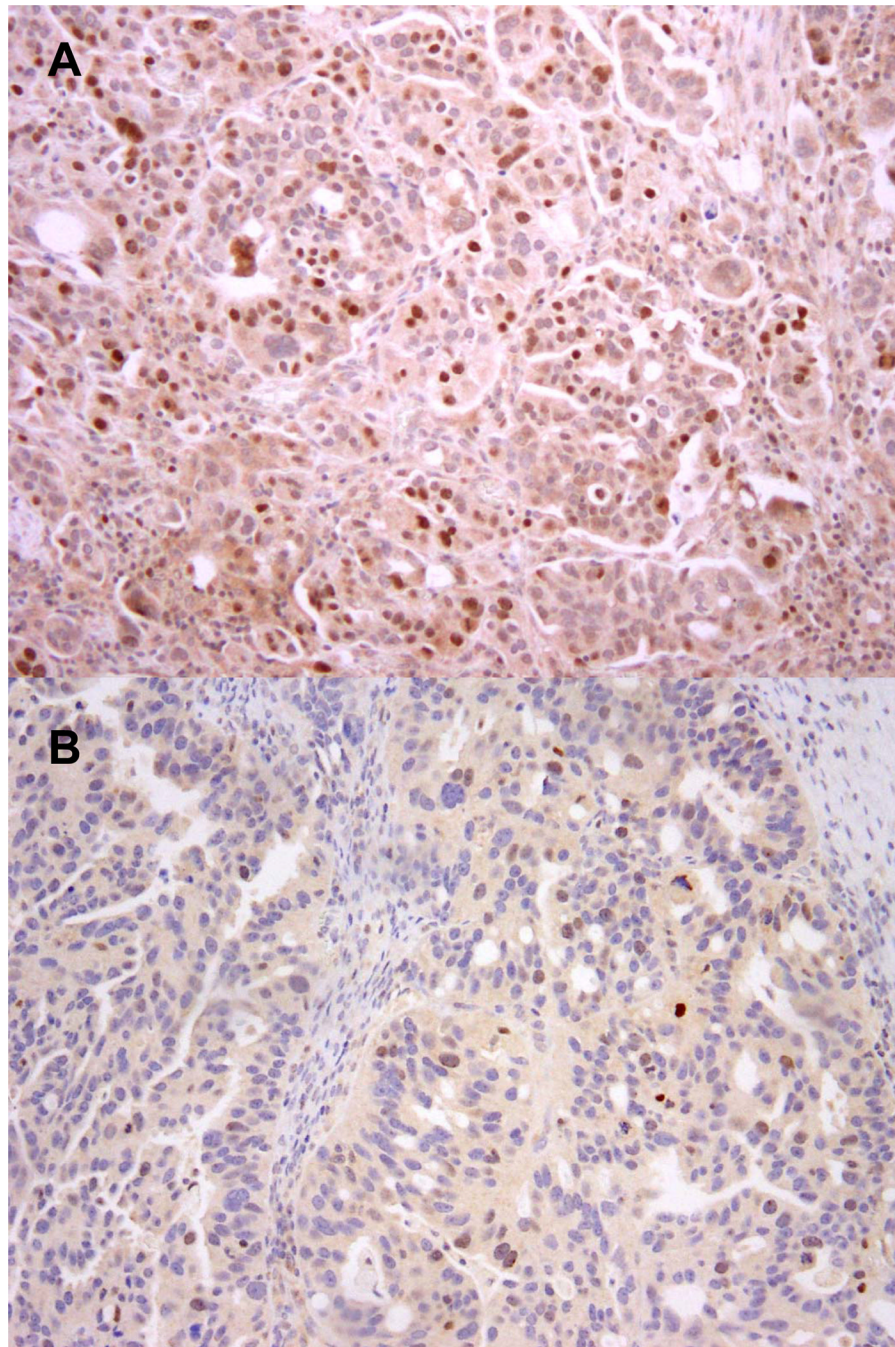
Figure 2.
A. High-grade serous carcinoma with numerous tumor cell nuclei that express BRCA1. This is interpreted as retained expression of BRCA1.
B. High-grade serous carcinoma with moderate intensity staining in 5–10% of tumor nuclei in a selected intermediate power field. There is a moderately intense internal control. This is interpreted as retained expression of BRCA1.
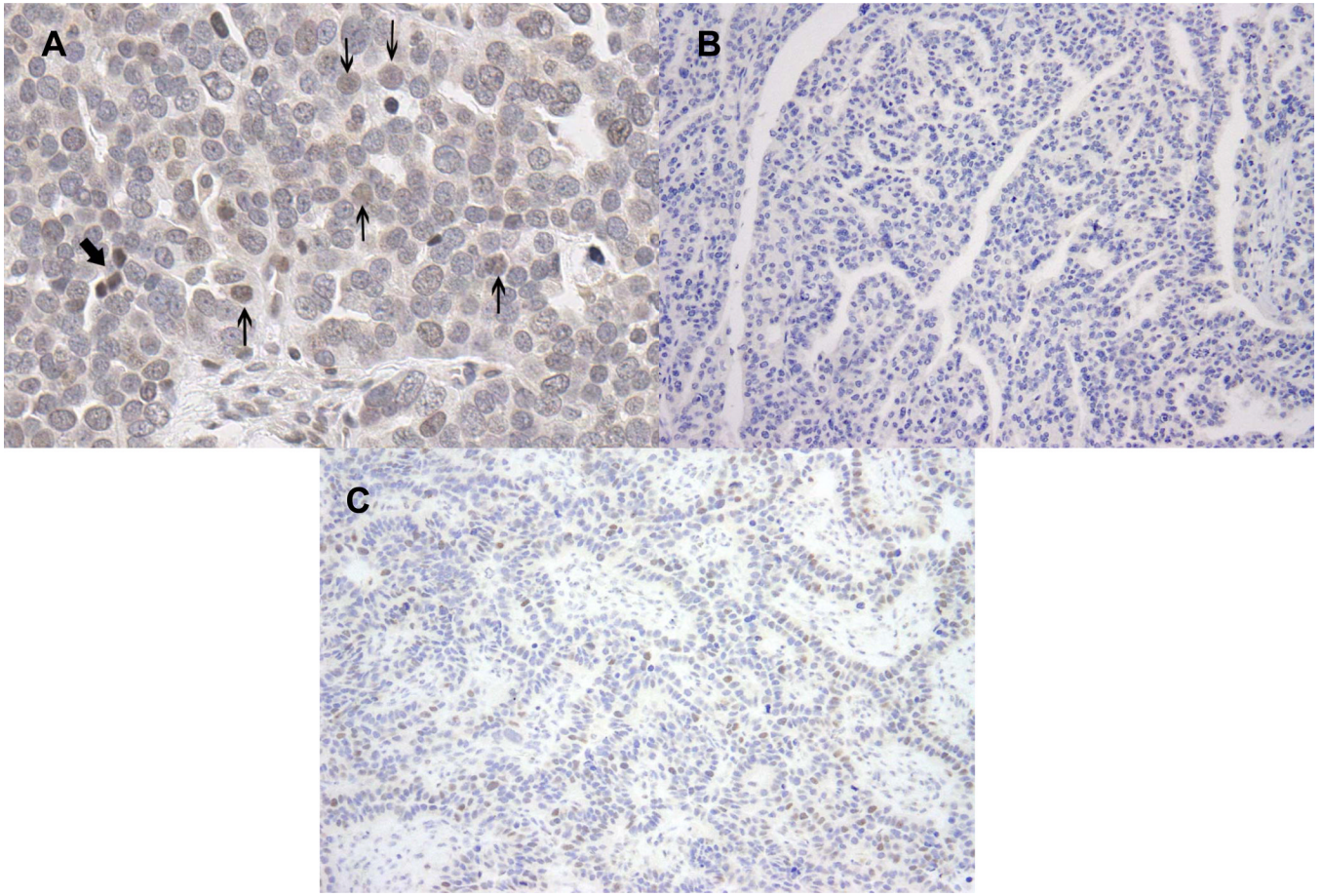
Figure 3.

A. High-grade serous carcinoma with weak staining in 5–10% of tumor cell nuclei, in presence of moderate to strong internal positive control. The thick arrow indicates a positive internal control with moderately intense staining. The thin arrows indicate examples of weakly stained tumor cell nuclei. The density of weakly stained nuclei is higher than in Figure 1B. This is interpreted as an equivocal result

B. High-grade serous carcinoma lacking nuclear expression of BRCA1. There is no positive internal control. This is interpreted as an equivocal result.

C. High-grade serous carcinoma with equivocal staining results. One pathologist scored this case as showing intact BRCA1 expression whereas the other pathologist scored the case as equivocal. Reasons to explain this discrepancy include differences in estimating the percentage of positive tumor cell nuclei and difficulties distinguishing tumor infiltrating lymphocytes from tumor cells. Compared to Figure 2B, staining intensity of tumor cell nuclei is very weak. Cases with this type of discrepancy were captured as "equivocal" for purposes of the study. The tumor had *BRCA1* promoter methylation.
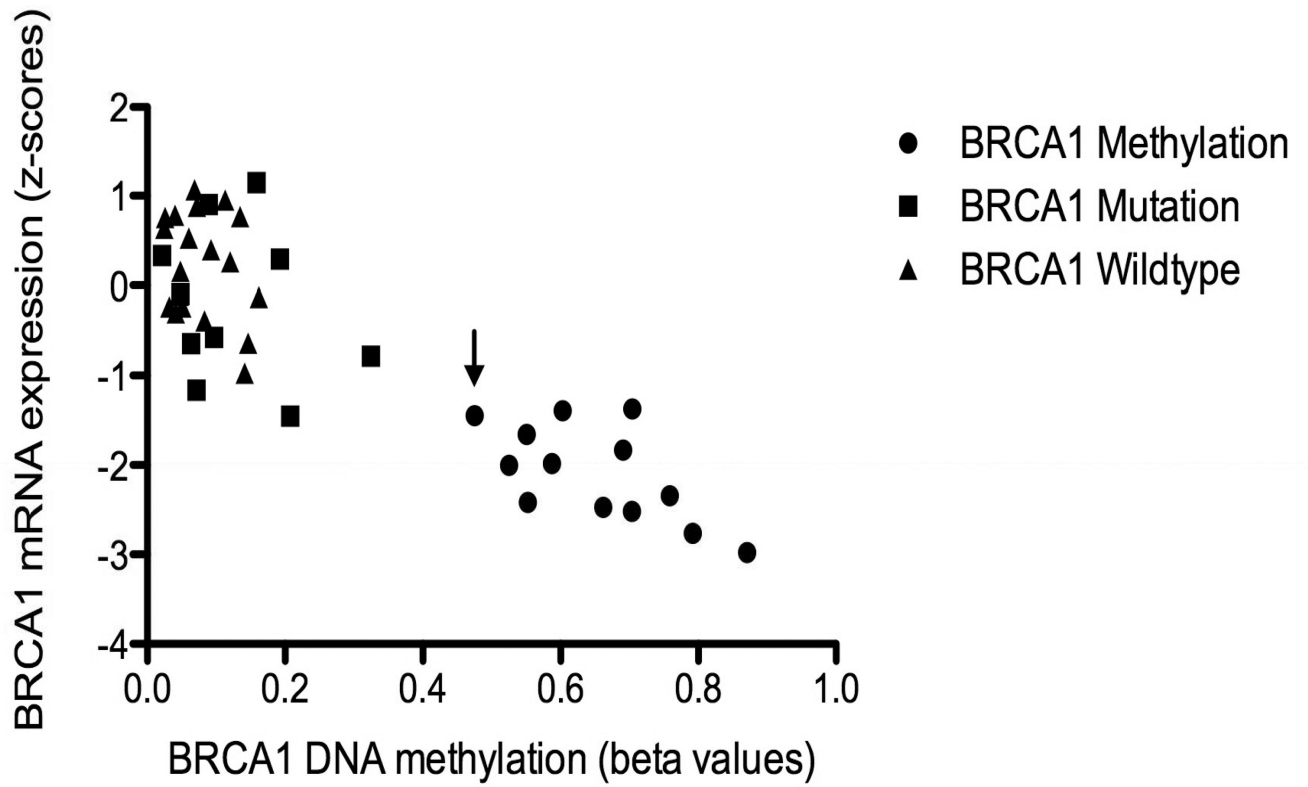
Figure 4.
BRCA1 gene expression and promoter methylation values in the training set (n=43). Arrow indicates methylated case with retained immunostaining.
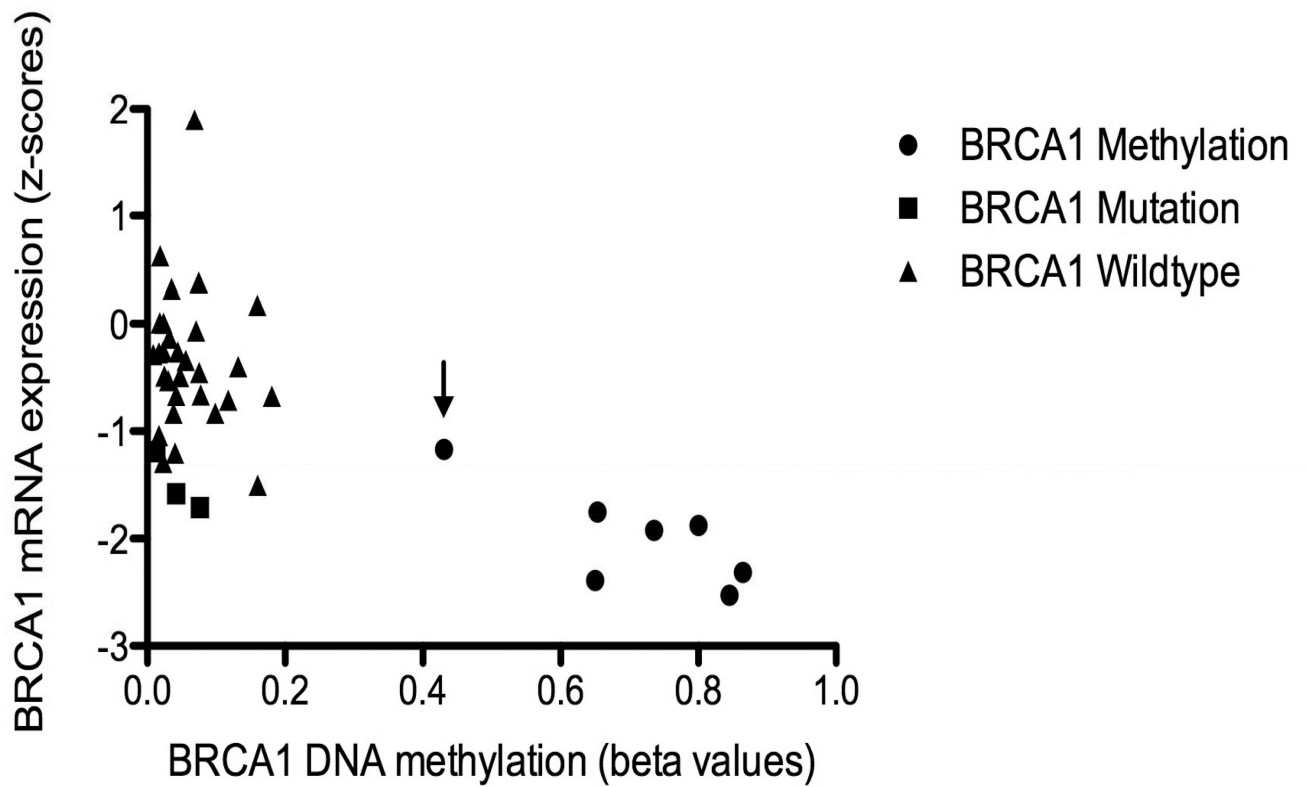
Figure 5.
BRCA1 gene expression and promoter methylation values in validation set 2 (n=39). Arrow indicates methylated case with retained immunostaining.

Table 1

Correlation between loss of BRCA1 IHC and BRCA1 genomic events for the training set (n=43)

	BRCA1 germline mutations (n=5)	*BRCA1* somatic mutations (n=5)	*BRCA1* promoter methylation (n=14)	*BRCA2* mutations (n=8)	*BRCA* unaffected (n=11)
BRCA1 IHC loss (n=20)	4 (80%)	3 (60%)	12 (86%)	1 (12%)	0
BRCA1 IHC equivocal (n=4)	1 (20%)	0	1 (7%)	0	2 (18%)
BRCA1 IHC intact (n=19)	0	2 (40%)	1 (7%)	7 (88%)	9 (82%)

Table 2

Correlation between loss of BRCA1 IHC and BRCA1 germline mutations for validation set 1 (n=31)

	BRCA1 **germline mutations (n=11)**	*BRCA2* **germline mutations (n=1)**	*BRCA* **unaffected / unknown (n=19)**
BRCA1 IHC loss (n=7)	7 (64%)	0	0
BRCA1 IHC equivocal (n=4)	4 (36%)	0	0
BRCA1 IHC intact (n=15)	0	1 (100%)	19 (100%)

Table 3

Correlation between loss of BRCA1 IHC and BRCA1 genomic events for validation set 2 (n=39)

	BRCA1 somatic mutations (n=3)	*BRCA1* promoter methylation (n=7)	*BRCA2* mutations (n=5)	*BRCA* unaffected (n=24)
BRCA1 IHC loss (n=6)	1 (33%)	4 (57%)	0	1 (4%)
BRCA1 IHC equivocal (n=7)	1 (33%)	2 (29%)	0	4 (17%)
BRCA1 IHC intact (n=26)	1 (33%)	1 (14%)	5 (100%)	19 (79%)

Table 4

Correlation between loss of BRCA1 IHC and BRCA1 genomic events for the training and validation Sets combined (n=113)

	BRCA1 germline mutations (n=16)	*BRCA1* somatic mutations (n=8)	*BRCA1* promoter methylation (n=21)	*BRCA2* mutations (n=14)	*BRCA* unaffected (n=54)
BRCA1 IHC loss (n=33)	11 (69%)	4 (50%)	16 (76%)	1 (7%)	1 (2%)
BRCA1 IHC equivocal (n=15)	5 (31%)	1 (12%)	3 (14%)	0	6 (11%)
BRCA1 IHC intact (n=65)	0	3 (38%)	2 (10%)	13 (93%)	47 (87%)

Table 5

Summary of performance metrics for BRCA1 immunostaining

Case set	PPV	NPV	Sensitivity	Specificity
Training set, equivocal cases excluded (n=40)	0.95	0.84	0.86	0.94
Training set, equivocal cases included [*] (n=43)	0.87	0.87	0.87	0.84
Validation set 1, equivocal cases excluded (n=27)	1	1	1	1
Validation set 1, equivocal cases included [*] (n=31)	1	1	1	1
Validation set 2, equivocal cases excluded (n=32)	0.83	0.92	0.71	0.96
Validation set 2, equivocal cases included [*] (n=39)	0.62	0.92	0.80	0.83
All data combined, equivocal cases excluded (n=99)	0.94	0.92	0.86	0.97
All data combined, equivocal cases included [*] (n=113)	0.83	0.92	0.89	0.88

Note: These data account for test performance in the detection of any genetic or epigenetic abnormality involving *BRCA1*. *BRCA2* results are combined with cases lacking *BRCA1* mutation or promoter methylation for the purposes of this calculation. NPV for BRCA1 germline mutation, specifically, was 100%.

[*] equivocal cases are considered abnormal