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Secondary mutations of *BRCA1/2* and drug resistance

Kiranjit K. Dhillon¹, Elizabeth M. Swisher², and Toshiyasu Taniguchi^{1,3}

¹Howard Hughes Medical Institute Divisions of Human Biology and Public Health Sciences Fred Hutchinson Cancer Research Center, Seattle, WA, USA

²Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Division of Medical Genetics, Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA

Abstract

Inherited mutations in the tumor suppressor genes *BRCA1* and *BRCA2* cause increased risk of developing various cancers, especially breast and ovarian cancers. Tumors that develop in patients with inherited *BRCA1/2* mutations are generally believed to be *BRCA1/2* deficient. Cancer cells with *BRCA1/2* deficiency are defective in DNA repair by homologous recombination and sensitive to interstrand DNA crosslinking agents, such as cisplatin and carboplatin, and poly(ADP-ribose) polymerase (PARP) inhibitors. Therefore, these agents are logical choices for the treatment for *BRCA1/2*-deficient tumors and have shown to be clinically effective. However, *BRCA1/2*-mutated tumors often develop resistance to these drugs. Restoration of *BRCA1/2* functions due to secondary *BRCA1/2* mutations has been recognized as a mechanism of acquired resistance to cisplatin and PARP inhibitors in *BRCA1/2*-mutated cancer cells. This indicates that even disease-causing inherited mutations of tumor suppressor genes can be genetically reverted in cancer cells, if the genetic reversion is advantageous for the cells' survival. In this review, we will discuss this drug resistance mechanism.

Drug resistance is a serious obstacle to effective cancer therapy. Though a number of drug resistance mechanisms have been identified^(1, 2), in this review, we mainly discuss resistance resulting from restoration of DNA repair. Specifically, we will focus on a recently recognized mechanism of resistance to cisplatin and poly(ADP-ribose) polymerase (PARP) inhibitors: restoration of *BRCA1/2* functions due to secondary mutations of *BRCA1/2* in *BRCA1/2*-mutated tumors^(3–6). Defects in DNA repair lead to cellular sensitivity to DNA damaging drugs and restoration of DNA repair results in acquired resistance to those drugs. This unexpected drug resistance mechanism has attracted attention from many researchers because of its clinical significance. In this review, we will discuss identification of *BRCA1/2* secondary mutation as a drug resistance mechanism, its clinical implications and future directions.

BRCA1, BRCA2 and Sensitivity of Cancer Cells to Cisplatin and PARP Inhibitor

Since DNA repair mechanisms play an important role in determining cellular sensitivity to DNA damaging agents, it is reasonable to speculate that DNA repair plays a critical role in development of resistance to anti-cancer DNA damaging agents (Fig. 1). Cells are equipped

³To whom corresponding should be addressed. ttaniguc@fhcrc.org Toshiyasu Taniguchi, MD, PhD Howard Hughes Medical Institute Divisions of Human Biology and Public Health Sciences Fred Hutchinson Cancer Research Center 1100 Fairview Ave. N., C1-015 Seattle, WA 98109-1024, USA Phone: 206-667-7283, Fax: (206) 667-5815.

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with multiple DNA repair mechanisms to deal with DNA damage caused by normal metabolic processes and exogenous agents. Loss of integrity of DNA repair promotes genetic instability, which can lead to tumorigenesis. An additional consequence of defective DNA repair is cellular hypersensitivity to DNA damaging agents, which has been exploited in the widespread use of DNA damaging anti-cancer agents in chemotherapy⁽⁷⁾. DNA damaging agents work especially well in individuals with known DNA repair defects in their cancer cells. A widely studied group of such are individuals with mutations in the *BRCA1* and *BRCA2* tumor suppressor genes.

Heterozygous germline mutations in *BRCA1/2* are responsible for a large fraction of hereditary breast and ovarian cancers. Additionally, *BRCA2* mutation carriers have increased risk of other types of cancer, such as pancreatic, and prostate cancers^(8, 9). The risk for developing breast cancer by age 70 is 50–70% for *BRCA1/2* mutation carriers, while the risk of developing ovarian cancer by age 70 is 40–50% for *BRCA1* mutation carriers and 10–20% for *BRCA2* mutation carriers^(10, 11). Though individuals are heterozygous for *BRCA1/2* mutations in normal cells, neoplastic cells show loss of the wildtype allele and retention of the mutated *BRCA1/2* allele; therefore, these tumors are considered to be *BRCA1/2* deficient^(12–14). In the case of pancreatic carcinomas in *BRCA2* mutation carriers, sometimes tumors can be *BRCA2* proficient⁽¹⁵⁾.

Biallelic germline mutations of *BRCA2* cause a severe form of a rare recessive genetic disease, Fanconi anemia (FA-D1 subgroup)⁽¹⁶⁾. Therefore, *BRCA2* is also called *FANCD1*. Fanconi anemia is characterized by bone marrow failure, cancer/leukemia susceptibility, multiple congenital abnormalities and cellular hypersensitivity to interstrand DNA crosslinking agents, such as cisplatin and mitomycin C⁽¹⁷⁾. Fanconi anemia can be divided into at least 13 complementation groups and all of the responsible genes have been identified. FA-D1 subtype patients are especially cancer prone, and the cumulative probability of any malignancy of FA-D1 subgroup is 97% by age 5.2 years⁽¹⁸⁾.

BRCA1 and *BRCA2* proteins play important roles in DNA double-strand break repair by homologous recombination (HR)^(19, 20). *BRCA1* directly binds to phosphorylated CtIP protein and localizes CtIP to DNA double-strand breaks (DSBs), which facilitates resection of DSB ends to create 3' overhangs of single-stranded DNA (ssDNA)^(21, 22). The ssDNA is then coated by RPA, which is subsequently displaced by the recombinase protein RAD51. *BRCA2* plays a critical role in this step: *BRCA2* directly binds to RAD51 and facilitates loading of RAD51 on ssDNA⁽²³⁾. RAD51 forms a nucleoprotein filament on ssDNA and catalyses the search for a homologous sequence and promotes strand invasion, which is followed by formation and resolution of intermediate recombination structures. *BRCA1* forms a protein complex with PALB2 and *BRCA2*, and regulates *BRCA2*⁽²⁴⁾. In addition, *BRCA1* has also been reported to be involved in checkpoint function, transcriptional regulation, chromatin remodeling, and ubiquitination⁽²⁵⁾.

Loss of *BRCA1/2* function leads to increased chromosomal instability, which can promote tumorigenesis. Importantly, *BRCA1/2* deficiency also results in cellular sensitivity to interstrand DNA crosslinking agents such as cisplatin^(26, 27), and a promising new class of anti-cancer agents called PARP inhibitors^(28, 29).

Cisplatin and its derivative, carboplatin, cause interstrand and intrastrand crosslinks which covalently link DNA, preventing transcription and replication⁽³⁰⁾. Interstrand crosslinks are mainly recognized during S phase as they can cause approaching replication forks to stall⁽³¹⁾, although interstrand crosslink repair also occurs in G1⁽³²⁾. Repair of the interstrand crosslink is a multistep processes⁽³³⁾, beginning with endonucleolytic excision on one strand, followed by translesion synthesis across the interstrand crosslink and removal by

excision. The resulting DSB must be resected to generate ssDNAs followed by repair by HR. As HR is defective in *BRCA1/2*-deficient cells, these cells are hypersensitive to interstrand crosslinking agents.

Platinum compounds (cisplatin and carboplatin) are effective chemotherapeutic agents for ovarian cancers⁽¹⁾. Patients with ovarian carcinomas and *BRCA1/2* mutations have longer recurrence-free intervals than patients with sporadic ovarian carcinomas, especially when treated with platinum-based therapy^(34–36). However, recurrence does occur in the majority of women with *BRCA1/2*-associated ovarian carcinoma and the recurrent carcinomas eventually acquire platinum resistance. Studies have suggested several mechanisms that can lead to cisplatin resistance including changes in transport of the drug via altered expression of copper transporters and increased glutathione expression⁽²⁾.

BRCA1/2-deficient cells are highly sensitive to PARP inhibitors^(28, 29), and many clinical trials of PARP inhibitors for the treatment of patients with *BRCA1/2*-mutated cancers are ongoing⁽³⁷⁾. PARP-1 attaches ADP-ribose to itself and other targets and facilitates repair of single strand breaks (SSBs). PARP inhibition results in the accumulation and persistence of SSBs, which then can be converted into DSBs during S phase. HR plays a critical role in the repair of DSBs in S phase. Therefore, inhibition of PARP in HR-deficient cells, such as *BRCA1/2*-deficient cells, results in synthetic lethality^(28, 29) and promising results of clinical trials in *BRCA*-associated carcinomas have been reported^(38–41). However, not all of the patients with *BRCA1/2*-mutated cancer respond to the PARP inhibitor, and even for patients who respond to the therapy, response duration is finite. Therefore, PARP inhibitor resistance is becoming a clinically important issue. The mechanism of clinical PARP inhibitor resistance is not clear, but in *BRCA1/2*-mutated ovarian carcinomas treated with a PARP inhibitor, there is an association between the clinical benefit rate and platinum-free interval, suggesting that there may be a common mechanism of resistance to cisplatin and PARP inhibitors⁽⁴⁰⁾. Data from a murine *Brca1/p53*-deficient mammary tumor model has shown that resistance to PARP inhibitors can result from upregulation of P-glycoprotein efflux pumps⁽⁴²⁾.

A Lesson from a Rare Genetic Disease, Fanconi Anemia

It is well known among researchers working on rare genetic diseases, such as Fanconi anemia, that spontaneous genetic alterations that compensate for inherited disease-causing mutations (genetic reversion) sometimes occur⁽⁴³⁾. These genetic alterations in Fanconi anemia include secondary genetic changes such as back-mutations to wildtype, compensatory mutations in *cis*, intragenic crossovers and gene conversion^(44, 45). This phenomenon is sometimes called “natural gene therapy”⁽⁴⁵⁾ because patients with genetic reversion in their hematopoietic cells tend to have milder clinical phenotype. Cells derived from Fanconi anemia patients are usually hypersensitive to interstrand crosslinking agents, but cells with genetic reversion are resistant. Furthermore, secondary mutation of *BRCA2/FANCD1* in a mitomycin C-resistant acute myelogenous leukemia cell line from a Fanconi anemia patient with biallelic mutations in *BRCA2/FANCD1* has been reported⁽⁴⁶⁾. Similarly, a Chinese hamster cell line with biallelic *Brca2* mutations, V-C8, is highly sensitive to mitomycin C, and *in vitro* mitomycin C selection of V-C8 led to generation of mitomycin C-resistant clones that acquired an additional *Brca2* mutation leading to the restoration of the open reading frame of one of the *Brca2* alleles⁽⁴⁷⁾.

This knowledge led us to hypothesize that secondary *BRCA1/2* mutations that cancel the effect of the inherited *BRCA1/2* mutations are a mechanism of acquired resistance to cisplatin and PARP inhibitor in *BRCA1/2*-mutated cancer.

BRCA2 Secondary Mutations Occur *In Vitro* in BRCA2-mutated Cancer Cell Line Models

This hypothesis is now supported by several lines of experimental and clinical evidence. Our group^(3, 5) and Ashworth's group⁽⁶⁾ have independently demonstrated that cisplatin/PARP inhibitor-resistant clones of *BRCA2*-mutated cancer cell lines can be generated *in vitro* and that restoration of functional *BRCA2* protein expression due to secondary *BRCA2* mutations is a major mechanism for the observed resistance. To date, *in vitro* models of secondary mutation have only been published for *BRCA2*-mutated cancer cell lines, not for *BRCA1*-mutated cancer cell lines.

In vitro models of drug resistance have been generated in two *BRCA2*-mutated cancer cell lines: a pancreatic cancer cell line, Capan-1^(3, 6), and an ovarian cancer cell line, PEO1⁽⁵⁾. Capan-1 harbors the mutant allele *BRCA2.6174delT*, but no wildtype *BRCA2* allele⁽⁴⁸⁾. PEO1 harbors the mutant allele *BRCA2.5193C>G* (Y1655X), but no wildtype *BRCA2* allele⁽⁵⁾. Therefore, these two cancer cell lines are *BRCA2*-deficient and sensitive to cisplatin and PARP inhibitors^(3, 5, 6).

We selected Capan-1 cells in the presence of cisplatin *in vitro* and obtained 14 cisplatin-resistant clones⁽³⁾. Interestingly, seven of the 14 cisplatin-resistant subclones harbored secondary *BRCA2* mutations that restored the reading frame, resulting in expression of almost full-length *BRCA2* protein (see Table 1 for mutation spectrum). The resulting *BRCA2* proteins all had intact C-terminal regions containing a DNA binding domain, a nuclear localization signal and a functionally important C-terminal RAD51 binding domain⁽⁴⁹⁾.

Ashworth's group selected Capan-1 cells in the presence of PARP inhibitor *in vitro* and obtained 12 PARP inhibitor-resistant clones⁽⁶⁾. All 12 of the PARP inhibitor-resistant clones had secondary mutations that remove the original 6174delT mutation and restored *BRCA2* protein with intact C-terminal nuclear localization signal and the RAD51 binding domain (Table 1). Intriguingly, some of the restored *BRCA2* proteins still lack the DNA binding domain, suggesting that the DNA binding domain may be dispensable for some functions of *BRCA2*.

We selected PEO1 cells in the presence of cisplatin or a PARP inhibitor *in vitro* and obtained 11 cisplatin-selected clones and 3 PARP inhibitor-selected clones⁽⁵⁾. Eight of 11 cisplatin-selected clones and all of the 3 PARP inhibitor-selected clones harbored the same secondary *BRCA2* mutation: a single-base substitution that altered the original nonsense mutation (Y1655X) to a missense mutation (Y1665L) and resulted in expression of full-length *BRCA2* (Table 1).

The *BRCA2* proteins resulting from secondary mutations have restored HR functions. First, most of Capan-1 and PEO1 clones with secondary *BRCA2* mutations restored ionizing radiation (IR)-induced RAD51 foci formation^(3, 5, 6), which is a marker of functional *BRCA2*⁽²⁶⁾. Second, the function of these *BRCA2* proteins resulting from secondary mutations was confirmed using an I-SceI-dependent DR-GFP recombination reporter assay^(3, 5, 6). Finally, siRNA-mediated depletion of *BRCA2* in *BRCA2*-restored cells sensitized the cells to cisplatin and PARP inhibitors, indicating that restored *BRCA2* is functional and responsible for the acquired drug resistance^(5, 6).

An unfortunate byproduct of resistance to cisplatin or PARP inhibitors due to secondary *BRCA2* mutation is cross-resistance to other DNA damaging drugs. Data from the *in vitro* models show that resistance to cisplatin mediated by *BRCA2* restoration also results in

resistance to PARP inhibitors and *vice versa*^(3, 5, 6). Not surprisingly, PARP inhibitor-resistant cells respond to docetaxel, an agent that inhibits mitosis by stabilizing microtubules, in a similar manner to parental Capan-1 cells, suggesting that resistance due to BRCA2 restoration modulates response to DNA damaging agents specifically and not chemotherapeutic agents that work via alternative mechanisms⁽⁶⁾. Intriguingly, cisplatin-resistant Capan-1 clones with BRCA2 secondary mutations are still sensitive to 6-thioguanine⁽⁵⁰⁾, suggesting that 6-thioguanine may be effective for the treatment of cisplatin/PARP inhibitor-resistant BRCA2-restored cancer. These findings indicate that restoration of BRCA2 by secondary BRCA2 mutation can be a mechanism of cisplatin/PARP inhibitor resistance at least in cell lines selected in the presence of cisplatin/PARP inhibitor *in vitro*. It is important to note that restoration of BRCA2 did not explain cisplatin resistance in all clones selected in cisplatin. Some of the cisplatin-resistant clones without secondary BRCA2 mutation remained sensitive to PARP inhibitor^(3, 5), indicating that platinum-resistant carcinomas without secondary BRCA2 mutations may still respond to PARP inhibitor therapy.

BRCA2 Secondary Mutations Can Occur *In Vivo* – Evidence in Cancer Cell Lines

Analysis of a BRCA2-mutated breast cancer cell line, HCC1428, provided evidence of secondary mutations occurring *in vivo*⁽³⁾. HCC1428 was isolated from the pleural effusion of a woman heterozygous for BRCA2_6174delT with breast carcinoma after she had undergone chemotherapy^(3, 51). Interestingly, the BRCA2 gene in HCC1428 has a 2135bp internal deletion that spanned the original 6174delT mutation and the exon 11/intron 11 junction (Table 1). This deletion leads to expression of two BRCA2 transcripts encoding intact DNA binding domain and the C-terminal nuclear localization signals. Consistently, HCC1428 is resistant to cisplatin and could be sensitized to cisplatin by depleting BRCA2⁽³⁾. Since HCC1428 was derived from the patient after chemotherapy, the internal deletion mutant may be a result of *in vivo* selection in the patient.

Another example of *in vivo* selection is seen with the PEO1/PEO4/PEO6 series of ovarian cancer cell lines derived from a woman heterozygous for BRCA2.5193C>G (Y1655X), with stage III ovarian adenocarcinoma^(5, 52). PEO1 was derived from ascites at the time of the first relapse when the disease was still sensitive to cisplatin. PEO4 was derived at time of second relapse when the disease was refractory to cisplatin and PEO6 was derived from ascites at the terminal phase. PEO1 is hemizygous for the original nonsense mutation (BRCA2.5193C>G) (Y1655X) and lacks full-length BRCA2 protein expression, and PEO4 and PEO6 had a secondary mutation at the same position (BRCA2.5193C>T) (Y1655Y) that restored BRCA2 protein expression (Table 1). Importantly, we confirmed the same secondary mutation in the clinical samples of the patient⁽⁵⁾. These data indicate that the secondary BRCA2 mutation occurred *in vivo* and may have contributed to the cisplatin resistance observed in the patient.

BRCA1/2 Secondary Mutations in Clinical Samples of Ovarian Cancer

Although cellular models to date have only shown evidence of a role for BRCA2 secondary mutation in drug resistance, clinical data indicate that secondary mutations of both BRCA1⁽⁴⁾ and BRCA2^(3, 5, 6) can occur in platinum-resistant ovarian carcinomas.

Our group and Ashworth's group reported analyses of a small number of platinum-sensitive and -resistant recurrent ovarian carcinomas from BRCA1/2 mutation carriers (Table 2)⁽³⁻⁶⁾. Interestingly, 7 of 10 platinum-resistant carcinomas had secondary BRCA1/2 mutations (3/5 BRCA1; 4/5 BRCA2) and 1 BRCA1-mutated primary platinum-resistant carcinoma had a

secondary *BRCA1* mutation, while none of the 5 platinum-sensitive recurrent ovarian carcinomas (3 *BRCA1*; 2 *BRCA2*) showed secondary *BRCA1/2* mutation. These studies suggest that *BRCA1/2* restoration is an important mechanism in clinical cases of platinum resistance in *BRCA1/2*-associated carcinomas. We are currently analyzing a larger number of cases in order to correlate secondary *BRCA1/2* mutations and clinical platinum resistance.

We also found a case with primary platinum resistance that showed a secondary mutation (back mutation) of *BRCA1* in the primary ovarian carcinoma⁽⁴⁾. This patient had a history of chemotherapy for breast cancer many years before she developed ovarian carcinoma. This anecdotal case suggests an interesting possibility that chemotherapy for prior malignancy may be involved in the occurrence of secondary mutation of *BRCA1/2* in subsequent cancers, but clearly we need a larger study to test this possibility.

Several reports have shown that the PARP inhibitor Olaparib shows effectiveness in ovarian and breast carcinomas in *BRCA1/2*-mutation carriers, though disease progression eventually occurs in most patients^(38–41). A recent clinical study evaluating efficacy of PARP inhibitor therapy for ovarian carcinomas in *BRCA1/2*-mutation carriers showed that best results were obtained in individuals that were still responsive to platinum therapy⁽⁴⁰⁾. Secondary *BRCA1/2* mutation is possibly an important mechanism of clinical resistance to PARP inhibitor and should be tested in future studies.

Animal Models of *Brca1/2*-mutated Cancer

Heterozygous deletion of *Brca1* or *Brca2* in mice fails to show a cancer phenotype and constitutional homozygous deletion of *Brca1* or *Brca2* generally results in embryonic lethality⁽⁵³⁾. In order to circumvent these problems, several tissue-specific conditional knockout models have been generated to mimic *Brca1*-^(54–57) and *Brca2*-^(58–61) associated breast carcinoma. Though most *Brca1/2* mouse models are generated using large deletions that do not result in expression of a transcript, a few do express mutant transcript (e.g. *Brca1* exon11 deletion variant), which may explain differences in phenotypes observed in these models⁽⁵³⁾. To date, murine models have not shown *Brca1/2* secondary mutation as a mechanism of drug resistance. However, the large deletions of *Brca1/2* used in these models preclude the possibility that secondary mutations can arise that will restore functional *Brca1/2* proteins.

Several groups have investigated resistance to cisplatin/carboplatin, PARP inhibitors, doxorubicin and docetaxel in *K14CreBrca1^{flox/flox}/p53^{flox/flox}*^(42, 62) and *blg-CreBrca2^{flox/flox}/p53^{flox/flox}*⁽⁶³⁾ mammary tumors by orthotopic transplantation of small tumors into mammary fatpads of wildtype females prior to challenge with drug. Treatment with doxorubicin and docetaxel resulted in variable response of carcinomas, although resistance always occurred to the maximum tolerable dose⁽⁶²⁾. Doxorubicin-resistant neoplasms upregulated the *Mdr1a* and *Mdr1b* genes that encode a P-glycoprotein, suggesting that efficient export of the drug out of cells results in the resistance. *Brca1/2*- and p53-deficient tumors also responded well to PARP inhibitor, although resistance always occurred due to upregulation of P-glycoprotein pumps^(42, 63).

Brca1-deleted neoplasms treated with cisplatin showed a significant reduction in size after treatment and eventually grew back⁽⁶²⁾. However, cisplatin-treated recurrent tumors were still sensitive to the drug⁽⁶²⁾. Moreover, PARP inhibitor-resistant *Brca1*-deleted tumors also had reduction in tumor volume after cisplatin treatment⁽⁴²⁾, consistent with the common notion that P-glycoprotein is not important for transport of cisplatin⁽⁶⁴⁾. The fact that cisplatin-treated tumors recurred and were subsequently sensitive to another round of treatment with cisplatin suggests that there is a subset of the tumor population that evades killing by cisplatin⁽⁶²⁾. In contrast, another report showed that cisplatin resistance in

mammary tumors in mice with *Brca1^{flox/flox}/p53^{flox/flox}* (Brca1 exon11 deletion variant) does occur⁽⁶⁵⁾. The difference of the two reports may be due to difference of *Brca1* alleles (null⁽⁶²⁾ vs. exon 11 deletion⁽⁶⁵⁾) or difference of cisplatin treatment regimens.

Murine models of Brca1/2-deficiency with large deletions of *Brca1/2* do not mimic the most common *BRCA1/2* mutations observed in patients and it is imperative to generate models that contain point mutations or smaller deletions that are more commonly observed in women with inherited *BRCA1/2* mutations. Nevertheless, findings from the current murine models provide insight into resistance observed in the clinic. As clinical trials testing efficacy of PARP inhibitors in *BRCA1/2*-deficient tumors are maturing, analysis of recurrent resistant tumors may yield both *BRCA1/2*-reexpression and increased export of the drug by P-glycoprotein as resistance mechanisms.

Mechanism of Secondary *BRCA1/2* Mutation

The work reviewed here demonstrates that secondary *BRCA1/2* mutations are an important mechanism of platinum resistance. How do these secondary mutations arise (Fig. 2)? We can easily imagine that at least 3 factors can contribute to this phenomenon. First, treatment with DNA damaging agents such as cisplatin or carboplatin could increase the mutation rate. Second, *BRCA1/2* deficiency could increase the mutation rate, since error-free HR DNA repair cannot work efficiently in the absence of *BRCA1/2* and cells rely on more error-prone mechanisms of DNA repair⁽⁶⁶⁾. Increased mutation rates could contribute to the occurrence of secondary mutations in *BRCA1/2* themselves. Third, treatment with DNA damaging agents or with PARP inhibitors could serve as a selective pressure for *BRCA1/2*-restored cells.

Secondary *BRCA1/2* mutations may be acquired during the course of chemotherapy. This can be a result of the increased amount of damaged DNA lesions caused by chemotherapy. Repair of these lesions would be error prone in the HR-defective, *BRCA1/2*-deficient cell background, which would increase the chance of occurrence of secondary *BRCA1/2* mutations. In this model, the *BRCA1/2*-deficient neoplasm would shrink upon treatment with chemotherapy, leaving cells with acquired *BRCA1/2* secondary mutations to expand to form a drug resistant malignancy.

Alternatively, resistance may result from the presence of a subset of *BRCA1/2*-expressing cells that may exist in the tumor prior to chemotherapy. Tumors can be very genetically heterogeneous in nature. It is possible that a *BRCA1/2*-deficient tumor comprises a small subset of cells that express functional *BRCA1/2* protein due to a reversion event that occurred during the expansion of the tumor population. A precedent for this explanation exists. Lung cancers may express the EGFR mutation T790M that confers resistance to EGFR tyrosine kinase inhibitors in rare cells prior to drug exposure if sensitive detection methods are used⁽⁶⁷⁾. Similarly, in some cases of chronic myelogenous leukemia, *BCR-ABL* mutations that confer resistance to imatinib therapy are detectable by PCR in rare neoplastic cells prior to imatinib exposure⁽⁶⁸⁾. Treatment of such a neoplasm with a platinum agent, PARP inhibitor, or other drug that selectively kills *BRCA1/2*-deficient cells would result initially in a decrease of tumor size. The few *BRCA1/2*-expressing cells in the tumor population would survive the treatment. These cells can proliferate to form a *BRCA1/2*-proficient tumor, which would be resistant to further treatment with cisplatin or PARP inhibitor. In this model, drug treatment simply selects for the survival of the population of *BRCA1/2*-expressing, drug-resistant cells. Further work is needed to distinguish between these two models.

At the moment, we do not know which error-prone DNA repair mechanisms are involved in generating secondary mutations, but microhomology-mediated end-joining⁽⁶⁹⁾ and

translesion synthesis⁽⁷⁰⁾ are obvious candidates. Some of the secondary *BRCA1/2* mutations are point mutations or back mutations to wildtype (Tables 1 and 2). Error-prone translesion synthesis polymerases may be involved for the generation of these types of mutations⁽⁷⁰⁾. Microhomology-mediated end-joining may be a mechanism that plays a major role in occurrence of secondary *BRCA2* mutations of *BRCA2*-deficient cells. Sequences surrounding the secondary *BRCA2* deletions in some of the drug-resistant Capan-1 clones and HCC1428 breast cancer cell line have short regions of sequence identity associated with the ends of the deleted region (Table 1)^(3, 6), suggesting involvement of microhomology-mediated end-joining. However, microhomology-mediated end-joining may not play a major role in secondary *BRCA1* mutations of *BRCA1*-deficient cancer cells, since *BRCA1* is reported to facilitate microhomology-mediated end-joining⁽⁷¹⁾.

Modification of Response to Chemotherapy in *BRCA1*-deficient Cells by 53BP1 Deficiency

Chemotherapeutic response of tumors with *BRCA1/2* deficiency may be affected by other genetic or epigenetic modifications. It is possible that suppressor mutations of other genes in *BRCA1/2*-deficient cells may be able to reverse sensitivity of *BRCA1/2*-deficient cells to cisplatin and PARP inhibitors, without restoring *BRCA1/2* themselves. Two recent reports have shown that loss of the DNA-damage response protein 53bp1 in mice with *Brcal*-null or *Brcal* exon11 deletion mutation can rescue the embryonic lethality of *Brcal*-deficient mice^(72, 73). *Brcal*/53bp1-deficient cells have increased proliferative potential, chromosomal stability and HR efficiency relative to *Brcal*-deficient mouse cells. Importantly, 53bp1 deficiency in *Brcal*-mutated cells also restored resistance to cisplatin and PARP inhibitor. It has also been shown that some estrogen receptor-negative, progesterone receptor-negative and HER2-non-overexpressing (triple negative) and *BRCA1/2*-associated breast cancers have reduced 53BP1 expression⁽⁷²⁾. These data suggest that suppression of *BRCA1* deficiency by the loss of 53BP1 may be a modifier of clinical progression of *BRCA*-associated neoplasms. Further studies are needed to determine if 53BP1 loss in *BRCA1*-deficient tumors can lead to clinical cisplatin/PARP inhibitor resistance.

Clinical Implications

The most profound implication of the work reviewed here on the clinical management of *BRCA1/2*-associated malignancies is that secondary *BRCA1/2* mutation may predict whether a patient will respond to platinum based chemotherapy. This may also be true for patients treated with PARP inhibitors, although there is the additional complication of upregulation of P-glycoprotein pumps contributing to resistance to this class of drugs.

This work also suggests that restoration of DNA repair plays an important role in acquired resistance to DNA damaging drugs. Therefore, drug-resistant cancers with secondary *BRCA1/2* mutations may be re-sensitized to those drugs if HR can be inhibited by another drug. For example, proteasome inhibitors⁽⁷⁴⁾, CDK inhibitors⁽⁷⁵⁾ and HSP90 inhibitors⁽⁷⁶⁾ have been reported to inhibit RAD51 foci formation. These drugs may be good candidates for the treatment of platinum/PARP inhibitor-resistant malignancies in combination with cisplatin or PARP inhibitors.

Future Directions

Here we have reviewed evidence for the contribution of secondary *BRCA1/2* mutations to resistance to cisplatin and PARP inhibitors in *BRCA1/2*-mutated cancers. There still remain many unanswered important questions related to this mechanism. First, we need to perform larger clinical studies in order to correlate secondary *BRCA1/2* mutations with clinical

outcomes, such as resistance to platinum agents, PARP inhibitors and other chemotherapeutics. Second, we need to clarify which error-prone DNA repair mechanisms (for example, microhomology-mediated end-joining⁽⁶⁹⁾ and translesion synthesis⁽⁷⁰⁾) are involved in the occurrence of secondary mutations. Third, it is unclear whether secondary mutations arise as a result of drug treatment or are pre-existing in the tumor cell population. Fourth, we want to know whether secondary mutations of other DNA repair genes or DNA damage response genes, such as ATM, NBS1, CHEK2, PALB2/FANCN, BRIP1/FANCI, RAD51C/FANCO, and other Fanconi anemia genes, are involved in resistance to DNA damaging agents. Fifth, we want to know whether re-expression of BRCA1/2 mediates therapeutic resistance in sporadic carcinomas with nonhereditary BRCA1/2 deficiency due to epigenetic silencing and other mechanisms. For example, in platinum-resistant recurrent ovarian cancer with *BRCA1* promoter methylation, re-expression of BRCA1 could result from partial demethylation of the promoter. Sixth, secondary *BRCA1/2* mutation is not the only mechanism of drug resistance, and we need to clarify other mechanisms, which may include a suppressor of BRCA1/2 deficiency that restores DNA repair even in the absence of functional BRCA1/2 protein. Efforts to understand the role of DNA repair in chemoresistance may eventually lead to novel strategies to prevent or overcome drug resistance in the future and improve patient survival.

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Fig. 1. Implications of DNA repair defects in cancer

DNA repair defects (for example, defect of homologous recombination due to BRCA1/2 deficiency) can contribute to genomic instability, which promotes malignant transformation of cells. They also lead to cellular sensitivity to certain DNA damaging agents. Restoration of DNA repair (for example, restoration of homologous recombination DNA repair due to functional restoration of BRCA1/2) can lead to acquired resistance to the DNA damaging agents.

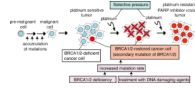


Fig. 2. Model for involvement of BRCA1/2 restoration in acquired resistance to platinum chemotherapy

(adapted with modifications from Sakai et al, Nature 2008;451(7182): 1116–20, supplemental material). Initially tumors are BRCA1/2 deficient and sensitive to platinum compounds (cisplatin and carboplatin). Rare cells restore functional BRCA1/2 due to secondary *BRCA1/2* mutation. The secondary mutation may occur during the course of chemotherapy or may pre-exist prior to chemotherapy. BRCA1/2 deficiency and treatment with DNA damaging agents can lead to increased mutation rate, which contribute to occurrence of secondary mutations in *BRCA1* and *BRCA2* themselves. Treatment with platinum compounds can also serve as a selective pressure for BRCA1/2-restored cells. BRCA1/2-restored cells survive the treatment and proliferate to form a BRCA1/2-proficient tumor, which would be resistant to both platinum compounds and PARP inhibitors.

Table 1
Secondary *BRCA2* mutations identified in *BRCA2*-mutated cancer cell line models

Deleted sequences are represented in red text and inserted sequences are represented by green text. Point mutations are shown in bold-faced black text. Blue text for clone C2-1 is a templated nucleotide sequence that is inserted downstream after deletion of a 7bp segment. Short sequences of homo logy that may contribute to deletion events via microhomology-mediated end-joining are underlined. (Data of PIR1-12 are adapted with permission from Edwards et al, Nature 2008;451(7182): 1111–5, Table 2 (reference 6).)

The table is a complex grid with multiple columns and rows, containing various mutation details such as cell line, mutation type, and sequence. The text is color-coded: red for deletions, green for insertions, and bold for point mutations. Some sequences are underlined to indicate microhomology.

Table 2

Secondary *BRCA1/2* mutations in clinical samples of recurrent ovarian carcinomas.

Patient	Mutated gene	Inherited mutation	Secondary genetic change	Platinum sensitivity at time of recurrence	Reference
UW91	<i>BRCA1</i>	185delAG	Yes (185insAG)	refractory	
UW208	<i>BRCA1</i>	E1250X	No	sensitive	
UW-F21	<i>BRCA1</i>	2594delC	Yes (2606_2628del23)	refractory	
UW9238	<i>BRCA1</i>	185delAG	Yes (185insAG)	refractory	
UW-LS317	<i>BRCA1</i>	3171insTGAGA	No	refractory	4
UW-F27	<i>BRCA1</i>	185delAG	Yes (185insAG)	refractory	
CS1	<i>BRCA1</i>	185delAG	No	sensitive	
CS4	<i>BRCA1</i>	185delAG	No	sensitive	
CS14	<i>BRCA1</i>	185delAG	No	refractory	
CS2	<i>BRCA1</i>	L598X	No	refractory	
	<i>BRCA2</i>	8765delAG	Lost mutated allele		
UW174	<i>BRCA2</i>	5578delAA	No	refractory	3
UW3548	<i>BRCA2</i>	6174delT	Yes (6174insT)	refractory	
CS9	<i>BRCA2</i>	6174delT	No	sensitive	
CS15	<i>BRCA2</i>	2699delT	No	sensitive	
UK1999	<i>BRCA2</i>	6174delT	Yes (137bp deletion)	refractory	
UK2223	<i>BRCA2</i>	6174delT	Yes (8bp deletion)	refractory	6