Abstract

Purpose: The importance of the BRCA gene products in maintaining genomic stability led us to hypothesize that BRCA-associated and sporadic ovarian cancers would have distinctive genetic profiles despite similarities in histologic appearance.

Experimental Design: A whole-genome copy number analysis of fresh, frozen, papillary serous ovarian cancer DNA was done using the Affymetrix 50K Xba Mapping Array using each patient’s normal genomic DNA as the matched control. Loss of heterozygosity and copy number abnormalities were summarized to define regions of amplification, deletion, or uniparental disomy (UPD), defined as loss of one allele and duplication of the remaining allele. Genomic abnormalities were compared between BRCA-associated and sporadic tumors.

Results: We compared 6 BRCA-associated with 14 sporadic papillary serous ovarian carcinomas. Genetic instability, measured by percentage of genome altered, was more pronounced in BRCA-associated tumors (median, 86.6%; range, 54-100%) than sporadic tumors (median, 43.6%; range, 2-83%; \( P = 0.009 \)). We used frequency plots to show the proportion of cases affected by each type abnormality at each genomic region. BRCA-associated tumors showed genome-wide loss of heterozygosity primarily due to the occurrence of UPD rather than deletion. UPD was found in 100% of the BRCA-associated and 50% of the sporadic tumors profiled.

Conclusions: This study reports on a previously underappreciated genetic phenomenon of UPD, which occurs frequently in ovarian cancer DNA. We observed distinct genetic patterns between BRCA-associated and sporadic ovarian cancers, suggesting that these papillary serous tumors arise from different molecular pathways.

Solid tumor DNA undergoes a wide range of genetic changes, including translocations, inversions, duplications, amplifications, deletions, and alterations in chromosome number (1–3). Like other solid tumors, ovarian cancer is genetically characterized by aneuploidy (4), cytogenetic chromosomal abnormalities (5), and deregulation of multiple genetic pathways (6). Among all of the gynecologic malignancies, ovarian cancer has the highest fatality-to-case ratio and it remains the fifth leading cause of all cancer-related mortality in women (7). This is due in large part to the advanced-stage, high-grade, papillary serous subtypes.

Approximately 10% of ovarian cancers are attributable to a hereditary predisposition from an inherited germ-line mutation in BRCA1 or BRCA2 (8). In the decade that has passed since these cancer susceptibilities genes were first identified, an increasing understanding of the molecular and cellular roles of the proteins they encode has emerged and their roles in maintaining genomic integrity and regulating cellular proliferation have been described (9–14). A female BRCA mutation carrier’s lifetime risk of developing ovarian cancer is ~16% to 44% with a BRCA1 mutation and 16% to 27% with a BRCA2 mutation (15–17). The majority of ovarian cancers arising in this high-risk subpopulation of women are advanced-stage, high-grade, papillary serous carcinomas (18).

The important role of the BRCA proteins in maintaining genomic stability led us to hypothesize that the molecular pathways leading to ovarian carcinogenesis in BRCA-associated tumors would differ compared with those in sporadic carcinomas even despite similarities in histologic appearance. A new array-based technology containing single nucleotide polymorphisms (SNP) has emerged, which allows for a high-density, genome-wide analysis of allelic imbalance and loss of heterozygosity (LOH) from a single DNA sample (19–21). This
Translational Relevance

Ovarian cancer continues to present significant clinical challenges. Despite initial success achieved with surgical debulking and platinum-based chemotherapy, the majority of epithelial ovarian cancers will recur and lead to death due to chemoresistant disease. There is a critical need to expand our treatment armamentarium against this highly fatal gynecologic malignancy. Toward this end, an understanding of the genetic events driving the growth of these tumors will ultimately lead to the development of more effective targeted therapies. The completion of the draft human genome has led to technologies that are accelerating the pace of these discoveries. In this study, we use a SNP-based mapping array to study the genetic events occurring in papillary serous ovarian cancers. Despite histologic similarities, we have found distinct genetic profiles that distinguish BRCA1/2-associated and sporadic ovarian carcinomas. The "BRCA signature" is defined by UPD, a genetic event that was underrecognized previously due to the difficulty of its identification with older genotyping technologies. BRCA-associated and sporadic ovarian cancers appear to arise out of distinct molecular pathways. These findings have important clinical implications for future studies defining the molecular oncogenesis of ovarian cancer and targeted treatment approaches.

Materials and Methods

Subjects. We identified 20 patients with papillary serous epithelial ovarian cancer who donated ovarian cancer tissue and blood at the time of primary cytoreductive surgery under an institutional review board-approved protocol at Cedars-Sinai Medical Center. Demographic, clinical, surgical, and histopathologic data were collected by review of medical records, inpatient and outpatient charts, operative reports, and pathology reports.

DNA isolation. DNA was isolated from fresh, snap-frozen, ovarian cancer samples collected during primary cytoreductive surgery using the Qiagen DNeasy Tissue Protocol (Qiagen). Briefly, 25 mg tumor tissue was sequentially treated with lysis buffer and proteinase K overnight at 55 °C, RNase A buffer (100 mg/mL), and 100% ethanol. Precipitated DNA was bound to DNeasy mini spin column, washed, and eluted. Genomic DNA was isolated from matched peripheral blood samples using a standard phenol-chloroform extraction method.

BRCA mutation analysis. Nine patients underwent commercial germ-line BRCA mutation testing based on characteristics of the clinical and/or family history that suggested an elevated risk for a hereditary ovarian cancer predisposition. For the remaining 11 cases, BRCA1 and BRCA2 mutation status were determined by laboratory evaluation of DNA isolated from peripheral blood. BRCA1 exon 11 and BRCA2 exons 10 and 11 were screened for mutations by protein truncation testing, which screens for truncating mutations of the two genes. This covers ~58% of the coding region of BRCA1 and 50% of the coding region of BRCA2. The three common Ashkenazi Jewish founder mutations (BRCA1 exon 2 185delAG, BRCA1 exon 20 5382insC, and BRCA2 exon 11 614delT) were tested by gel electrophoresis. The BRCA1 exon 13 ins6kb mutation was also tested by gel electrophoresis. As part of the protocol, all mutations detected by screening protocols were confirmed by DNA sequencing. Laboratory mutation analysis was done at the Center for Research in Women’s Health at the Sunnybrook Research Institute and/or the University of Toronto.

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Women’s College Health Science Center, University of Toronto under the direction of S.A.N.

**Mapping 50K array hybridization.** For each case, tumor DNA and matched normal DNA were each hybridized to a Mapping 50K Xba array (Affymetrix). Briefly, 250 ng genomic DNA was digested with XbaI restriction enzyme and ligated to common adaptors, allowing one primer PCR amplification of the entire genome. PCR products were fragmented, end-labeled, and hybridized to the array.

**Data analysis.** Data were processed and visually displayed using the Copy Number Analyzer for GeneChip (University of Tokyo) software program (27). Figure 1 shows an example of the Copy Number Analyzer for GeneChip visual output for chromosome 17 of a representative case. LOH and allele-specific plot data are available because matched peripheral blood DNA was used as the reference for each tumor DNA sample. The sum of these plots were summarized and genomic regions were classified as normal, amplified, deleted, or as an area of normal copy number LOH (UPD). UPD with amplification (copy number >2) was found in areas of LOH with deletion of one allele and greater than two copies of the remaining allele. Each genomic abnormality was measured, classified, and schematically represented at its location along the genome. All graphs and statistical analyses were done with Stata version 8 (Stata).

A variable was created to represent the percentage of the genome altered in each tumor. The length of each abnormality was summed and the total was divided by 2917.4 Mb, the length of genome coverage on the 50K Array. Frequency plots were generated to display the frequency of each type of genomic abnormality along each chromosome. The genomic abnormalities occurring in ovarian cancer DNA were compared between sporadic and BRCA-associated groups.

### Results

**Patient characteristics.** Among 20 patients with papillary serous epithelial ovarian cancer, 18 (90%) patients were Caucasian and 9 (45%) were Jewish, and median age at diagnosis was 62 years (range, 45-72). All patients underwent surgical cytoreduction; 19 of 20 patients were optimally cytoreduced to <1 cm residual disease. Stage distribution included 2 stage IIC, 3 stage IIIB, 11 stage IIIC, and 4 stage IV ovarian cancers. Following surgery, all patients received systemic adjuvant chemotherapy with a platinum-based regimen (18 carboplatin/paclitaxel and 2 cisplatin/cytoxan). All 20 patients recurred with a median progression-free survival of 14 months (range, 0.1-39.2). Median overall survival was 42.9 months (range, 11.5-90.5).

Nine patients with suspicious clinical or family histories underwent commercial BRCA mutation testing and 6 tested positive. None of the 11 remaining cases tested in the laboratory were found to have an additional germ-line mutation. Among the 6 patients, there were 3 with BRCA1 mutations, 2 with BRCA2 mutations, and 1 with a mutation in both BRCA genes. The spectrum of BRCA mutations are detailed in Table 1.

**Genetic instability of ovarian cancer DNA.** Ovarian cancer DNA was found to have a great degree of genetic instability as well as heterogeneity between cases. The degree of genetic instability was measured as a percentage of the genome altered inclusive of all areas with copy number changes and/or LOH. Among the 20 cases, the percent of genome altered varied from 4% to 100%, with a median of 67.4%. Patients with germ-line BRCA mutations had tumors with more genetic alterations (median, 86.6%; range, 54-100%) compared with BRCA-negative patients (median, 43.6%; range, 2-83%; \( P = 0.009 \)).

**Patterns of genomic abnormalities differ among BRCA-associated tumors.** We created a frequency plot summarizing the specific types of genetic changes occurring in sporadic versus BRCA-associated tumors (Fig. 3). LOH (shown in Fig. 3A, pink columns) occurs in genomic regions that have undergone hemizygous deletion (Fig. 3B, green columns) or uniparental disomy (Fig. 3C, blue columns).

Sporadic tumors undergo a lower level of LOH compared with the BRCA tumors and the areas undergoing LOH are largely represented by hemizygous deletions. In contrast, the BRCA-associated tumors have a dramatic degree of LOH affecting the majority of the genome. With the exception of chromosomes 7 and 20, every other chromosome is affected by LOH in at least 50% of the cases. In the BRCA cases, LOH occurs 100% of the time on regions of chromosomes 17 and 22 and the X-chromosome. This increased level of LOH is largely due to UPD rather than deletion. Among the sporadic tumors, UPD occurs at a lower frequency along the entire genome, with the exception of a higher frequency of UPD occurring on chromosome 17.

Amplifications (Fig. 3D, red columns) occur at greater frequency among the BRCA-associated tumors and are relatively...

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### Table 1. Spectrum of BRCA mutations

<table>
<thead>
<tr>
<th>Case</th>
<th>BRCA1 mutation</th>
<th>BRCA2 mutation</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>185delAG</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>185delAG</td>
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</tr>
<tr>
<td>3</td>
<td>185delAG</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2699delT</td>
<td>2185delAG</td>
</tr>
<tr>
<td>5</td>
<td>5674delT</td>
<td>185delAG</td>
</tr>
<tr>
<td>6</td>
<td>5674delT</td>
<td>185delAG</td>
</tr>
</tbody>
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http://www.aacrinefs.org
evenly distributed across the genome. Exceptions occur at chromosomes 13, 16, and 17, where very rare amplifications occur in the BRCA-associated tumors.

**Genetic alterations in higher resolution.** We mapped the specific types of abnormalities occurring on chromosomes 13 and 17 (Fig. 4). Cases 1 to 6 correspond to the tumors from BRCA mutation carriers (case number corresponds to BRCA mutations described in Table 1), whereas cases 7 to 20 represent the sporadic tumors. Among the 6 BRCA-associated cases, the entirety of chromosomes 13 and 17 are affected almost exclusively by UPD or deletion. We also determined the genetic alterations occurring at the loci containing important tumor suppressor genes. On chromosome 13, BRCA2 (located at 31.7 Mb) and RB1 (located at 47.7 Mb) are affected by deletion/UPD in 5 of 6 (83%) BRCA-associated cases compared with 3 of 14 (21%) sporadic cases \( (P = 0.01, \text{ Fisher’s exact test}) \). On chromosome 17, TP53 (located at 7.5 Mb) and BRCA1 (located at 38.5 Mb) are affected by deletion/UPD in 6 of 6 (100%) of the BRCA-associated cases compared with 9 of 14 (64%) of the sporadic cases \( (P = 0.1) \).

Analysis of the genomic events occurring at the BRCA1 and BRCA2 loci among BRCA1/2 mutation carriers shows UPD to be a frequent cause of LOH (Fig. 4). Among the 4 patients with germ-line BRCA1 mutations (cases 1-3 and 6), LOH at the BRCA1 locus (chromosome 17, 38.5 Mb) occurs by UPD in 3 cases and by deletion in 1 case. Among the 3 patients with germ-line BRCA2 mutations (cases 4-6), LOH at the BRCA2 locus (chromosome 13, 31.7 Mb) occurs by UPD in 1 case and by deletion in 2 cases.

Details of the genetic alterations occurring on all 22 autosomal chromosomes and the X chromosome can be found in the Supplementary Materials. Visual comparison of the 6 BRCA-associated tumors compared with the 14 sporadic tumors further shows the higher degree of genetic instability in the BRCA-associated cases. Additionally, color changes represent copy number transitions, showing multiple areas of chromosomal breakage but no areas representing recurrent breakpoints in either group.

**Patterns of UPD.** Using the SNP-based array platform, we found UPD in 13 of the 20 (65%) ovarian cancer genomes

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**Fig. 3.** Frequency plot summarizing the specific types of genetic changes occurring in sporadic versus BRCA-associated tumors: LOH (A, pink columns), deletions (B, green columns), UPD (C, blue columns), and amplifications (D, red columns). The percentage of cases affected by a specific type of genomic abnormality at each region is represented as a vertical column along the Y-axis. The genomic location is represented along the X-axis with chromosome 1 to the left and the X chromosome on the right.
LOH and UPD in BRCA1/2-Associated Ovarian Carcinomas

There are substantial differences in the frequency and types of genetic abnormalities occurring in BRCA-associated versus sporadic ovarian cancer. Compared with sporadic ovarian cancers, BRCA-associated tumors are characterized by increased genomic instability. The types of genomic alterations also differ between tumor types. BRCA-associated ovarian cancers show more frequent amplifications, as well as LOH due to UPD, but do not appear to have a greater frequency of allelic loss due to deletions.

These findings are compatible with the known function of the BRCA proteins in maintaining genomic integrity. The BRCA1 and BRCA2 genes encode large nuclear proteins that are widely expressed during the S and G2 phases of the cell cycle (12). BRCA1 has been implicated in a wide number of cellular processes, including chromatin remodeling, transcriptional regulation, and DNA repair (9, 13, 28). BRCA2 has been found to have a more limited role in DNA recombination and repair, mainly acting as a regulator of RAD51 activity (12, 28, 29).

Deficiency in the BRCA1 or BRCA2 proteins results in the loss of homologous recombination, a conservative DNA repair process that repairs double-strand DNA breaks during the S-G2 phases of the mitotic cell cycle (12). BRCA1 has been implicated in a wide number of cellular processes, including chromatin remodeling, transcriptional regulation, and DNA repair (9, 13, 28). BRCA2 has been found to have a more limited role in DNA recombination and repair, mainly acting as a regulator of RAD51 activity (12, 28, 29).

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Discussion

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a SNP-based array platform, which combines LOH with copy number data, has allowed us to identify UPD as a novel finding within the ovarian cancer genome, most significantly in those tumors arising on the background of a germ-line BRCA mutation.

Since the advent of the SNP array technology, several other groups have shown UPD to be a frequent somatic event occurring in various cancer types (25, 26, 36, 37). The distribution of UPD was found to frequently extend to the telomere, implying a mechanism of somatic recombination as the cause of normal copy number homozygosity (26, 38). We have found a similar high occurrence of UPD extending to the telomere, occurring in 41% of the UPD events in the tumors of BRCA mutation carriers and 44% of the UPD events in sporadic tumors. However, we also found a high frequency of UPD events buried within chromosomes as well as tandemly arranged with other UPD events with various copy number changes. The complexity of the UPD patterns seen in ovarian carcinoma signals the possibility of other mechanisms underlying these events, such as structural alterations resulting from interchromosomal recombinations due to defects in the homologous recombination DNA repair process.

Different regions that undergo UPD are likely to contain important genes that drive the carcinogenic process. One of the two daughter cells may acquire a growth advantage due to differences in alleles between the involved gene copies, resulting in a tumor cell population dominated by the descendents of the selected clone (41). Indeed, this has been shown to be the case in several hematologic processes. In myeloproliferative disorders, the acquired activating mutation of the JAK2 tyrosine kinase is homozygous in a subset of patients due to somatic recombination (42), and in acute myeloid leukemia, homozygous mutations of the WT1, FLT3, CEBPA, and RUNX1 genes have been found, implying that mutation precedes mitotic recombination, which acts as the “second-hit” that removes the normal, wild-type allele (43).

This study shows the complexity of the genomic abnormalities occurring during the ovarian cancer carcinogenic process. Distinct differences exist in the molecular pathways leading to BRCA-associated versus sporadic ovarian cancers. In tumors arising from a background of BRCA deficiency, the loss of the homologous recombination DNA repair process results in genomic instability. Use of the 50K SNP array to profile the genetic changes occurring in ovarian cancer DNA shows frequent UPD in BRCA-associated tumors, supporting a link between loss of BRCA function and the occurrence of somatic recombination events. Further work in a larger data set may penetrate the background noise generated by genomic instability to reveal the nonrandom genetic events occurring in BRCA-associated ovarian cancers and lead to the identification of critical genes in the carcinogenic process.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


*Clin Cancer Res 2008;14(23) December 1, 2008 7650 www.aacrjournals.org*
Clinical Cancer Research

Genome-Wide Loss of Heterozygosity and Uniparental Disomy in BRCA1/2-Associated Ovarian Carcinomas

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