Nonequivalent Gene Expression and Copy Number Alterations in High-Grade Serous Ovarian Cancers with BRCA1 and BRCA2 Mutations

Joshy George1,3, Kathryn Alsop1,3, Dariush Etemadmoghadam1,2,4, Heather Hondow1, Thomas Mikeska1,4, Alexander Dobrovic1,2,4, Anna deFazio7,8, for the Australian Ovarian Cancer Study Group1,7,8,9, Gordon K. Smyth5,6, Douglas A. Levine10, Gillian Mitchell1,2, and David D. Bowtell1,2,3,4

Abstract

Purpose: High-grade serous carcinoma (HGSC) accounts for the majority of epithelial ovarian cancer deaths. Genomic and functional data suggest that approximately half of unselected HGSC have disruption of the BRCA pathway and defects in homologous recombination repair (HRR). Pathway disruption is regarded as imparting a BRCA1/2 phenotype. We explored the molecular changes in HGSC arising in association with specific BRCA1/BRCA2 somatic or germline mutations and in those with BRCA1 DNA promoter methylation.

Experimental Design: We describe gene expression and copy number analysis of two large cohorts of HGSC in which both germline and somatic inactivation of HRR has been measured.

Results: BRCA1 disruptions were associated with the C2 (immunoreactive) molecular subtype of HGSC, characterized by intense intratumoral T-cell infiltration. We derived and validated a predictor of BRCA1 mutation or methylation status, but could not distinguish BRCA2 from wild-type tumors. DNA copy number analysis showed that cases with BRCA1 mutation were significantly associated with amplification both at 8q24 (frequencies: BRCA1 tumors 50%, BRCA2 tumors 32%, and wild-type tumors 9%) and regions of the X-chromosome specifically dysregulated in basal-like breast cancer (BLBC; BRCA1 62%, BRCA2 34%, and wild-type 35%). Tumors associated with BRCA1/BRCA2 mutations shared a negative association with amplification at 19p13 (BRCA1 0%, BRCA2 3%, and wild-type 20%) and 19q12 (BRCA1 6%, BRCA2 3%, and wild-type 29%).

Conclusion: The molecular differences between tumors associated with BRCA1 compared with BRCA2 mutations are in accord with emerging clinical and pathologic data and support a growing appreciation of the relationship between HGSC and BLBC. Clin Cancer Res; 19(13); 1–11. ©2013 AACR.
Translational Relevance
Disruption of the BRCA pathway is a feature of approximately half of all high-grade serous ovarian carcinomas (HGSC), but it is unclear whether aberrations in the pathway are functionally equivalent. This study for the first time systematically investigates differences in gene expression, in combination with DNA copy number, in HGSC arising in association with BRCA1 or BRCA2 mutations. We show that BRCA1-mutant tumors are associated with a specific molecular subtype of HGSC and have a distinct gene expression signature, which is heavily influenced by specific amplification events at 8q24 and on the X chromosome. In contrast, BRCA2-mutant tumors more closely resemble "wild-type" HGSC. High frequency of amplification involving 8q24 and loci on the X chromosome in BRCA1 HGSC resembles basal-like breast cancer (BLBC). Our work has important implications for the design of clinical trials in mutation carriers and in understanding the molecular features of HGSC and BLBC.

we recently reported a higher combined BRCA1/2 germline mutation frequency in HGSC (17%). When germline mutations were associated with other histotypes, this was probably due to pathologic misclassification of tumors at initial diagnosis (8). Consistent with the importance of BRCA1/2 in the genesis of HGSC, somatic point mutations are seen in these genes in approximately 5% to 7% of these tumors (7) and promoter methylation of BRCA1 is found in a further 11% of HGSC (7). Inactivation of both BRCA1 or BRCA2 is rarely seen together (7), suggesting that disruption of either gene is functionally equivalent or lethal in combination. Collectively, changes in BRCA1/2, together with germline or somatic mutation, methylation, or amplification of other members of the HRR pathway including EMSY, FANC-family genes, RAD51C, and PTEN occur in approximately 50% of HGSC, a figure that accords with functional assays of defective HRR in HGSC (10).

Little is known of the molecular differences that underlie clinical and pathologic variation between BRCA1- and BRCA2-associated HGSC. A supervised analysis of microarray-based gene expression data identified distinct gene expression profiles of BRCA1- and BRCA2-mutated tumors and suggested that sporadic HGSC resembled one or the other germline-mutated samples (11). These data were subsequently used to generate a classifier of BRCA1, which could predict response to platinum-based therapies or PARP inhibitors (12). More recently, germline mutation in BRCA1 or BRCA2 was found to be anticorrelated with amplification of the CCNE1 gene, which encodes the cell-cycle regulator cyclin E1 (7).

Here, we explore the molecular biology of HGSC arising in association with BRCA aberrations, finding further evidence of fundamental differences between BRCA1-mutated/methylated and BRCA2-mutated tumors or their wild-type counterparts.

Materials and Methods

Patient samples and associated genomic information
Previously published gene expression data were obtained from 3 independent ovarian cancer cohorts: The Cancer Genome Atlas (TCGA) dataset of 316 HGSC (7), a cohort of 132 HGSC from the Australian Ovarian Cancer Study (AOCS; ref. 2), and 61 ovarian tumors of mixed histologies from the Memorial Sloan-Kettering Cancer Center, which we refer to as the Jazaeri dataset (11, 12). Further information about the cohorts and their respective genomic datasets are provided in Supplementary Methods.

Bioinformatic analyses
Methodology for evaluation of the gene expression and DNA copy number data, including the generation of a gene expression–based classifier, is provided in the Supplementary Methods. In addition, to facilitate reproducibility of the research, a Sweave formatted file, capable of reproducing all the figures and tables, can be provided on request.

qPCR validation of copy number associations
Gene copy number for MYC, PTK2, and PYCRL were assessed by quantitative real-time PCR (qRT-PCR) using the 7900HT Fast Real-Time PCR system (Applied Biosystems) as described previously (13). Primers were designed to amplify exonic regions, avoiding known single-nucleotide polymorphisms (SNP) and amplification of homologous sequences, using Primer3 (14). Further information, including primer sequences, can be found in the Supplementary Methods.

Results

High frequency disruption of the BRCA1/2 pathway in HGSC tumors
To explore the molecular features of tumors arising in BRCA mutation carriers and noncarriers, we first screened for germline (8) and somatic BRCA1/2 mutation information in a subset of 132 women recruited to the AOCS for which we had previously obtained Affymetrix U133+2.0 gene expression data on tumor samples (2). Germline mutations were identified 15.9% of cases (14 BRCA1, 7 BRCA2; Table 1), a similar frequency to that reported previously for HGSC (7, 9) and slightly below that seen for the overall AOCS cohort (8). A further 6.1% (8 of 132) of patients had a germline BRCA1/2 sequence variant of unknown, but likely low, pathogenic significance and were considered wild-type (Supplementary Table S1). Consistent with previous reports (7, 15, 16), pathogenic somatic mutations in BRCA1/2 were found in 6.1% of our samples (4 BRCA1, 4 BRCA2; Table 1). Methylation of the BRCA1 promoter (17) and several other members of the HRR pathway have been described previously, including PALB2 (18) and FANCF (19). Extensive BRCA1 promoter methylation was observed in 15.9% of AOCS samples.
(Supplementary Table S4), however, no significant methylation of either the PALB2 (20) or FANCF promoter regions was observed, a finding that was consistent with independent TCGA data (7). Overall, 37.9% of the AOCS samples showed evidence of disruption of the BRCA1/2 pathway by either BRCA1/2 germline or somatic mutation or BRCA1 methylation, with the different type of disruption being almost completely mutually exclusive types (Fig. 1A).

Carcinomas associated with BRCA1-mutant tumors cluster with the C2 molecular subtype of HGSC

We previously described 4 molecular subtypes (C1, C2, C4, and C5) of HGSC (2) that were subsequently validated in the TCGA analysis (7). One hundred and eleven of the AOCS tumors profiled for BRCA pathway disruption, and 210 of those from the TCGA analysis were subsequently classified as being HGSC, and were included in one of these 4 subtypes. BRCA1-disrupted tumors (methylated or somatically/germline mutated) were observed to be markedly enriched in the C2 (immunoreactive) molecular subtype for both the TCGA (Fig. 1B and Supplementary Table S6) and AOCS datasets (Fig. 1C and Supplementary Table S7). A focused statistical test designed to detect this enrichment was strongly significant for both the TCGA ($P = 0.0002$; Fig. 1B) and AOCS cohorts ($P = 0.017$; Fig. 1C). In contrast, BRCA2-mutant tumors were not significantly associated with any of the molecular subtypes in either the AOCS or TCGA datasets. The C2 subtype is characterized by an intense infiltration of T cells in the epithelial fraction of the tumor and generally favorable patient overall survival (OS; refs. 2, 21).

Gene expression distinguishes BRCA-mutated and wild-type tumors

A microarray-based gene expression profile has been previously described that distinguished BRCA1- and BRCA2-mutant tumors and classified sporadic cancers as either BRCA1- or BRCA2-like (11). We were unable to

Table 1. Pathogenic mutations identified (germline or somatic) in the 132 AOCS serous ovarian cases included in this analysis

<table>
<thead>
<tr>
<th>AOCS study ID</th>
<th>Gene</th>
<th>Exon</th>
<th>Nomenclature</th>
<th>Location</th>
<th>Molecular subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>6399</td>
<td>BRCA1</td>
<td>2</td>
<td>c.68_69het_delAG</td>
<td>Germline</td>
<td>3</td>
</tr>
<tr>
<td>6708</td>
<td>BRCA1</td>
<td>2</td>
<td>c.68_69het_delAG</td>
<td>Germline</td>
<td>2</td>
</tr>
<tr>
<td>1880</td>
<td>BRCA1</td>
<td>3</td>
<td>c.131G&gt;T</td>
<td>Germline</td>
<td>4</td>
</tr>
<tr>
<td>8515</td>
<td>BRCA1</td>
<td>11</td>
<td>c.1961het_delA</td>
<td>Germline</td>
<td>2</td>
</tr>
<tr>
<td>2595</td>
<td>BRCA1</td>
<td>11</td>
<td>c.1961het_delA</td>
<td>Germline</td>
<td>1</td>
</tr>
<tr>
<td>1563</td>
<td>BRCA1</td>
<td>11</td>
<td>c.2504_2505het_insAAGTATCCATTTGGGACA</td>
<td>Germline</td>
<td>4</td>
</tr>
<tr>
<td>4112</td>
<td>BRCA1</td>
<td>11</td>
<td>c.2716A&gt;T</td>
<td>Germline</td>
<td>1</td>
</tr>
<tr>
<td>2846</td>
<td>BRCA1</td>
<td>11</td>
<td>c.2836_2837delinsC</td>
<td>Germline</td>
<td>2</td>
</tr>
<tr>
<td>4065</td>
<td>BRCA1</td>
<td>11</td>
<td>c.3627_3682het_insA</td>
<td>Germline</td>
<td>1</td>
</tr>
<tr>
<td>5874</td>
<td>BRCA1</td>
<td>11</td>
<td>c.3756_3759het_delGTCT</td>
<td>Germline</td>
<td>5</td>
</tr>
<tr>
<td>3158</td>
<td>BRCA1</td>
<td>11</td>
<td>c.3756_3759delGTCT</td>
<td>Germline</td>
<td>4</td>
</tr>
<tr>
<td>4947</td>
<td>BRCA1</td>
<td>11</td>
<td>c.4065_4068delTCAA</td>
<td>Germline</td>
<td>2</td>
</tr>
<tr>
<td>4194</td>
<td>BRCA1</td>
<td>11</td>
<td>c.4066_4069het_delCAAG</td>
<td>Germline</td>
<td>4</td>
</tr>
<tr>
<td>2039</td>
<td>BRCA1</td>
<td>14</td>
<td>c.4484G&gt;A</td>
<td>Germline</td>
<td>1</td>
</tr>
<tr>
<td>4956</td>
<td>BRCA1</td>
<td>11</td>
<td>c.3645delG</td>
<td>Somatic</td>
<td>2</td>
</tr>
<tr>
<td>435</td>
<td>BRCA1</td>
<td>11</td>
<td>c.3762delGAACA</td>
<td>Somatic</td>
<td>1</td>
</tr>
<tr>
<td>9046</td>
<td>BRCA1</td>
<td>11</td>
<td>c.3827T&gt;A</td>
<td>Somatic</td>
<td>n/a</td>
</tr>
<tr>
<td>2378</td>
<td>BRCA1</td>
<td>22</td>
<td>c.5380G&gt;T</td>
<td>Somatic</td>
<td>4</td>
</tr>
<tr>
<td>958</td>
<td>BRCA2</td>
<td>11</td>
<td>c.2456het_delA</td>
<td>Germline</td>
<td>1</td>
</tr>
<tr>
<td>3202</td>
<td>BRCA2</td>
<td>11</td>
<td>c.3136G&gt;T</td>
<td>Germline</td>
<td>n/a</td>
</tr>
<tr>
<td>5129</td>
<td>BRCA2</td>
<td>11</td>
<td>c.4163delCTinsA</td>
<td>Germline</td>
<td>5</td>
</tr>
<tr>
<td>1251</td>
<td>BRCA2</td>
<td>11</td>
<td>c.5350_5351het_delAA</td>
<td>Germline</td>
<td>4</td>
</tr>
<tr>
<td>6867</td>
<td>BRCA2</td>
<td>11</td>
<td>c.5576_5579het_delTTAA</td>
<td>Germline</td>
<td>4</td>
</tr>
<tr>
<td>3142</td>
<td>BRCA2</td>
<td>11</td>
<td>c.5946het_delT</td>
<td>Germline</td>
<td>1</td>
</tr>
<tr>
<td>9540</td>
<td>BRCA2</td>
<td>16</td>
<td>c.7757G&gt;A</td>
<td>Germline</td>
<td>5</td>
</tr>
<tr>
<td>1819</td>
<td>BRCA2</td>
<td>6</td>
<td>c.476_1G&gt;A</td>
<td>Somatic</td>
<td>1</td>
</tr>
<tr>
<td>8794</td>
<td>BRCA2</td>
<td>11</td>
<td>c.4357A&gt;T</td>
<td>Somatic</td>
<td>1</td>
</tr>
<tr>
<td>1603</td>
<td>BRCA2</td>
<td>11</td>
<td>c.4540G&gt;T</td>
<td>Somatic</td>
<td>1</td>
</tr>
<tr>
<td>1511</td>
<td>BRCA2</td>
<td>11</td>
<td>c.4945A&gt;T</td>
<td>Somatic</td>
<td>1</td>
</tr>
</tbody>
</table>

NOTE: Germline pathogenic mutations identified using Sanger sequencing of peripheral blood DNA (8). Somatic mutations identified using a high-resolution melt analysis (Supplementary Methods). Abbreviation: n/a, not available.
replicate these findings within the same dataset (Jazaeri) or with the TCGA dataset (described in Supplementary Methods and Supplementary Figs. S1–S3). We were also unable to distinguish BRCA1/2-mutated tumors from those arising in noncarriers in either the AOCS or TCGA datasets when we used a more recently reported BRCA-like gene expression signature, also derived from the Jazaeri dataset (Supplementary Fig. S5; ref. 12). We therefore sought to develop a novel classifier that could identify ovarian tumors carrying any mutation in BRCA1 or BRCA2. We made use of the TCGA expression dataset for gene discovery (200 expression profiled cases with known BRCA status; 27 BRCA1, 28 BRCA2, and 145 wild-type) and then validated findings in the AOCS cohort.

It is unclear whether BRCA1 methylation impacts on HGSC biology to the same extent as somatic or germline mutations, as patients with BRCA1 methylation have been reported to have similar clinical outcomes to those with wild-type HGSC (7). Therefore, to maximize the opportunity to discover a signature associated with either BRCA1 or BRCA2 mutation, we initially excluded BRCA1-methylated samples and focused on BRCA germline or somatically mutated tumors for our analyses. Sixty-five genes were identified that were differentially expressed between BRCA-mutated and wild-type samples, after correcting for multiple testing and allowing for a false discovery rate of less than 5% (Supplementary Methods and Supplementary Table S8). The differences in expression of individual genes were modest, in most cases involving less than a 2-fold change in expression between mutated and wild-type cancers (Supplementary Table S8). There was no overlap between the 65 genes identified here and those associated with the previously described BRCA-like signature (12).

A classifier was created using the differentially expressed gene-list derived from TCGA data (Supplementary Methods) and applied to the AOCS cohort. The distribution of scores in tumors with either germline or somatic BRCA1/2 mutations and BRCA1 promoter–methylation samples was
Figure 2. Gene expression signatures associated with BRCA1/2 mutation status. A, validation of a 65-gene classifier developed using TCGA data and tested using AOCS samples. Classifier scores for wild-type samples were significantly different to those with BRCA-pathway inactivation (BRCA = germline/somatic BRCA mutation; BRCA1 promoter methylation; non-BRCA = wild-type). B, scores obtained with the 65-gene BRCA classifier and samples segregated by mutation type. C, distribution of scores obtained with a BRCA1 classifier, based on 34 genes identified as differentially expressed between BRCA1-mutated and wild-type tumors in the TCGA dataset, and applied to AOCS tumors. D, scores obtained with the 34-gene BRCA1 classifier and applied to AOCS samples, segregated by mutation type. P values reported in each case obtained with Student t test.
highly significantly different to wild-type AOCS tumors (Fig. 2A; \( P < 0.0001; \) Student \( t \) test). A receiver operating characteristic (ROC) curve was computed for the TCGA, AOCS, and Jazaeri datasets, showing that our classifier outperformed the previously described BRCA-like signature in all 3 instances (Supplementary Table S10).

A distinct pattern of gene expression distinguishes BRCA1 from BRCA2 and wild-type tumors
Separation of the tumors by mutation type showed that median classifier values of BRCA1-mutated or methylated samples were significantly different to wild-type tumors, however, there was less discrimination between wild-type and BRCA2-mutated samples (Fig. 2B). As these findings suggested that BRCA1-mutated samples contributed most of the discriminatory power of the classifier, we repeated the gene selection process, but this time seeking markers specifically associated with either BRCA1 or BRCA2 mutation when compared with wild-type tumors. Within the TCGA data, we identified 34 genes that were differentially expressed between BRCA1-mutated and wild-type tumors, 24 of which were common to the initial BRCA1/2 signature (Supplementary Table S9). No differentially expressed genes were associated with BRCA2-mutated tumors, at a false discovery rate of less than 5\% and after correcting for multiple testing. Collectively, these findings suggest that BRCA1-mutant and methylated tumors have a common distinct pattern of gene expression, whereas BRCA2-mutant tumors more closely resemble those arising in a wild-type background.

Independent validation of the 34 gene-classifier using AOCS samples showed clear separation of BRCA1-mutant and methylated tumors from wild-type samples, with BRCA2-mutant tumors having intermediate values (Fig. 2C and D). Using the 34-gene list and a k-nearest neighbor classification method to predict the BRCA1 status, we achieved a positive predictive value of 0.77 and a negative predictive value of 0.92, with an overall accuracy of 89\% (Supplementary Table S12).

Women with tumors deemed to be BRCA1-like according to the classifier were shown to have a longer progression-free (PFS) and OS compared with wild-type, in both the TCGA and AOCS cohorts in univariate analyses (log-rank test: TCGA PFS \( P = 0.027; \) OS \( P = 0.027; \) AOCS PFS \( P = 0.010; \) OS \( P = 0.008; \) Supplementary Fig. S7), supporting distinct underlying biology or chemoresponsiveness of BRCA-like tumors.

Chromosomal alterations at 8q24, 19q12, and X are associated with BRCA1 disruption
The inability to identify significantly differentially expressed genes between BRCA1 and BRCA2 tumors, and between BRCA2 and wild-type tumors prompted us to consider a gene set analysis (22). Gene set analysis identified genes associated with chromosomal regions 8q24 and Xq28 as being differentially expressed between BRCA1-mutated and other samples (Supplementary Table S14). In contrast, there was no obvious enrichment of genes associated with specific chromosomal loci among BRCA2-mutated samples (Supplementary Table S15). HGSC are characterized by genomic instability, including amplifications and deletions (23), and we therefore considered whether copy number differences in the BRCA1-associated tumors contributed to their specific gene expression signature. We made use of the TCGA cohort, for which there were 204 HGSC samples available with annotated BRCA-pathway events and copy number data (34 BRCA1-mutated, 30 BRCA2-mutated, and 140 wild-type). The proportion of samples with genomic copy number changes in BRCA1/2 carriers and wild-type patients were compared and \( P \) values estimated after correcting for the false discovery rate. We observed a general increase in amplifications in BRCA1-mutant tumors and deletions in tumors from BRCA2 carriers (Supplementary Fig. S8). Importantly, several chromosomal regions were significantly differentially amplified, including 8q24, 19q12-13, and regions on the X chromosome, in tumors arising in BRCA1-mutant samples versus those in noncarriers (Fig. 3A). The 8q24 amplicon is gained in 63.4\% of TCGA HGSC samples and amplified in 23.7\% of cancers (7, 23), however, amplification was much more common in BRCA1-mutant compared with wild-type cancers (\( P < 0.0001 \)). Amplification of 8q24 is the most common copy number variant in HGSC (7) and the MYC proto-oncogene is a putative driver of the amplification.

We wanted to exclude the possibility that the increase in the frequency of 8q24 amplification simply reflected a general increase in DNA copy number in BRCA1-associated tumors. We therefore examined amplification on chromosome 3, involving another commonly gained region at 3q26. The 3q26 amplicon is gained in 64.9\% of TCGA samples, and amplified in 17.2\%. Unlike the 8q24 and X-chromosome loci, there was no significant difference in the level of amplification at 3q26 in BRCA1-mutated HGSC when compared with BRCA2-mutated or wild-type HGSC (Fig. 3A). As a further control, the BRCA1-mutated tumors were stratified by their MYC status, and the extent of overall genomic alterations compared. There was no significant relationship between the degree of 8q24 gain/amplification and the extent of overall genomic alteration in individual samples (Supplementary Fig. S14). The control data confirm that aberrations at 8q24 and the X-chromosome are specifically enriched in tumors associated with BRCA1 mutations. We have previously shown that MYCN amplification is specifically associated with the C5 molecular subtype of HGSC (21), but unlike MYC, we found no evidence of an association between MYCN copy number and BRCA1/2 status (data not shown).

Amplification of the 8q24 locus is broad and complex and, in addition to involving MYC, it frequently involves genetic risk loci associated with colorectal and breast cancer (24). For example, the noncoding RNA, Pvt-1, which is adjacent to MYC also seems to contribute to the oncogenic effects of amplification at 8q24 (25). We plotted the strength of association of copy number variation at 8q24 with BRCA1 mutation status, finding that the strongest association lay telomeric to MYC, adjacent to the PTK2 gene (Fig. 3B)
Our analysis of TCGA and AOCs datasets revealed that amplifications at 8q24, 19q12, and Xq28 significantly associated with BRCA mutation status. 

**Interaction between BRCA1/2 mutation and regions in chromosome 19 and the X chromosome**

Amplifications at 19q12 and on the X chromosome were also significantly differentially altered in BRCA-mutated tumors. As reported previously (7), amplification of CCNE1 at 19q12 was mutually exclusive to BRCA-pathway disruption (Fig. 3A) and this extended to 19q13, which is partially coamplified with 19q12 (26). In addition, amplification of several regions on the X chromosome (Xq21, Xq25, Xq26, Xq27, and Xq28) were also associated with BRCA1 mutation, and genes associated with these regions were enriched among the list of 34 genes we had found to be BRCA1-specific (Supplementary Table S9).

Amplification at Xq28 seems to be even more specific to BRCA1 mutation than the association with 8q24 (Fig. 3A). Uniparental X-chromosomal isodisomy, gain of Xq28, and over expression of a subset of X chromosome genes has been reported previously (27). Similar to the findings in basal-like cancers, we found no evidence of a global change in expression of X chromosome genes in BRCA1- or BRCA2-mutant HGSC (Supplementary Fig. S15). We did observe, however, that a subset of X-chromosome genes that were previously identified as over expressed in BLBCs (27) were also significantly over expressed in BRCA1-mutation–associated HGSC (Supplementary Table S19).

**Discussion**

Consistent with previous reports, we observed somatic or germline mutations in the BRCA1 and BRCA2 genes associated with a large proportion of HGSC tumors, and these were almost completely mutually exclusive. Mutual exclusivity may reflect a functional equivalence of the mutations, in which there is no selective advantage to a tumor cell by possessing more than one defect in the BRCA pathway. Sensitivity to platinum-based therapy in the primary (28) and relapse setting (8), as well as significant responses to PARP inhibitors (29) are all consistent with the notion of a
shared BRCA1/2 BRCAness phenotype of tumors arising in BRCA1/2 carriers (30). However, recent evidence points to important clinical and pathologic differences in the behavior of tumors arising in women with BRCA1 compared with BRCA2 mutations.

Although both genes encode proteins that participate in the HRR pathway, BRCA1 has both an earlier and wider role in DNA damage response (31–33) and additional cellular functions, including cell-cycle regulation (32). BRCA1 loss may therefore have more extensive molecular and clinical consequences when compared with a BRCA2 mutation. Indeed, germline BRCA1 mutation confers a higher risk of developing ovarian cancer than germline BRCA2 mutation (34) and on average, ovarian tumors arise a decade earlier in BRCA1 carriers compared with those in women with BRCA2 mutations or with wild-type BRCA1/2 genes (8). Women with either BRCA1 and BRCA2 germline mutations generally have a better response to therapy and a longer OS compared with patients with noncarrier ovarian cancer, and some have reported that those with a BRCA2 mutation survive longer than BRCA1 carriers despite usually being older at diagnosis (35).

Differences are also observed between BRCA1- and BRCA2-associated breast cancers. Distinct pathologic features, including high rates of mitosis and pushing margins, are seen in BRCA1-associated breast cancers (36), whereas tumors arising in BRCA2 carriers more closely resemble those of noncarriers. In addition, germline mutations in BRCA1 are strongly associated with basal-like, estrogen-receptor (ER)–negative breast cancers, whereas
both ER-positive and -negative tumors are seen in BRCA2 carriers. Recently, necrosis, high-mitotic counts, prominent intraepithelial lymphocytes, and nuclear atypia have been specifically associated with BRCA1 rather than BRCA2 mutation in HGSCs (37, 38). Our finding of a strong association of BRCA1 inactivation with the C2 molecular subtype of HGSC is consistent with these reports, as this subtype is characterized by intense intraepithelial T-cell infiltration (2). Not all C2 tumors in our set had detectable inactivation of BRCA1, and it is possible that other mechanisms of BRCA1/HRR deficiency are operative in these tumors. It is unclear whether BRCA1-associated tumors tend to be more strongly immunogenic and/or are less capable of suppressing a cytotoxic immune response, however, these findings suggest that knowledge of BRCA mutation status should be considered in the design of future immunotherapy trials in HGSC.

BRCA1- and BRCA2-associated gene expression signatures have been reported previously (11, 12), yet we were unable to validate these signatures in independent datasets. Furthermore, the previous observation that BRCA1 and BRCA2 tumors have distinct patterns of expression, and that wild-type tumors resembled one or the other profile, was not supported by our study. By using a more homogenous tumor cohort, well annotated for BRCA status, we found that BRCA1-mutated tumors are the outlier in gene expression, with BRCA2 and wild-type tumors being more closely related. Interestingly, both the signature and the association with C2 molecular subtype were also observed in BRCA1-methylated cancers, even though patients with methylated BRCA1 alleles apparently do not share the same survival advantage of HGSC patients with germline BRCA1 mutations (7). The similar molecular phenotype of germline and methylated BRCA1 HGSC is consistent with recent results in breast cancer (39). A number of genes associated with the BRCA1 gene expression signature in HGSC are associated with DNA damage and/or BRCA1, including Bmi1 and CDKN1C (40, 41), and HSF1, possibly reflecting genomic stress and altered multiprotein complex stoichiometry (42). The tumor suppressor CDKN1C has previously been identified in a gene expression signature found in the fallopian tubes of BRCA1 mutation carriers with preneoplastic lesions (41). In conjunction with BRCA-loss being an initiating event in the development of HGSC, the downregulation of CDKN1C may also be important for tumorigenesis in mutation carriers.

A gene-expression signature that can help identify mutation carriers could be clinically useful in several ways. Patients with germline BRCA1/2 mutations have high response rates to PARP inhibitors but responses are also seen in noncarriers; therefore, identifying biomarkers of patients with HGSC, who are likely to respond to therapy or have a BRCA2 phenotype, is a high priority. A signature that can interrogate the overall activity of the BRCA pathway, rather than the need to conduct a series of gene-specific tests, would be desirable. At present, this signature is not sufficiently reliable to be used as a surrogate for genetic testing of probands, however, the classifier may complement other tools for assessing the likely pathogenicity of BRCA sequence variants of unclassified significance uncover during diagnostic BRCA testing. Reversion of germline BRCA1 and BRCA2 alleles and partial restoration of HRR following platinum treatment is associated with resistance to platinum-based therapies and PARP inhibitors (12, 43, 44). It is important to know whether reversion of BRCA1 alleles is associated with a gene expression signature more typical of wild-type tumors.

Gene set enrichment analysis showed that the BRCA1-specific gene signature was substantially driven by chromosomal aberrations at 8q24 and on the X chromosome, and these regions were also identified by a supervised analysis of copy number data. The strongest association between 8q24 amplification and BRCA1 mutation localized to PTK2 within the TCGA dataset, however, this shifted to MYC in validation studies with the AOCS dataset. Functional studies are needed to further refine the contribution of one or more genes in the 8q24 locus to an interaction with BRCA1 mutation.

We have previously noted a relationship between HGSC and BLBC with both tumor types sharing a propensity for widespread chromosomal copy number change, almost ubiquitous TP53 mutation, frequent disruption of the BRCA pathway, MYC gain, and CCNE1 amplification together with RB loss (45). Our findings suggest that MYC amplification is particularly a feature of BRCA1-mutated or BRCA1-methylated HGSC and is less common in BRCA2 germline mutant or nonmutant HGSC. Genomic amplification of MYC has previously been linked to BRCA1-mutated and -methylated breast tumors (46) and BLBC (45). The significance of a special relationship between BRCA1 protein loss and MYC amplification is unclear, however, we note that these proteins have been shown to physically interact with BRCA1-repressing MYC-mediated transcription (47). In the absence of BRCA1 function, cells may enjoy a selective advantage from MYC amplification. Although we did not observe a difference in survival of BRCA1 carriers with or without amplification of the MYC locus, this analysis made use of a limited number of tumor samples and is worthy of further consideration.

We also identified specific chromosomal aberrations and over expression of a subset of X chromosome genes in HGSC that were previously identified in BLBC (27), providing a further parallel between these tumor types and implying a common molecular relationship between specific X chromosome loci and BRCA1 (48–50).

Although EOC is still largely treated as a single entity, molecular and pathologic studies of the last decade have underscored the diverse nature of the disease (2, 4–6, 37). Here, we provide additional evidence of this molecular diversification, segregating even those tumors that share a common pathway deficiency. Understanding the molecular changes associated with tumors arising in distinct genetic backgrounds will help provide an integrated picture of their circuitry and thereby offer novel approaches to therapeutic intervention.
Disclosure of Potential Conflicts of Interest
A. deFazio has honoraria from Speakers Bureau of Roche and ownership interest (including patents) in patent unrelated to this article. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: J. George, K. Alsop, G. Mitchell, D. Bowtell
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Alsop, D. Etemadmoghadam, T. Miokesa, A. Dobrovic, A. deFazio, D. A. Levine, G. Mitchell, D. Bowtell
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. George, K. Alsop, D. Etemadmoghadam, T. Miokesa, G. K. Smyth, D. A. Levine, D. Bowtell
Writing, review, and/or revision of the manuscript: J. George, K. Alsop, D. Etemadmoghadam, H. Hondow, T. Miokesa, A. Dobrovic, A. deFazio, G. K. Smyth, D. A. Levine, G. Mitchell, D. Bowtell
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. George, D. A. Levine, G. Mitchell, D. Bowtell
Study supervision: D. Bowtell

Acknowledgments
The authors thank the cooperation of the participating institutions in Australia and worldwide, and also acknowledge the contribution of the participating institutions in Australia and worldwide, and also acknowledge the contribution of the participating institutions in Australia and worldwide.

References


Grant Support
This study was supported by the Ovarian Cancer Research Program of the U.S. Department of Defense [W81XWH-08-1-0684 and W81XWH-08-1-0685]; Cancer Australia and the National Breast Cancer Foundation [IDJ639303, CG-08-07, IDJ59366]; the Peter MacCallum Cancer Centre Foundation and the Cancer Council Victoria. AOCs was supported by the U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0729. The Cancer Council Victoria, Queensland Cancer Fund, the Cancer Council New South Wales. The Cancer Council South Australia. The Cancer Foundation of Western Australia, The Cancer Council Tasmania and the National Health and Medical Research Council of Australia [NHMRC; ID400413, ID400281].
Nonequivalent Gene Expression and Copy Number Alterations in High-Grade Serous Ovarian Cancers with BRCA1 and BRCA2 Mutations

Joshy George, Kathryn Alsop, Dariush Etemadmoghadam, et al.

Clin Cancer Res Published OnlineFirst April 30, 2013.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-0066

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2013/04/30/1078-0432.CCR-13-0066.DC1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.