ABSTRACT

Ovarian cancer is the leading cause of death among gynecological cancers. It exhibits great heterogeneity in tumor biology and treatment response. Germline mutations of DNA repair genes BRCA1/2 are the fundamental defects in hereditary ovarian cancer that expresses a distinct phenotypic high response rates to platinum agents, improved disease-free intervals and survival rates, and high-grade serous histology. The term “BRCAness” describes the phenotypic traits that some sporadic ovarian tumors share with tumors in BRCA1/2 germline mutation carriers and reflects similar causative molecular abnormalities. BRCA pathway studies and molecular profiling reveal BRCA-related defects in almost half of the cases of ovarian cancer. BRCA-like tumors are particularly sensitive to DNA-damaging agents (e.g., platinum agents) because of inadequate BRCA-mediated DNA repair mechanisms, such as nucleotide-excision repair and homologous recombination (HR). Additional inhibition of other DNA repair pathways leads to synthetic lethality in HR-deficient cells; this has been employed in the treatment of BRCA-like ovarian tumors with poly(ADP-ribose) polymerase inhibitors with promising results. This article presents a comprehensive review of the relevant literature on the role of BRCAness in ovarian cancer with respect to BRCA function, methods of BRCA epigenetic defect detection and molecular profiling, and the implications of BRCA dysfunction in the treatment of ovarian cancer.

INTRODUCTION

Ovarian malignancies are a group of heterogeneous tumors that express diverse pathologic characteristics and biological behavior. Hereditary ovarian cancer comprises 10%–15% of all cases of ovarian malignancies and is mainly associated with germline mutations in the BRCA1 and BRCA2 DNA repair genes [1]. Ovarian tumors in BRCA-mutated patients have relatively uniform behavior with high overall response rates to first-line platinum-based treatment [2–4], high response rates to platinum-based chemotherapy at first and subsequent relapses [2, 3], long disease-free intervals [2–4], improved overall survival rates (especially in more advanced stages) [2–5], possibly higher incidences of visceral metastases [6], and usually (but not exclusively) high-grade serous histology [5–9]. In a multivariate analysis for independent predictors of survival, BRCA status was one of three parameters (together with patient age and extent of surgery) associated with patient survival rates both in the subgroup of patients with stage III disease and in the entire study population [2].

The term “BRCAness” has been used to describe the phenotypic characteristics that some sporadic ovarian cancers share with tumors found in the setting of BRCA germ-line mutations. The term also reflects that this common biologic behavior comes from molecular defects in the cellular machinery similar to the ones caused by BRCA mutation [10, 11]. The notion began to form in 1996 after studies of BRCA1/2 genes in sporadic ovarian cancer showed multiple defects in the BRCA1/2 pathway that would explain a BRCA-like phenotype [12–17].
been shown to lead to decreased methylation in cytosine residues of CpG dinucleotides has up to 82% of ovarian tumors [17].

A variety of mutations have been reported in different epigenetic processes. The pattern of biological and clinical behavior seems to be the result of approximately 20% of all ovarian tumors [16]; the importance of this mechanism has not been verified in ovarian cancer.

BRCAness could also emerge from defects in genes whose function either affects or is affected by normal BRCAness function. A typical example is the amplification of EMSY that leads to BRCAness silencing. The EMSY gene is amplified in about 20% of cases of high-grade serous ovarian carcinomas [27] and disrupts BRCA2 participation in DNA damage response, rendering the cell prone to genomic instability [28, 29]. BRCAness cooperates with the proteins of the Fanconi anemia (FA) complex in the pathway of DNA repair and thus defects in members of the FA complex reproduce the BRCAness-deficient phenotype [30]. Methylations of the FA complex gene FANCF are found in 21% of ovarian cancers and ovarian cell lines with FANCF methylation demonstrated high sensitivity to platinum agents that was reversed with FANCF demethylation [31].

Defects in proteins involved in DNA repair besides BRCA could theoretically also lead to BRCAness. In a large-scale genomic analysis of ovarian cancer cases, hypermethylation of Rad51C, a protein that locates DNA repair machinery to the damaged strand, was found in 2% of the cases. In addition, mutations of the DNA-damage sensory proteins ATM and ATR were found in 3% of the cases [23]. In the same study, PTEN was deleted or mutated in 7% of the cases [23]. PTEN involvement in transcription regulation of Rad51 and genomic integrity maintenance [32]; a BRCAness-like phenotype could emerge when the function of either one of these is disrupted.

### BRCA Gene and Molecular Defects in BRCAness

The phenotypic traits of BRCAness are reflective of defective function of the BRCA pathway in the affected cancer cells (Table 1). The BRCA1 and BRCA2 tumor suppressor genes are implicated in cell proliferation, DNA damage response, and DNA repair. DNA is under constant stress during replication, transcription, and exposure to harmful agents such as ionizing radiation, oxygen radicals, and genotoxic chemical compounds including antitumor drugs. When DNA damage occurs, sensory proteins, such as the kinases ATM and ATR that participate in cell cycle checkpoints, activate DNA repair pathways that vary according to the kind and extent of the damage inflicted [18]. Knowledge of DNA damage response pathways and their status in cancer facilitates prediction of the sensitivity of healthy and neoplastic tissues to chemotherapeutic agents and radiation and permits exploitation of the defects of these pathways in favor of the patient.

BRCA1 and BRCA2 germline mutations are the fundamental defect in hereditary ovarian cancer where the normal allele of the carrier is inactivated in cancer cells [17, 19]. On the contrary, BRCA1/2 somatic mutations are generally rare in the sporadic forms [12, 15, 17, 20–22] but still are a significant causative gene defect as shown in extensive genomic analyses of ovarian carcinoma by the Cancer Genome Atlas Research Network [23]. Higher incidence of somatic mutations is found in patients with specific characteristics, such as Italian or Jewish origin, serous histology, and younger age [24]. Either genetic or somatic mutations of BRCA1 and BRCA2 are found in approximately 20% of all ovarian tumors [16]; BRCA1/2 alterations of all kinds, including mutations, have been reported in up to 82% of ovarian tumors [17].

In cases other than BRCA mutations, the BRCAness pattern of biological and clinical behavior seems to be the result of different epigenetic processes. The BRCA1 promoter aberrant methylation in cytosome residues of CpG dinucleotides has been shown to lead to decreased BRCA1 expression in 5%–30% of ovarian tumors, resulting in BRCAness [13–15, 23]. A subsequent study indicated that BRCA1 promoter methylation can be a particularly adverse prognostic factor compared to either BRCA1 germline mutation or no loss [25]. A more recent report found epigenetic silencing of BRCA1 and BRCA1/2 mutations to be mutually exclusive; patients with epigenetic BRCA1 silencing were found to have similar prognosis with wild-type carriers [23]. Although loss of heterozygosity for the BRCA locus has been noted in sporadic breast cancer [26], the importance of this mechanism has not been verified in ovarian cancer.

### BRCA Defect Detection and Molecular Profiles

Screening for mutations is impractical for large populations and also not informative for other kinds of defects in the BRCA pathway that can lead to BRCAness. Loss of heterozygosity (LOH) caused either by a germline mutation or an epigenetic change may be a better way to identify tumors that behave in a BRCA-like way. In one study, the presence of LOH was frequently associated with BRCA somatic or germline mutation, especially in the presence of family history [15]. In another study, immunohistochemistry for BRCA1 demonstrated a sensitivity of 80%, specificity of 93%, and positive predictive value of 73% for detecting a BRCA1 mutation [33]. Of note, high BRCA1 protein expression detected with immunohistochemistry had a negative prognostic value for progression-free survival in patients with ovarian cancer and minimal residual disease [34]. BRCA1 loss was assessed in breast cancer via a comparative genome hybridization classifier; a positive result had positive predictive value for the efficacy of DNA damage-inducing chemotherapy [35]. Certain morphologic patterns in tumor specimens may predict for a BRCA-defective genotype. More specifically, the BRCA1/2 genotype in high-grade serous ovarian carcinoma was found to be associated with solid, pseudo-endometrioid, or transitional cell carcinoma-like mor-

<table>
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<th>Table 1. Some molecular defects that can lead to BRCAness</th>
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<td>Defective mechanism</td>
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</tr>
<tr>
<td>BRCA1/2 germline mutation</td>
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<tr>
<td>BRCA1/2 somatic mutation</td>
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<tr>
<td>BRCA promoter methylation</td>
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<tr>
<td>EMSY amplification</td>
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<td>Fanconi anemia complex defects</td>
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<td>PTEN focal deletion/mutation</td>
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<td>Rad51C hypermethylation</td>
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BRCA1 and BRCA2 are found in 3% of the cases [23]. In the same study, PTEN was deleted or mutated in 7% of the cases [23]. PTEN involvement in transcription regulation of Rad51 and genomic integrity maintenance [32]; a BRCAness-like phenotype could emerge when the function of either one of these is disrupted.

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phology; higher mitotic indices; increased tumor-infiltrating lymphocytes; and necrosis. In fact, these characteristics could predict its presence with 100% sensitivity and 57% specificity [36]. BRCA2-mutated tumors seem to have necrosis and tumor-infiltrating lymphocytes to a lesser extent than those with a BRCA1 mutation [36], although a study in a much greater population showed no statistically significant differences between BRCA1- and BRCA2-mutated ovarian tumor pathology [37].

An early attempt to describe the BRCA1ness pathway explored the molecular profiles of nonredundant, significantly expressed genes of BRCA1- and BRCA2-mutated ovarian tumors and then used them to segregate sporadic cancers in two BRCA1 or BRCA2-like groups [38], suggesting that BRCA1-like and BRCA2-like molecular profiles are expressed in some sporadic ovarian tumors. More recently, a BRCA1ness gene signature was developed from samples of BRCA1/2-mutated tumors, which successfully predicted platinum responsiveness in tumor specimens. The presence of a BRCA1ness profile also carried strong independent prognostic value for patients with sporadic ovarian cancers [39]. Interestingly, in these studies the gene signatures of sporadic BRCA1-like tumors were much more like those of BRCA tumors than non-BRCA-like sporadic cancers. Additionally, a BRCA-like profile was associated with longer survival times. In fact, there were some hereditary BRCA tumors that expressed a signature similar to the nonhereditary, non-BRCA-like tumors, whereas the BRCA-like tumors clustered with most hereditary BRCA tumors. Furthermore, there were more similarities between BRCA1 and BRCA1-like tumors and between BRCA2 and BRCA2-like tumors, respectively, than between BRCA1 and BRCA2 tumors [39].

Of note, the primary BRCA defect in BRCA1-deficient tumors may correlate with alterations of the molecular profile. BRCA genetic loss relates to decreased PTEN mRNA levels, whereas epigenetic loss of BRCA1 is related to copy number gain of PIK3CA [40]. It is well known that both of these defects lead to the activation of the PI3K/Akt pathway. Also, accumulation of mutated p53 protein, which is the most common somatic genetic event in ovarian cancer, was found in the same frequency in BRCA1/2 mutated and nonmutated cases [41]. However, overexpression of p53 with loss of p21 expression is significantly more frequent in high-grade serous carcinomas with epigenetic loss of BRCA1 compared with high-grade serous tumors without loss of BRCA1 or with BRCA1 somatic and germline mutations [40].

**BRCA1ness in Treatment**

BRCA1 and BRCA2 are mainly involved in the path of homologous recombination (HR) that repairs DNA interstrand crosslinks and double-strand breaks [42]. BRCA1 also participates in nonhomologous end joining of double-strand breaks and nucleotide excision repair of DNA adducts [43]. Double-strand breaks and DNA adducts are the typical DNA damage caused by DNA alkylating agents such as cisplatin and mitomycin. BRCA-deficient cells are highly sensitive to these agents in vitro [44, 45]. Many clinical studies in patients with BRCA-deficient ovarian cancer have demonstrated high sensitivity of these tumors to platinum-based therapy [3, 11, 46] that leads to long disease-free intervals and improved overall survival rates [2–5, 9]. A recent study of women with high-grade serous ovarian cancer revealed significant chemosensitivity and survival benefit only in BRCA2 mutation carriers compared with BRCA1 mutations and BRCA1/2 wild types [47], but the study received strong criticism for its low statistical power. Nonetheless, these conflicting results stress the need for evaluation of BRCA1ness status as a stratification factor in large phase III studies, especially in light of new targeted therapies.

BRCA status also appears to affect the efficacy of mitotic spindle poisons, such as the taxanes. BRCA1 participates in the mitotic checkpoint at the metaphase-anaphase transition and controls the proper segregation of chromosomes between daughter cells [48, 49]. Spindle disruption leads to apoptotic cell death that involves the JNK pathway [50]. BRCA1 activates the JNK pathway [51]; in BRCA1-deficient breast cancer cells, paclitaxel treatment led to reduced JNK activation and lower apoptosis [50]. These findings suggest that BRCA1 directs cells towards apoptotic death after spindle poison-based treatment, in contrast to its protective role in DNA repair and cell survival after treatment with DNA-damaging treatment.

Studies in breast cancer cell lines showed that loss of BRCA1 function leads to taxane resistance [52, 53], but a clinical study showed that decreased expression of BRCA2 mRNA predicted a favorable response to docetaxel in breast cancer [54]. PI3K/Akt activation in BRCA1-deficient ovarian cancer [40] could also contribute to taxane-resistance as overexpression of activated Akt has been shown to decrease apoptosis induced by paclitaxel in ovarian cancer cells [55]. Overall, there have been conflicting reports on the role of BRCA in taxane-sensitivity in ovarian cell lines [56–58] with the latest showing that inhibition of endogenous BRCA1 expression results in increased sensitivity to platinum therapy and decreased sensitivity to spindle poisons. In the same report, high BRCA1 mRNA expression levels were associated with increased overall survival rates for ovarian cancer after taxane-containing chemotherapy. This BRCA1-related binary behavior could be used in treatment planning for ovarian cancer.

**Poly(ADP-Ribose) Polymerase Inhibitors**

BRCA-deficient cells have defective HR capacities and are thus dependent on other pathways to repair DNA damage. The interruption of those pathways is likely to be deleterious for those cells, while leaving cells with adequate HR function unaffected. This is the thinking behind synthetic lethality—a term referring to the targeted exploitation of genes relating to functions that are already defective in a particular cell. The two insults together are lethal for the cell, whereas cells with one or the other defect remain unaffected [59, 60]. The term was first used in 1946 by Dobzhansky in *Drosophila* studies [61].

BRCA1-deficient cancers are ideal targets for synthetic lethality, which has been accomplished by targeting another DNA repair pathway called base excision repair (BER) through poly(ADP-ribose) polymerase (PARP) inhibition. PARPs are a family of enzymes that play a key role in the re-
pair of single-strand breaks through BER. PARP1 is the most abundant member of this family and is the main target of a novel category of molecules called PARP inhibitors [62]. PARP inhibition causes accumulation of DNA single-strand breaks, which, when left unpaired, lead to potentially lethal double-strand breaks. In normal cells, the latter can be repaired through HR. However, in BRCA-deficient cells, the combined inadequacy of HR and BER leads to cell death—a typical example of synthetic lethality [63, 64].

In a very impressive example of translational research, PARP inhibitors were rapidly taken to phase I studies demonstrating considerable antitumor activity against BRCA1/2-related tumors of ovarian, breast, and prostate origin with acceptable toxicity [65, 66]. The following proof-of-concept phase II studies of the PARP1 inhibitor olaparib [67] in BRCA1/2-mutated patients with advanced chemorefractory breast cancer and recurrent platinum-treated ovarian cancer [68, 69] showed dose-related response rates and good tolerability.

In concordance with the BRCA1/2 theory, PARP inhibition proved to be synthetically lethal for cells lacking other proteins involved in homologous recombination besides BRCA1/2, such as RAD51, ATM, ATR, CHK1, and FANCA or FANCC [70]. This is important because homologous recombination seems to be defective in almost half of ovarian cancers [23]. Olaparib was tested as monotherapy in a phase 2 study in patients with high-grade serous and/or undifferentiated ovarian cancer or triple-negative breast cancer. Patients were stratified according to BRCA status; high response rates in both BRCA-mutated and nonmutated ovarian tumors were observed. However, the same study failed to show any benefit for patients with triple-negative breast cancer [71].

Similar outcomes were reported for a phase III study of the addition of iniparib to gemcitabine and carboplatin treatment for patients with triple-negative breast cancer. That study failed to show significant improvement in the coprimary endpoints of overall and progression-free survival rates, although patients receiving iniparib as second- or third-line treatment had a modest but still not significant benefit [72]. However, the results of this study could be misleading; iniparib’s primary mechanism of action may be the modification of cysteine-containing proteins and not PARP inhibition [73]. Furthermore, iniparib failed to kill homologous recombination-deficient cells or inhibit PARP activity in vitro compared with better characterized PARP inhibitors such as olaparib in a recent study [74].

Most recently, olaparib was found to have equivalent efficacy with liposomal doxorubicin in patients with recurrent BRCA1/2-mutated ovarian tumors [75]. It must be noted that the outcome in the liposomal doxorubicin arm of this study was significantly superior to what would be anticipated based on historical data; this may be coincidental, but it could also be due to the increased sensitivity of BRCA1-mutated tumors to DNA poisons.

The first explanation for the conflicting results of PARP inhibition between breast and ovarian BRCA1-mutated tumors that comes to mind is that “triple negativity” is not an appropriate surrogate marker for BRCA1/2 in breast cancer. On the other hand, BRCA1/2 seems to be fairly well defined and convincingly documented in ovarian cancer. However, better markers of BRCA1/2 are still needed, especially in the light of potential therapeutic gain from PARP inhibition.

A list of ongoing trials of PARP inhibitors in ovarian cancer can be found on the site www.clinicaltrials.gov. Searching for the terms “PARP inhibitors” and “ovarian cancer” recalls 28 studies, of which six are completed. Results have been published for four of the completed studies [65, 69, 71, 75]. The other two completed trials of PARP inhibitors in ovarian cancer are a single-arm study of iniparib in patients with BRCA1/2-associated ovarian cancer (ClinicalTrials.gov identifier NCT00677079) and a phase I study of the PARP inhibitor veliparib in combination with temozolomide in patients with various cancer types, including ovarian cancer (NCT00526617). The PARP inhibitor olaparib is used in eight of the ongoing studies, administered either alone, with chemotherapy agents (in combination or sequentially with carboplatin with or without paclitaxel) or with antiangiogenic agents (cediranib) in BRCA1-deficient or sporadic ovarian cancer.

The PARP inhibitor veliparib is studied in five phase I trials that include patients with ovarian cancer, given alone or with irinotecan, topotecan, pegylated liposomal doxorubicin, carboplatin, paclitaxel, bevacizumab or temozolomide, as well as four phase II studies with pegylated liposomal doxorubicin, topotecan, temozolomide and cyclophosphamide. Iniparib is being studied in three phase II studies of recurrent ovarian cancer—as a single agent in one study and in combination with gemcitabine/carboplatin in the other two studies. Two more trials are investigating PARP inhibitors AG014699 (phase II) and MK4827 (phase I) in ovarian cancer [77]. Data on clinical trials was current as of April 10, 2012, updated from [77]. Results from these trials should clarify the role of PARP inhibitors in ovarian cancer and the need to identify BRCA-like cases.

**What Happens After PARP Inhibition?**

Tumor behavior after PARP inhibition therapy is also interesting. Preliminary analysis of olaparib-treated patients with chemorefractory ovarian cancer showed remarkable response to carboplatin and/or paclitaxel-based treatment after disease progression, although a causative relationship between olaparib and the subsequent enhanced chemosensitivity cannot be established [78]. Finely targeted therapy provokes critical, equally precise resistance mechanisms in the constantly changing cancer cell.

A report from 2008 revealed a reversion of BRCA2 mutation in BRCA2-mutated platinum-sensitive ovarian cancer as a platinum resistance gaining mechanism [79]; similar restoring mutations in both BRCA1 and BRCA2 were recently described in primary and recurrent patients with BRCA1/2-mutated ovarian cancer who had previously received chemotherapy. These mutations appeared in 28% of recurrent ovarian carcinomas and 46% of the platinum-resistant cases and were predictive of platinum chemotherapy resistance [80, 81].

Reversion of the BRCA2 mutation also confers resistance...
to PARP inhibition therapy [82]. However, PARP inhibitor resistance can potentially occur through other mechanisms as well, such as upregulation of other DNA repair pathways, activation of cell proliferation pathways, or mutations in the PARP gene that alter the interaction of PARP with its inhibitor [83]. As it seems, BRCA-deficient ovarian cancer would be platinum-sensitive and taxane-resistant, so under the pressure of platinum-based therapy a BRCA-restoring mutation in these cells could reverse the sensitivity-resistance relationship, perhaps rendering the cells taxane-sensitive again. Alternating BRCA function could be of use in optimizing therapeutic gain from variants of taxane/platinum regimens [84].

In an attempt to predict sensitivity to PARP inhibition, si-RNA screen studies showed that defects in genes involved in DNA repair pathways other than HR can be used with PARP inhibition for synthetic lethality and revealed novel targets such as CDK5, MAPK12, PLK3, and the transcription coupled DNA repair proteins DDB1 and XAB2–9 [85, 86]. More recently, BRCA1 CpG island hypermethylation was also proposed as another predictive factor of PARP inhibition sensitivity [87]. Rad51 nuclear foci, which are formed when BRCA1 senses DNA double-strand breaks, were studied as a marker for adequate HR in ovarian cancer samples and correlated with in vitro response to PARP inhibition [88].

Synthetic lethality in BRCA-deficient tumors could be achieved with other DNA repair-disrupting molecules. ATM inhibition in FA pathway-deficient cells was shown to result in DNA breakage, cell cycle arrest, and apoptotic cell death [89]. Furthermore, ATM kinase inhibitor use sensitized mantle lymphoma cells to PARP inhibitors [90], underlining the potential of multitargeted DNA repair inhibition in already DNA-repair defective tumors. Decreased PTEN levels in BRCA-defective cells could enhance synthetic lethality with PARP inhibition, as PTEN-deficient cells were 20-fold more sensitive to PARP inhibition and showed decreased levels of Rad51 foci formation [32, 91, 92].

FOXMI transcriptional factor network is upregulated in 84% of high-grade serous ovarian cancer [23] and is related to tumorigenesis and tumor proliferation [93]. FOXMI cross-talks with the BRCA pathway [94] and could represent a novel therapeutic target in ovarian cancer. The role of hypoxia in this setting should also be assessed because the latter inhibits its DNA repair mechanisms [95] and has been reported to lead to decreased expression of BRCA1/2 and Rad51 [96, 97] and, therefore, to increased PARP inhibition sensitivity in tumor cells [98].

SUMMARY
Ovarian cancer is the fifth most common female cancer in the western world. BRCA1/2 germline mutations are the most common defect that gives rise to hereditary ovarian cancer, accounting for about 10% of cases. Further study implicated these genes in sporadic ovarian cancer via multiple deactivating mechanisms that all lead to impaired function of BRCA1/2 and thus to a distinct phenotype called BRCAness. The central role of BRCA1 in DNA damage response and repair renders BRCA-defective cells sensitive to DNA damaging agents. Further inhibition of other DNA repair pathways can be deleterious for BRCA-defective cells. This synthetic lethality is employed in new targeted treatments in ovarian cancer, such as PARP inhibition, thus leading to the best example of personalized therapy in ovarian cancer to date.

Improved BRCA defect characterization and detection will allow better patient selection and possibly improved clinical outcomes for a disease that is still the leading cause of death among gynecological malignancies. This type of extensive and in-depth rational understanding of a biological variant is rare and warrants further development—not only for its obvious, inherent therapeutic implications, but also as a model of studying and understanding core processes in cancer cells.

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