BRCA1: A Missing Link in the Fanconi Anemia/BRCA Pathway

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Summary: Domchek and colleagues provide a case report of a 28-year-old woman with congenital abnormalities, inherited ovarian cancer, and carboplatin hypersensitivity. Interestingly, the woman had validated germline mutations in both BRCA1 alleles. These findings further implicate BRCA1 in the Fanconi anemia/BRCA pathway and have important implications for BRCA1 genetic testing. Cancer Discov; 3(4): 376–8. © 2013 AACR.

See related article by Domchek et al., p. 399 (6).

Significant advances in our understanding of breast and ovarian cancer have resulted from the genetic linkage mapping and cloning of 2 major tumor suppressor genes, BRCA1 and BRCA2. Mutations in BRCA1 and BRCA2 account for approximately 16% of the familial risk of breast cancer (1). Moreover, heterozygous carriers of mutations in BRCA1 and BRCA2 have an 82% lifetime risk of breast cancer and a 54% and 23% risk of ovarian cancer, respectively (2). Biallelic germline mutations in BRCA2 account for a rare and highly cancer-prone subtype of Fanconi anemia (3); however, humans with validated biallelic germline mutations in BRCA1 have not been identified, suggesting that at least one functional allele of BRCA1 is required for human embryogenesis and development.

Fanconi anemia is an autosomal-recessive or X-linked-recessive genetic disease characterized by multiple congenital anomalies, bone marrow failure, cancer predisposition, and cellular hypersensitivity to DNA cross-linking agents, such as mitomycin C and cisplatin. There are 15 known Fanconi anemia genes, and the encoded 15 Fanconi anemia proteins (Fig. 1), which is required for the repair of DNA cross-links (ref. 5; Fig. 1). Additional proteins (shown in red in Fig. 1) such as BRCA1, while not bona fide Fanconi anemia proteins (i.e., not encoded by genes known to have germline mutations in patients with Fanconi anemia), are also required for the activity of the pathway and are therefore candidate Fanconi anemia proteins. Interestingly, at least 4 of the Fanconi anemia genes, FANCD1/BRCA2, FANCN/PALB2, FANCF/BRIP1, and FANCO/RAD51C, are also breast/ovarian cancer susceptibility genes. Heterozygote carriers of germline mutations in these genes carry an increased cancer risk, albeit at highly variable penetrance. A new study by Domchek and colleagues (6) shows that the BRCA1 protein is also a critical component of this pathway and that BRCA1 may itself be a Fanconi anemia gene.

The interaction of BRCA1 with other proteins in the Fanconi anemia pathway has been suspected for several years. BRCA1, like the bona fide Fanconi anemia proteins, has a well-known function in the maintenance of genomic stability through homologous recombination repair and in the promotion of DNA repair of interstrand cross-links (7). Moreover, knockdown of BRCA1 in tumor cells results in a reduction of FANCD2 monoubiquitination and nuclear FANCD2 DNA repair foci (8), suggesting that BRCA1 is an amplifier of the Fanconi anemia/BRCA pathway. Perhaps most interestingly, BRCA1 is a component of a large nuclear protein complex (Fig. 1) consisting of at least 3 other bona fide Fanconi anemia proteins, BRCA2 (FANCD1), PALB2 (FANCN), and BRIP1 (FANCJ). PALB2 was originally identified in a screen of BRCA2-binding proteins (9), and it binds to the extreme N-terminus of BRCA2. PALB2 also binds to the C-terminal BCRT repeats of BRCA1, and BRCA1, in turn, binds directly to BRIP1 (J; Fig. 1). This intimate interaction of BRCA1 with other bona fide Fanconi anemia proteins, which are themselves breast/ovarian cancer susceptibility proteins, further suggests that BRCA1 directly participates in the Fanconi anemia/BRCA pathway and that biallelic BRCA1 mutations might result in Fanconi anemia or a Fanconi anemia–like syndrome.

Indeed, in their case report, Domchek and colleagues (6) determined that the proband with ovarian cancer had biallelic mutations in BRCA1. Although one mutant allele was clearly deleterious (the known c.2457delC allele), the other allele was a variant of unknown significance (VUS), encoding a BRCA1 protein with a V1736A amino acid substitution. The authors provide several lines of reasoning to determine that this VUS allele is also deleterious and encodes a dysfunctional BRCA1 protein. First, they examined other kindreds that carry this variant allele. Two kindreds have family members with breast and ovarian cancer, and the combined OR in favor of the variant allele being pathogenic was elevated (ratio of 234:1). Second, the V1736A mutation falls in one of the C-terminal BRCT domains of BRCA1. Although the mutation did not directly disrupt the BRCT-binding surface of BRCA1, it indirectly altered the binding affinity of the BRCT domain for...
Figure 1. BRCA1 cooperates in the Fanconi anemia/BRCA1 pathway. The fifteen known Fanconi anemia proteins (A, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P) cooperate in a common DNA repair pathway (green). The pathway is activated when a replication fork stalls at a cisplatin interstrand cross-link (ICL).  The Fanconi anemia core complex binds to the stalled fork, leading to monoubiquitination and recruitment of the L and D2 proteins.  The downstream Fanconi anemia proteins (D2, N, J, and O) are recruited to DNA repair complexes.  Additional proteins (red), while not bona fide Fanconi anemia proteins, are also required for function of the Fanconi anemia pathway and for repair of DNA cross-links.  The BRCA1 protein is a Fanconi anemia-like protein and binds in a complex with at least 3 bona fide Fanconi anemia proteins.  Biallelic mutations in BRCA1 (see text) result in an inherited ovarian cancer (Fanconi anemia-like) syndrome.

RAP80, another BRCA1-interacting protein.  Furthermore, the presence of the V1736A mutation reduced BRCA1 localization to genomic sites of DNA double-strand breaks.  Interestingly, recent studies indicate that mice with homozygous mutations corresponding to this BRCT region of BRCA1 yield viable animals, albeit with increased cancer risk (10).  Third, and most convincingly, the ovarian tumor derived from the proband did not have loss of heterozygosity at the BRCA1 locus.  This finding is in contradistinction to the ovarian tumors derived from other family members that did exhibit loss of the wild-type BRCA1 allele and persistence of the V1736A-mutant allele.

Importantly, this case report does not indicate that BRCA1 is a Fanconi anemia gene, per se.  The proband had a Fanconi anemia–like syndrome (some congenital abnormalities, ovarian cancer, carboplatin hypersensitivity) but some clear differences from Fanconi anemia.  The patient did not exhibit spontaneous bone marrow failure, though she did exhibit significant mucositis and enhanced bone marrow toxicity (prolonged neutropenia) following carboplatin treatment.  Interestingly, carboplatin hypersensitivity is not a feature of BRCA1 heterozygote carriers (11), further supporting the argument that this patient had biallelic deleterious BRCA1 mutations.  The patient died before the definitive diagnostic test for Fanconi anemia, named the diepoxybutane (DEB) chromosome breakage test (12), could be conducted.  If the patient had survived, the DEB test could have been conducted on the primary peripheral blood lymphocytes and on a patient-derived ovarian tumor cell line.  There is a high probability that these patient-derived cells would have been hypersensitive to DEB or carboplatin in vitro and that transfection with the wild-type BRCA1 cDNA would have rescued this cellular phenotype.  Still, the cells of the patient also carried a variant allele of BRCA2, which may have contributed, at least in part, to the hypersensitivity to carboplatin.

Although patients with Fanconi anemia are prone to myeloid leukemia and squamous cell carcinomas, they generally do not develop breast or ovarian cancer.  Several explanations may resolve this paradox.  First, women with Fanconi anemia are hypogonadal, and their low serum estrogen level may protect them from these cancers.  Second, women with Fanconi anemia have decreased breast and ovarian tissue mass, perhaps further reducing their cancer risk.  Third, women with Fanconi anemia often die young from complications of bone marrow failure or other cancers.  Because women with Fanconi anemia live longer, due to improved supportive care, careful screening for breast and ovarian cancer in these patients may be warranted.  Recently, there have been anecdotal reports of women with subclinical Fanconi anemia (i.e., FANCA−/− patients) who have presented with breast cancer, despite the absence of more obvious Fanconi anemia phenotypic features.  On the basis of these findings, any women with ovarian or breast cancer who have a suspicious phenotype (i.e., very early onset of cancer, characteristic developmental abnormalities, or profound carboplatin sensitivity) should be screened for mutations in BRCA1 or BRCA2 and perhaps other genes in the Fanconi anemia/BRCA pathway.  Similarly, presentation with a Fanconi anemia phenotype (i.e., bone marrow failure and developmental defects) should warrant investigation for mutations in these same Fanconi anemia/BRCA genes.

In summary, this important new work by Domchek and colleagues (6) identifies a new autosomal recessive genetic syndrome (developmental defects, inherited ovarian cancer susceptibility, and cross-linker hypersensitivity caused by biallelic mutations in BRCA1).  The work also describes a
systematic approach for testing variant BRCA1 proteins for functional activity in vitro. As more variant BRCA1 alleles are identified through expanding genome-sequencing efforts, there will be a growing need for better functional assays to distinguish deleterious from benign variants.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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REFERENCES