A Revertant of the Pathogenic Germline Mutation in BRCA1 as a Possible Cause of Breast Cancer Chemoresistance

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Abstract

Inherited mutations in BRCA genes increase the risk of developing both breast and ovarian cancers. Wild-type BRCA proteins are involved in DNA repair mechanisms, and DNA damaging chemotherapeutics take advantage of these repair deficiencies in BRCA mutated cancers. However, chemoresistance often develops and currently represents one of the most significant barriers to effective breast and ovarian cancer therapy. Secondary somatic BRCA mutations that restore functional BRCA proteins could contribute to the development of chemoresistance, but clinical case reports demonstrating these mutations are lacking. The following case report outlines a BRCA1 somatic mutation that could rescue the function of BRCA1 proteins and provides clinical evidence of the role BRCA1 plays in the development of chemoresistance.

The clinical relevance of BRCA1 and BRCA2 are well established in the biology of breast and ovarian cancers. Working through related mechanisms, these proteins are involved in DNA repair and the maintenance of chromosomal stability.\(^1,2\) Mutation of the wild-type BRCA1 or 2 allele results in the accumulation of DNA damage in healthy cells facilitating cellular transformation and ultimately tumor formation. BRCA1, the focus of the following report, participates in the repair of double-strand DNA breaks by mediating homologous recombination through multiple distinct mechanisms.\(^2\)

While a lack of BRCA activity plays an integral role in tumor formation, this characteristic also leaves tumor cells vulnerable to DNA-damaging chemotherapeutic agents. Indeed, DNA-damaging agents have been a mainstay in therapy for BRCA-deficient breast and ovarian cancers. Additionally, poly (ADP-ribose) polymerase-1 (PARP) inhibitors are being added to this treatment arsenal. This novel class of chemotherapeutics works through inhibition of a pathway that repairs single-strand DNA breaks and indirectly exploits the deficiency of the cancer cell to repair DNA damage.\(^3\) Unfortunately, cancer cells often become refractory to these therapies. The mechanisms through which tumors develop this chemoresistance are an area of great interest, and the role of BRCA1 genes in this process is under investigation.

Secondary mutations in BRCA genes have been implicated in the development of chemoresistance in cancer cell lines and clinical specimens.\(^4,5\) A recent review suggests a model in which platinum-sensitive tumors treated with platinum-based chemotherapeutics are placed under a selective pressure that favors the survival of cells harboring secondary BRCA4 mutations.\(^6\) These secondary mutations potentially rescue BRCA protein function, producing tumor cells that can now escape cell death caused by DNA cross-linking agents and/or PARP inhibitors.

The development of this model is largely based on in vitro studies utilizing cancer cell lines, as well as clinical samples of ovarian cancer. The current literature distinctly lacks clinical case reports detailing specific secondary mutations in BRCA genes associated with the development of chemoresistance in patients. Such reports are needed to validate models of BRCA-related chemoresistance,
and to present examples of specific secondary mutations with clinical relevance. The following report will begin to address this need through the presentation of a potentially novel BRCA1 rescue mutation in a patient with breast cancer.

Case Description

A 27-year-old Colombian woman without family history of breast or ovarian malignancy presented for evaluation of a palpable left breast mass. She denied any skin or nipple changes but her perception was an enlargement of the left breast compared to the right. Initial breast imaging revealed no conclusive findings and short-term follow-up was recommended. Over the next several weeks, the breast mass enlarged and she returned for interval assessment. At that time, the mass expanded to over 5 cm and now had suspicious imaging characteristics prompting urgent biopsy. The mass proved to be an invasive ductal cancer, grade II, which was negative for estrogen receptor (ER = 0%), progesterone receptor (PR = 1%, intensity 1+), and was HER2 negative by immunohistochemistry (IHC). Given the locally advanced nature of the lesion, she was counseled for neoadjuvant chemotherapy for 6 cycles. Following 2 cycles, clinical examination and a repeat breast MRI demonstrated a marked interval diminution in tumor burden from pre-treatment (7.1 cm x 6.8 cm x 5.5 cm to 2.1 cm x 2.4 cm x 2.7 cm). While additional cycles were administered, her clinical examination was not further changed. During her neoadjuvant chemotherapy, genetic counseling and germline testing revealed a single-base substitution resulting in a pathogenic BRCA1 nonsense mutation (p.W1508X, c.4523G>A); on this basis, bilateral mastectomy was planned as her definitive surgical procedure. Prior to surgery, a repeat MRI was performed which suggested that the tumor had grown, now reflecting multiple foci of tumor, although the overall burden of disease was decreased. At surgery, she was found to have a 4.5 cm residual tumor, now grade III (undifferentiated), with multiple foci of lymphovascular invasion, and 1 of 10 axillary lymph nodes involved with metastatic disease.

Out of concern for the emergence of resistance, repeat molecular analysis of the tumor was performed demonstrating the persistence of the native BRCA1 mutation in 87% of sequencing reads analyzed and a new, presumably somatic substitution mutation, W1508Q (Trp1508Gln), in 25% of the reads. Further investigation revealed that the secondary substitution is in “cis” configuration (on the same allele) with the germline variant (p.W1508Q, c.4522_4523TG>CA). This observation is consistent with 2 separate mutations affecting the wild type allele in which the wild type sequence initially mutated from tryptophan to a stop codon (TGG → TAG) and the secondary mutation reverted the stop codon to glutamine (TAG → CAG) (Fig. 1). This secondary mutation is predicted to reverse the loss of function seen with the germline mutation. It should be noted that the 87% allele frequency found for W1508X was obtained in the background of 70% tumor. According to the loss of heterozygosity model, if a variant is germline, we would expect 85% frequency when testing a sample that contains 70% tumor. Thus the allele frequency obtained during tumor testing corroborates and supports that W1508X was in fact a germline variant.

Discussion

The BRCA1 gene product is composed of 1863 amino acids, contains an N-terminal RING domain, 2 C-terminal BRCT domains, and nuclear localization signals.2 Mutations affecting both the RING domain and BRCT domains are known to compromise the tumor suppressive activities of BRCA1.7 The W1508X nonsense mutation

Figure 1

A) General structure of BRCA1 protein is shown with a focus on the wild-type (WT) DNA and amino acid sequences. B) Pathogenic BRCA1 germline mutation detected during neoadjuvant chemotherapy demonstrating the nonsense mutation leading to a premature stop codon. C) Revertant mutation resulting in glutamine in place of the stop codon potentially restoring the DNA repair function of the BRCA1 protein.
sequenced from this patient’s initial biopsy would result in a truncated protein product lacking structurally sound C-terminal BRCT domains. Consistent with the germline testing results, W1508X mutations have also been documented as a class 5 germline variant in 23 cases in the Breast cancer Information Core (BIC) database.

As described above, repeat molecular analysis revealed a subpopulation of cells harboring a rare W1508Q mutation. The conversion of tryptophan to glutamine requires a double substitution in DNA, which in our case possibly resulted from 2 distinct mutational events. The first event, which was inherited and was detected during neoadjuvant chemotherapy, converted tryptophan to a stop codon. The second event converted the stop codon to glutamine, which was the secondary mutant discovered on subsequent analysis. The effects of W1508Q have not been fully characterized and have not been published in the literature or reported in mutation databases such as BIC, Biobase, LOVD, ClinVar, and Catalogue of Somatic Mutations in Cancer (COSMIC).

We utilized 3 algorithms to predict the effect of Glutamine substitution for Tryptophan at residue 1508. In our in silico analysis, 2 of the 3 algorithms predicted neutrality: SIFT algorithm predicted that the substitution is “damaging” (SIFT score of 0.01) and Polyphen2 and Align GVGD predicted neutrality, suggesting that this variant is more likely to not affect the BRCA1 function. In addition, the presence of W1508Q results in removal of the premature stop codon and a full-length protein product containing both C-terminal BRCT domains. The structural restoration of BRCA1 in the cells containing the W1508Q mutation would then allow these cells to survive the DNA-damaging chemotherapeutics intended to take advantage of deficient DNA repair mechanisms. We believe this rescue mutation allowed a subpopulation of cells to evade chemotherapy-induced cell death in this patient, which potentially accounts for the observed tumor growth during neoadjuvant chemotherapy. Future in vitro experiments, such as those used to investigate the role of secondary BRCA2 mutations in ovarian cancer, will assist in determining the true effect of W1508Q variant on BRCA1 function and resolve the inconsistent in silico prediction results.

The clinical course and molecular analysis described in this case present an example of BRCA1-mediated chemoresistance in a breast cancer patient. These observations were made possible by the repeat molecular analysis performed following tumor growth suggesting that serial molecular profiling of tumors before, during, and after treatment may lead to novel genetic and epigenetic findings. Additionally, serial molecular profiling could improve outcomes by allowing clinicians to adapt treatment regimens to both respond to and avoid potential chemoresistance. The main limitation to this suggestion is the availability of chemotherapeutics with diverse mechanisms of action that could target signal transduction pathways still relevant to the survival of tumor cells. Countless studies currently underway are designed to investigate these survival pathways and to design novel therapeutics targeting specific signal mediators. The introduction of PARP inhibitors into the treatment arsenal is a prime example of the success of such efforts.

Case reports such as this are crucial to inform the design of translational studies investigating the development of chemoresistance. This report details a secondary somatic mutation that potentially rescues the function of BRCA1. Analysis of the functional relevance of this mutation is warranted and has the potential to further our understanding of in vivo mechanisms of chemoresistance.

References