Ovarian Cancer: BRCA Genetics Reveals Targets for New Therapies

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Abstract

Ovarian cancer is the most lethal gynecological malignancy and the fifth cause of cancer mortality in women in the Western Countries. Advanced genomic analysis showed that most hereditary and a large proportion of sporadic ovarian cancers are associated with genetic and epigenetic aberrations either in BRCA1 and/or BRCA2 genes or in other genes involved in DNA repair and genomic stability. BRCA dysfunction identifies a major subset of ovarian cancers with peculiar epidemiological and clinical features, such as higher incidence in younger females, high-grade serous phenotype, better chemosensitivity and outcome. Being particularly sensitive to DNA-damaging agents, such as platinum compounds, these tumors are suitable for new therapeutic options that represent future challenges for oncologists. In this article we review the known molecular dysfunction in hereditary ovarian cancers and BRCA1/2 and discuss the implications of new advances for more personalized treatments.

Keywords: Ovarian Cancer (OC); BRCA1; BRCA2; BRCA1/2; BRCA1/2-Related OCs; BRCA1/2-related OCs; Synthetic lethality; Platinum-based chemotherapy; poly (ADP-ribose) polymerases (PARP) inhibitors; Taxanes; Trabectedin; Pegylated-Liposomal-Doxorubicin (PLD)

Introduction

Ovarian cancer (OC) is the leading cause of gynecological cancer-related death and the fifth cause of cancer mortality in women in the Western Countries [1,2]. Most patients present at diagnosis with an advanced stage III and IV, which are managed by surgical optimal resection and systemic platinum-based chemotherapy. Although high response rate and significant improvement of median survival were observed, the plateau of survival rates has changed little in the last decade [3]. Only one third of patients can be cured with this approach and the majority will eventually relapse with a median progression-free survival of 18 months [4]. At present, five-year overall survival for stage III and IV OC accounts for approximately 45% [5,6].

In the last decade a major effort has been made to elucidate the development of OC, not only from a cell biology viewpoint (a cellular origin from Fallopian tube has been proposed) [5], but also from a genetic and biochemical perspective. The main contribution came from the discovery of breast cancer associated (BRCA) genes BRCA1 and BRCA2. Germline mutations in BRCA1 and BRCA2 are responsible for 90% of hereditary OCs [6], the remaining 10% being due to mutations in mismatch-repair genes MSH1 and MSH2 [7]. Hereditary OCs collectively account for 10%-15% of all OC cases. Female carriers of BRCA1 mutations have 16% to 60% lifetime risk of developing epithelial ovarian carcinoma, whereas female carriers of BRCA2 mutations have a 16% to 27% risk [8,9]. BRCA1-related OCs present peculiar epidemiological and clinical features, such as a higher incidence in specific populations (younger females, Jewish and Italian race), better chemosensitivity and outcome, with BRCA2-mutated carriers having the best prognosis [10].

Some 85%-90% of OCs are sporadic forms that very rarely harbor BRCA1/2 mutations [11-13]. Surprisingly, a large proportion of sporadic OCs with wild-type BRCA genes displays a BRCA-like phenotype called "BRCA1-ness" [14-16]. The discovery of BRCA1-ness has important clinical implications because it refines stratification of OCs and provides the rationale for extending therapeutic options for BRCA1/2-mutated OCs to a larger number of patients.

Investigating the genetic and clinical profile of the BRCA1-ness is now crucial for understanding why BRCA1/2 mutations and BRCA1/2-ness correlate with better chemosensitivity and outcome even if both conditions exhibit high-grade serous (HGS) histopathology and aggressive proliferation identical to the majority of sporadic, non-BRCA1/2-related OCs.

The present work reviews the molecular profile of BRCA1/2-mutated OCs and BRCA1/2-ness. Furthermore, evidences and controversies on their clinical behavior in terms of chemosensitivity and outcome are presented. To conclude, we discuss how the in-depth understanding of the molecular signature of BRCA1/2-mutated OCs and BRCA1/2-ness is providing the rationale for new therapeutic strategies.

Genetic Profile of OC

BRCA1 and BRCA2 in OC

The transmission of an autosomal dominant trait predisposing women to the development of breast cancer and OC was first reported in the early 1970s [17]. Twenty years later the genetic basis for this predisposition was established with the cloning of the genes BRCA1 (located at chromosome 17q21) [18] and BRCA2 (located at chromosome 13q12.3) [19]. BRCA1 and BRCA2 are tumor suppressor genes whose most notable function is to maintain genomic stability during meiosis and mitosis. Mutation of either BRCA gene in the germline predisposes to tumorigenesis, but is still compatible with normal phenotype. Cancer development depends upon a second BRCA mutation in somatic cells and BRCA loss of function. As typical of autosomal dominant inheritance, one mutation segregates in each...
family, but rare examples of carriers with simultaneous mutation in BRCA1 and BRCA2 or two BRCA2 mutations have been also described [20].

Absence of BRCA proteins results in an inefficient cell capacity to repair DNA damages produced by stress and genotoxic agents. This condition generates the common ground for the onset of other mutations and genomic instability. BRCA1 and BRCA2 control different stages of DNA repair and have complementary functions. This explains why loss of either BRCA1 or BRCA2 proteins is not fully compensated by the other. BRCA1 senses DNA damage and repairs double-strand breaks (DSBs) and single strand breaks (SSBs). DSBs are repaired through homologous recombination (HR) and non-homologous end joining (NHEJ) pathways. HR repairs DNA breaks with high fidelity whereas NHEJ is imprecise and results in DNA deletions (exhaustively reviewed in 21,22). Lack of BRCA1 inhibits HR and may also impair NHEJ and SSB repair [23].

BRCA1 is also involved in other cell functions, such as activation of G1/S and G2/M checkpoints, regulation of telomere length and apoptosis [22] and mitotic spindle assembly [24].

In contrast to the multiple functions of BRCA1, BRCA2 mediates the core mechanism of HR by loading other proteins to the sites of DSBs and stalled DNA replication forks. BRCA2-recruited partners include RAD51, PALB2 and BRCA1 [25]. Abrogation of the BRCA1-RAD51-PALB2-BRCA2 complex in vivo, though with intact BRCA1 and BRCA2 genes, impairs HR repair [26] and explains why mutations in PALB2 and RAD51 are also implicated in breast and OC predisposition [27,28].

Given that BRCA1 and BRCA2 are essential for a generic biological function such as DNA repair it is reasonable to wonder why mutations in BRCA genes preferentially induce gynecologic tumors. One theory is that each menstrual cycle produces an excess of free oxygen radicals followed by DNA damage and impaired repair in BRCA-mutated cells [22]. Alternatively, BRCA1 might function as a regulator of estrogen receptor signaling and suppress estrogen-dependent transcriptional pathway related to mammary and ovarian cell proliferation, thus leading to tumorigenesis [29]. The site of mutations in BRCA1 or BRCA2 may also influence tropism, as suggested by the finding that breast and OC risk correlates with distinct clusters of mutations [30,31]. These results require further validation in a larger number of patients.

**BRCA1 and BRCA2 Functions**

BRCA1 and BRCA2 functions were also described. An example is amplification of EMSY, an inhibitor of BRCA2 that causes BRCA2 silencing and genomic instability. Amplified EMSY were detected in 17% OCs and were associate to worse outcome [34].

**BRCA1 Loss**

BRCA1 loss may be more prevalent than originally assumed. New susceptibility genes and pathways responsible for genomic instability (TP53 and FOXM1), proliferation and apoptosis, (PI3K/Akt/mTOR, RB and NOTCH), are emerging. The potential of new sequencing technologies and the large cohorts of patients enrolled in clinical studies are likely to provide more reliable links between molecular data and phenotypic characterization of BRCA1.

**Synthetic lethality**

In 2005 Ashworth’s and Hellday’s laboratories first demonstrated that BRCA1 and BRCA2-deficient cells were hypersensitive to pharmacological blockade of poly(ADP-ribose) polymerases (PARPs) [35,36]. PARPs are a family of enzymes involved in DNA repair, gene transcription, chromatin architecture and apoptosis [37]. The most abundant and well-studied member, PARP1, polymerizes poly(ADP-ribose) on substrate proteins to regulate repair of SSBs. PARP inhibition leads to accumulation of SSBs, followed by collapse of replication forks and subsequent formation of DSBs (reviewed in [38,39]). In normal cells DSBs are repaired via HR, but tumor cells lacking BRCA1 or BRCA2 cannot activate HR and do not tolerate inhibition of PARP. The synergistic cytotoxicity of PARP inhibitors and mutated BRCA1 genes was described as “synthetic lethality”, namely a cell death produced by two enzymatic dysfunctions, one produced genetically and the other achieved pharmacologically. The finding that BRCA1/2-mutated cells can be killed using PARP inhibitors has opened a new era for antitumor therapies. Several PARP inhibitors have been produced and are currently under application in clinical trials (www.clinicaltrials.gov [37,40]).

Preclinical studies are now providing insights for a wider utilization of synthetic lethality as therapeutic strategy. Not only BRCA1- and BRCA2-mutated OCs are eligible, but also tumor variants displaying BRCA1, if we consider that cells lacking other proteins involved in HR may become sensitive to PARP inhibitors [32,41].

**Clinical Findings**

**Platinum-based chemotherapy: the mainstay of treatment**

A major clinical point in the management of OC is whether tumor variants (BRCA-mutated, BRCA1, sporadic) have common or distinct clinicopathological features, prognostic outcome and drug sensitivity. Hereditary and sporadic OCs harbor HGS histopathology and advanced stage at diagnosis. However, hereditary BRCA–mutated BRCA1 and OCs have an improved overall survival compared to sporadic carcinomas [42]. It is difficult to determine whether such an improved prognosis is due to biological differences, greater chemosensitivity or both. A potential indolent clinical behavior due to a lower mitotic index was hypothesized. However, the majority of data agree that the determinant is the higher sensitivity to DSBs or to adds of chemotherapy generated by chemotherapeutic agents. Platinum compounds (cisplatin and carboplatin), are the mainstay treatment in ovarian cancer, demonstrating an antiblastic activity as common alkylating agents that interact with DNA and form intra-strand adducts and inter-strand cross links [33,34,42].

The high sensitivity to platinum-based therapy is also maintained through multiple relapses of disease and is therefore essential for better response to chemotherapy.
disease-free and overall survival in OCs. Several clinical studies, though mainly retrospective and based on a small number of patients, support this conclusion [42-46]. Cass et al. (2003) were the first who correlated platinum-based chemosensitivity to prolonged survival of hereditary vs. sporadic OCs (91 months vs. 54 months) [46]. This result has been confirmed in a recent study in which 22 patients with BRCA1/2 mutations and BRCAness with high family risk were compared to 44 sporadic epithelial OC patients treated with platinum-based therapy. BRCA group maintained better response rate either at first and second-line treatment with significant long time to first relapse (5 vs. 1.6 years, p=.001) and improved overall survival (8.4 years vs. 2.9, p=.002) [15].

In the standard platinum-based first line chemotherapy, platinum compounds are associated with mitotic spindle poisons, such as paclitaxel. The role of taxanes in BRCA1-mutated OCs remains unclear and conflicting reports are still published [47]. An initial experimental study has demonstrated no sensitivity of BRCA1-mutated breast epithelial cell line HBL100 to taxanes [48], but recent clinical studies on BRCA2 hormone sensitive breast [49,50] and prostate [51] cancer have provided preliminary evidences that sensitivity to taxanes (docetaxel) may be associated to BRCA2 mutations. We also reported a case of complete remission in a BRCA2-mutated metastatic breast cancer treated with cisplatin and taxane (nab-paclitaxel) [52]. However, there is no conclusive evidence of a link between BRCA2 mutations and sensitivity to mitotic spindle poisons.

At present, clinical oncologists are struggling with two main concerns: platinum sensitivity of OCs and time point of relapse. The longer the interval, the longer the duration of response to be achieved by subsequent platinum treatment. Based upon this scheme three patient groups have been identified: platinum-sensitive relapsed disease (relapse >12 months after first line platinum delivery), refractory disease (relapse < 6 months), partially platinum-sensitive disease (relapse between 6-12 months). Patients with refractory and partially sensitive disease may benefit from the use of DNA-damaging agents that prolong platinum-free interval. Under the pressure of these agents cell clones with higher genomic instability proliferate and re-gain platinum-sensitivity [53]. This property helps designing a synergistic pharmacologic approach to the disease (Figure 1).

**Beyond platinum-based chemotherapy: towards a personalized therapy?**

Among alternative treatments in BRCA1 and BRCA2 OCs, PARP inhibitors, pegylated liposomal doxorubicin (PLD) and trabectedin have been considered either in preclinical and clinical settings. In BRCA-related OCs, PARP inhibitors represent the best example of

![Figure 1](image-url)
targeted translational research to date and a successful therapeutic application of the synthetic lethality. Phase I-II studies with single agent PARP inhibitor olaparib have shown dose-related response and acceptable safety profile in BRCA1/2-related OCs [54,55]. A recent phase II multicentre open-label non randomized study of olaparib in HGS-OC with or without BRCA1/2 mutations, has shown better response in BRCA-mutated compared to non mutated patients (41% vs. 24%) [56]. Other PARP inhibitors, alone or in combination with chemotherapeutic or anti-angiogenic agents, are under clinical trial in hereditary and sporadic OCs (www.clinicaltrials.gov).

A major advantage in the use of PARP inhibitors is that they can restore chemosensitivity in chemorefractory OCs. After relapse, patients treated with olaparib experienced disease progression, but then re-gained the potential to respond to platinum chemotherapy [57]. Interestingly, revertant phenotypes with restored BRCA function were identified in OC patients with BRCA1/2 mutations. These cases accounted for 28% platinum-sensitive relapses and 46% platinum-resistant cases and were predictive for resistance to platinum and PARP-inhibition [58,59]. The mechanism of reversion is still unclear, but it can be hypothesized that under pressure of chemotherapies, somatic cells with high genomic instability accumulate additional BRCA1/2 mutations that restore wild-type phenotype. PARP inhibitors could restore BRCA1/2 function through genetic or epigenetic mechanisms [60]. Long-term PARP inhibition per se is also cause of tumor resistance, possibly through PARP gene mutations that alter interaction with inhibitors, or up-regulation of alternative DNA repair or cell proliferation pathways [61]. In summary, PARP inhibitors, the leading agents of synthetic lethality, are precious tools for killing tumor cells after the acquisition of resistance to previously employed therapies.

Targeting recurrent OCs benefit from other DNA-damaging agents as pegylated liposomal doxorubicin (PLD) that is a pegylated (polyethylene glycol coated) liposome-encapsulated form of doxorubicin that delivers higher drug concentration in cancer cells, resulting in a more favourable efficacy with less toxicity. Doxorubicin interferes with topoisomerase 2 and produces DNA breaks that cannot be repaired in cells lacking HR, thus causing cytotoxicity [62]. The efficacy of PLD in OC has been reported in a phase III study (MITO2) designed to compare the combination of carboplatin plus paclitaxel vs. carboplatin plus PLD in first line treatment. This study demonstrated similar progression-free survival, but better toxicity profile, for carboplatin plus PLD [63] and similar data were provided for platinum sensitive OCs. In platinum-resistant relapsed OC, PLD used alone provide better outcome over other single chemotherapeutic agents [64].

A few retrospective studies have demonstrated that BRCA mutations are predictor of improved outcome in relapsed hereditary OCs treated with PLD [62,65,66]. In particular, family history or BRCA1/2 mutations predict better outcome as compared to sporadic OCs [62], both in median time to treatment failure (15.8 months vs. 8.1 months, p=0.009) and overall survival (56.8 months vs. 22.6months, p=0.002) [65], providing further a compelling rationale for a more personalized treatment based on the BRCA status. A randomized phase II study, involving 97 patients with partially platinum-sensitive hereditary OCs, compared olaparib vs. PLD obtaining a satisfactory, though non-statistically significant, median progression free survival [67].

Trabectedin, an anti-tumor compound originally extracted by the marine organism Tunicate Ecteinascidia, and now synthetically produced for pharmacological purposes, is another promising option for OCs resistant to previous therapies [68]. Trabectedin has a unique mechanism of action based on interaction with the minor groove of the DNA double helix generating adducts that bend DNA and induce DSBs, it affects gene transcription and DNA repair pathways, resulting in G2-M cell cycle arrest and ultimately apoptosis [69]. Trabectedin cytotoxicity is determined by the functional nucleotide excision repair (NER) and a deficient homologous recombination repair (HRR) machinery. Consequently, trabectedin shows decreased activity (from 2- to 8-fold) in NER-deficient cell lines, while cells deficient in HRR are approximately 100 times more sensitive to the drug [70,71].

Trabectedin is demonstrated to be active in breast, ovarian, non-small cell lung cancers, melanoma and sarcoma and several reports showed that it could be safely combined with other agents such as gemcitabine and PLD [72-74]. Monk reported a phase III study on recurrent, partially platinum-sensitive OCs treated with trabectedin plus PLD. Combined agents revealed better platinum-free survival over PLD alone (7.3 versus 5.8 months; p=0.019) with 46% vs. 21% reduced risk of progressive disease [75]. Taking into account these results and the remarkable reviewed data on survival (23 months, 41% reduction in the risk of death) [76], trabectedin in combination with PLD received approval for the treatment of recurrent partially-platinum sensitive OC.

Based on this strong preclinical rationale and the previous results of phase III study in unselected OCs, a Phase II multicentre study (MITO 15) is actually ongoing to evaluate the activity of trabectedin in BRCA1/2 mutation carriers or BRCA-ness phenotype (clinicaltrials.gov).

Concluding Remarks and Future Directions

OC due to BRCA dysfunction depends upon a complex genetic profile, whose common determinant is the lack of efficient DNA repair, the disruption of genomic stability and consequent tumorigenesis. Genetic and epigenetic abnormalities identified three different genotypes, BRCA1- and BRCA2-mutated OCs and BRCA1ness. The mutation spectrum reveals that 20% of HGS-OC have germline or somatic mutations in BRCA1/2, 11% have lost BRCA1 expression through hypermethylation and that BRCA1 is inactivated by mutually exclusive genomic and epigenetic mechanisms. On the other hand, BRCA1ness, which should be considered separately from non BRCA-related cases, lack a complete genotypic profile.

At present, it is difficult to classify hereditary or sporadic OCs based upon genetic counseling and family history, due to selection bias. Thus, a detailed molecular characterization of the different OC genotypes is needed. In addition, protein profiling of BRCA-related tumors and BRCA1ness would help understanding how gene transcription, post-translational modifications, proteins dynamic, signaling and metabolic pathways, change under the pressure of gene mutations and genomic instability.

Genomics

Laboratory tests to determine gene mutations and promoter hypermethylation are still difficult to apply on a large scale, and extremely expensive. However, these data should be made available to clinicians before treatment decision, particularly to distinguish BRCA1ness from other OC variants and to aid molecular subtype stratification of BRCA-related OCs. In addition, some peculiar genomic features, such as the methylation state of genes responsible for BRCA1ness should be monitored over time, since they seem to undergo changes during cell life [77].
Proteomics

Protein profiling of OCs is equally important because proteins are the functional effectors of gene expression and because proteomics analysis offers the opportunity for finding new biomarkers of the disease and new molecular targets for more personalized therapy. A proteomic profile that distinguishes BRCA-related tumors and BRCA-ness from the other HGS-OC variants is still lacking. To gain insight into this issue, protein microarray and tissue immunohistochemistry were used to detect proteins involved in homologous recombination (FANCDD2, BRCA1, PARP, H2AX, ATM, PTEN and p53) over a cohort of 186 patients with sporadic OC. This study identified a BRCA-ness protein profile that is not associated with BRCA1 and linked triple positive FANCDD2+/PARP+/p53+ patients with very early recurrence of the disease [78].

Over the last decade, high-throughput technologies have allowed identification of novel biomarkers in serum, ascites and tissue samples derived from OC patients and OC cell lines on a large scale [79]. Since OC represents a complex and heterogeneous disease, it is unlikely that a single biomarker provides specificity and sensitivity for all OC types. Thus, integrative proteomics for the identification of biomarkers of BRCA-related tumors and BRCA-ness are promising venues, but should be designed carefully taking into account that the bottleneck of this issue, protein microarray and tissue immunohistochemistry were used to detect proteins involved in homologous recombination (FANCDD2, BRCA1, PARP, H2AX, ATM, PTEN and p53) over a cohort of 186 patients with sporadic OC. This study identified a BRCA-ness protein profile that is not associated with BRCA1 and linked triple positive FANCDD2+/PARP+/p53+ patients with very early recurrence of the disease [78].

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