Published in final edited form as:
Cancer Biol Ther. 2008 July ; 7(7): 1004–1005.

Secondary *BRCA1* and *BRCA2* alterations and acquired chemoresistance

Weixin Wang and **William D. Figg**
Medical Oncology Branch, National Cancer Institute, Bethesda, Maryland USA

Abstract

Tumor suppressor *BRCA1* and *BRCA2* are frequently mutated in familial breast and ovarian cancer. More than ten percent of women with breast or ovarian cancer carry *BRCA1* or *BRCA2* (*BRCA1/2*) mutations. Cancers that arise in mutation carriers have often lost the wild-type allele through somatic alterations during tumor progression. BRCA1/2 play important roles in homologous recombination repair of DNA double-strand breaks. Because of this, *BRCA1/2*-deficient cancers often have a better response to DNA cross-linking agents such as platinum analogues and to poly(ADP-ribose) polymerase (PARP) inhibitors. However, over time, the majority of these *BRCA1/2*-deficient cancers become resistant and patients die from refractory diseases. Three recent studies demonstrated that acquired resistance to platinum analogues or PARP inhibitors in tumors carrying frame-shift *BRCA1/2* mutations came from restored BRCA1/2 expression and HR function due to secondary intragenic mutations that corrected the open reading frames of mutated *BRCA1/2*.

Keywords

BRCA1; BRCA2; resistance; mutation; PARP; cisplatin; inhibitor

Tumor suppressor *BRCA1* and *BRCA2* are frequently mutated in familial breast and ovarian cancer. More than ten percent of women with breast or ovarian cancer carry *BRCA1* or *BRCA2* (*BRCA1/2*) mutations. Cancers that arise in mutation carriers have often lost the wild-type allele through somatic alterations during tumor progression [1,2]. BRCA1/2 play important roles in homologous recombination (HR) repair of DNA double-strand breaks [1, 2]. Because of this, *BRCA1/2*-deficient cancers often have a better response to DNA cross-linking agents such as platinum analogues and to poly(ADP-ribose) polymerase (PARP) inhibitors [3-5]. However, over time, the majority of these *BRCA1/2*-deficient cancers become resistant and patients die from refractory diseases.

Three recent studies demonstrated that acquired resistance to platinum or PARP inhibitors in tumors carrying frame-shift *BRCA1/2* mutations came from restored BRCA1/2 expression and HR function due to secondary intragenic mutations that corrected the open reading frames (ORFs) of mutated *BRCA1/2* [6-8]. Pancreatic cancer cell line CAPAN1 lacks a wild-type *BRCA2* but carries a *BRCA2* with a 6174delT frame-shift mutation, a founder mutation often detected in the Ashkenazi Jewish population [9,10]. This 6174delT mutation results in expression of a truncated BRCA2 protein that lacks two BRC repeats, the DNA-binding/DSS1 (DBD) interaction domain, the second RAD51-binding domain (TR2) and the nuclear localization sequences (NLS) [6,7,10]. Ashworth's and Taniguchi's groups isolated PARP inhibitor-resistant [6] and cisplatin-resistant [7] clones from *BRCA2*-deficient CAPAN1 cells.

Correspondence: William D. Figg, Medical Oncology Branch, National Cancer Institute, Building 10, Room 5A01, Bethesda, Maryland 20892, USA, Tel.: 301.402.3622, Fax: 301.402.8606, Email: wdfigg@helix.nih.gov.

These clones were resistant to both PARP inhibitors and cisplatin, but not to the microtubule-stabilizing agent, docetaxel [6,7]. Most resistant clones possessed multiple copies of the *BRCA2* gene. All resistant clones still carried the original 6174delT allele and the allele with secondary mutations [6,7]. Ashworth's group found that the PARP inhibitor-resistant clones expressed *BRCA2* alleles missing the region containing 6174delT mutation due to deletions ranging from 458 bp to 58 kb, and most of these deletions occurred in regions with small tracts of homology, possibly due to error-prone repair caused by *BRCA2* deficiency. As a result, *BRCA2* ORF was restored that included five BRC repeats, the C-terminal NLS and the TR2 RAD51 interaction domain [6].

Taniguchi's group found that, in cisplatin-resistant clones with restored BRCA2 expression, the *BRCA2* ORF was restored by deletion, insertion, or deletion/insertion at sites close to the original 6174delT mutation site, or by in-frame deletions flanking the original mutation site [7]. They also found a 2,135 bp in-frame deletion flanking the 6174delT mutation in one cisplatin-resistant breast cancer cell line, HCC1428, that resulted in alternative splicing and expression of truncated BRCA2 proteins and restoration of BRCA2 function [7]. However, not all the cisplatin-resistant clones were found to carry the secondary *BRCA2* mutations. In a half of resistant clones, there were no secondary mutations in *BRCA2* and no restored BRCA2 expression and HR function were observed. These clones were also not resistant to PARP inhibition. This suggested that these clones acquired cisplatin resistance through mechanisms other than the restoration of HR function [7].

The HR function was restored in resistant clones expressing BRCA2 isoforms [6,7]. By performing RNA interference experiments knocking down the expression of variant BRCA2 in resistant clones, and by reconstituting variant BRCA2 expression in *BRCA2*-deficient CAPAN1 and VC8 cells, they demonstrated that the restoration of HR function and acquired chemoresistance did indeed result from the expression of these variant BRCA2 proteins [6,7].

Sequencing of DNAs from recurrent platinum-resistant ovarian tumors indicated that *BRCA2* ORF was restored by deletions downstream of 6174delT mutation [6], or by reversion of 6174delT mutation back to wild-type [7].

In another study, Taniguchi's group showed that the acquired cisplatin resistance in recurrent *BRCA1*-deficient ovarian cancers resulted from the restoration of BRCA1 expression [8]. They analyzed cisplatin-resistant ovarian cancer tissues with a frame-shift 185delAG mutation in *BRCA1*, a founder mutation commonly found in the Ashkenazi Jewish population [11], and observed that recurrent tumors regained wild-type *BRCA1* by genetic reversion of 185delAG to wild-type. In another case, the primary tumor was detected carrying a wild-type *BRCA1* resulting from genetic reversion of a frame-shift 2594delC mutation in *BRCA1*, making this primary tumor resistant to cisplatin. In a recurrent tumor derived from this patient, the genetically reverted wild-type allele was lost, but a secondary deletion (2606-2628del23) was found in sequence with the inherited mutation (2594delC), resulting in the restoration of BRCA1 expression [8]. As expected, in recurrent cisplatin-sensitive tumors, no secondary genetic changes in *BRCA1* were observed [8].

These studies provided insights on mechanisms of acquired chemoresistance to platinum analogues and PARP inhibitors in tumors carrying frame-shift *BRCA1/2* mutations. It remains to be seen how resistance might develop in tumors carrying other types of *BRCA1/2* mutations such as large segments of deletions or truncations. It is also unclear what roles other DNA repair mechanisms might play in acquired chemoresistance in *BRCA1/2*-defective cancers since increased nucleotide excision repair and loss of mismatch repair proteins have been associated with cisplatin-resistant ovarian cancers [12-16].

References

1. Narod SA, Foulkes WD. BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer 2004;4(9):665–76. [PubMed: 15343273]
2. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 2002;108(2): 171–82. [PubMed: 11832208]
3. Farmer H, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434(7035):917–21. [PubMed: 15829967]
4. Bryant HE, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005;434(7035):913–7. [PubMed: 15829966]
5. Ratnam K, Low JA. Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology. Clin Cancer Res 2007;13(5):1383–8. [PubMed: 17332279]
6. Edwards SL, et al. Resistance to therapy caused by intragenic deletion in BRCA2. Nature 2008;451 (7182):1111–5. [PubMed: 18264088]
7. Sakai W, et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature 2008;451(7182):1116–20. [PubMed: 18264087]
8. Swisher EM, et al. Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. Cancer Res 2008;68(8):2581–6. [PubMed: 18413725]
9. Neuhausen S, et al. Recurrent BRCA2 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. Nat Genet 1996;13(1):126–8. [PubMed: 8673092]
10. Goggins M, et al. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. Cancer Res 1996;56(23):5360–4. [PubMed: 8968085]
11. Struewing JP, et al. The carrier frequency of the BRCA1 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. Nat Genet 1995;11(2):198–200. [PubMed: 7550349]
12. Ferry KV, Hamilton TC, Johnson SW. Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells: role of ERCC1-XPF. Biochem Pharmacol 2000;60(9):1305–13. [PubMed: 11008124]
13. Reed E. Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. Cancer Treat Rev 1998;24(5):331–44. [PubMed: 9861196]
14. Aebi S, et al. Loss of DNA mismatch repair in acquired resistance to cisplatin. Cancer Res 1996;56 (13):3087–90. [PubMed: 8674066]
15. Brown R, et al. hMLH1 expression and cellular responses of ovarian tumour cells to treatment with cytotoxic anticancer agents. Oncogene 1997;15(1):45–52. [PubMed: 9233776]
16. Drummond JT, et al. Cisplatin and adriamycin resistance are associated with MutLalpha and mismatch repair deficiency in an ovarian tumor cell line. J Biol Chem 1996;271(33):19645–8. [PubMed: 8702663]