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FANCF methylation contributes to chemoselectivity in ovarian cancer

A new model of ovarian cancer tumor progression implicates aberrant *FANCF* promoter methylation that is associated with gene silencing and disruption of the Fanconi-anemia-BRCA pathway. Disruption of the pathway occurs de novo in ovarian cancers and may contribute to selective sensitivity to platinum salts.

Ovarian cancer cells accumulate genetic changes that allow them to evade chemotherapeutic drugs and become increasingly dangerous. In view of the high mortality rates associated with ovarian cancer, a better understanding of the molecular mechanisms underlying tumor progression in the disease could reveal novel pathways of high clinical relevance. A key feature of ovarian cancer is its sensitivity to platinum salts such as Cisplatin (CDDP) and Carboplatin, two drugs that have been the mainstay of therapy for decades. Unfortunately, ovarian cancer cells, with their unstable genomes, are initially sensitive to this class of drugs, but the cells invariably become resistant.

In a recent study, Taniguchi et al. (2003) describe a model for ovarian tumor progression in which the initial methylation of *FANCF*, a gene associated with Fanconi anemia, is followed by *FANCF* demethylation and CDDP resistance. *FANCF* is one of seven recently cloned Fanconi anemia genes whose protein products were found to interact with proteins involved in DNA repair pathways, including BRCA1, RAD51, ATM, and NBS1 (D'Andrea and Grompe, 2003). Five of the *FANC* gene products (FANCA, FANCC, FANCE, FANCF, and FANCG) are subunits of a nuclear complex (FA complex) that is required for the monoubiquitination of the downstream FANCD2 protein (Figure 1A). The seventh gene, *FANCD1*, was recently shown to be identical to *BRCA2* (Howlett et al., 2002). Defects in the Fanconi-anemia-BRCA (FA-BRCA) pathway are associated with genomic instability and increased sensitivity to DNA-damaging agents such as ionizing radiation (IR), mitomycin C (MMC), and CDDP. In response to ionizing radiation-mediated double-strand breaks, ATM phosphorylates the NBS1 protein. Phosphorylation of NBS1 is required for FANCD2 phosphorylation at serine 222, leading to activation of an S phase checkpoint. In response to DNA damage, the FA complex mediates ubiquitination of FANCD2 at lysine 561. Activated FANCD2 is translocated to chromatin and DNA repair foci, which contain the BRCA1 protein and BRCA2/FANCD1 protein complex. BRCA2/FANCD1 binds to RAD51 and to DNA, promoting a DNA repair response. The ubiquinated FANCD2 also colocalizes with NBS-MRE11-RAD50 complex in DNA damage nuclear foci. Germline mutation of several genes in the pathway result in impaired response to DNA damage and increased cancer susceptibility.

FANCD2 exists as two isoforms in normal cells, nonubiquitinated FANCD2-S and monoubiquitinated FANCD2-L. Inducible expression of monoubiquitinated FANCD2 in response to DNA damage requires an intact FA-BRCA pathway. Taniguchi et al.(2003) screened 25 ovarian cancer cell lines with varying sensitivities to CDDP and found two cell lines, 2008 and TOV-21G, without the FANCD2-L isoform. Compared to other ovarian cancer cell lines, both 2008 and TOV-21G are hypersensitive to CDDP with half maximal inhibitory concentration (IC50) less than 1.0 μm of CDDP. TOV-21G cells were retrovirally transduced with various *FANC* cDNAs (*FANCA*, *FANCC*, *FANCE*, *FANCF*, *FANCG*) in an attempt to correct any

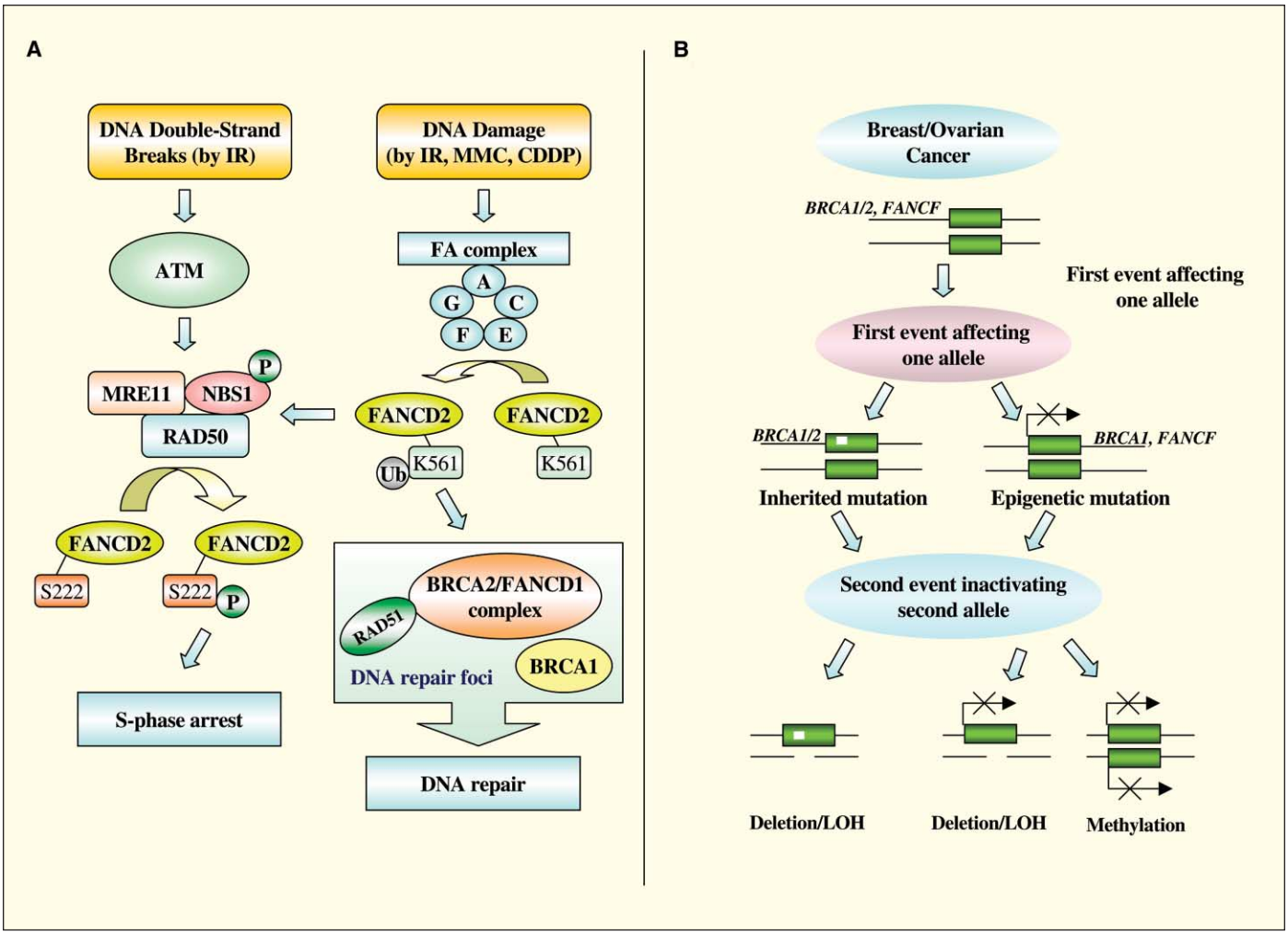

Figure 1. The Fanconi anemia/BRCA pathway and inactivation of BRCA1 in breast/ovarian cancer

A: FANCD2 protein functions at the intersection of two signaling pathways. In response to ionizing radiation-mediated double-strand breaks (DSB), ATM phosphorylates the NBS1 protein. Phosphorylation of NBS1 is required for FANCD2 phosphorylation at serine 222 (S222), leading to activation of an S phase checkpoint. In response to DNA damage, the FA complex mediates the Ub of FANCD2 at lysine 561. Activated FANCD2 is translocated to chromatin and DNA repair foci, which contain the BRCA1 protein and BRCA2/FANCD1 protein complex. BRCA2/FANCD1 binds to RAD51 and to DNA, promoting a DNA repair response. The Ub-FANCD2 also colocalizes with NBS-MRE11-RAD50 complex in DNA damage nuclear foci.

B: The mechanism of inactivation of BRCA1 in breast/ovarian cancer. Hypermethylation of the *BRCA1* promoter was detected in sporadic breast and ovarian cancer samples with proportions ranging from 11% to 31% and from 5% to 15%, respectively. This model shows that promoter methylation can serve as a "first hit" in the *BRCA1* and *FANCF* genes just as inherited mutation can serve as a "first hit" in the *BRCA1* and *BRCA2* genes. In cases where the first copy is methylated, the second copy may be inactivated by LOH or methylation.

abnormalities in proteins upstream of FANCD2 in the FA-BRCA pathway. Only FANCF was able to correct the defect in FANCD2 monoubiquitination in the transfected cells. The FANCF-corrected TOV-21G cells became resistant to MMC and CDDP with IC50 > 1 μm CDDP. No *FANCF* gene mutations were found in TOV-21G, but its promoter was densely methylated. Two additional cell lines, C13*, a CDDP-resistant derivative of 2008 with low levels of FANCF protein and OAW42, a cell line derived from the ascites of a woman previously exposed to CDDP, were also methylated. When C13* was treated with 5-aza-2'-deoxycytidine, a demethylating agent, *FANCF* mRNA expression and monoubiquination of FANCD2 were restored. The treated C13* cells then became less sensitive to CDDP. The authors established a more general role for *FANCF* promoter methylation in ovarian cancer with the observation that 4 of 19 (21%) primary ovarian cancers, not previously exposed to CDDP, also demonstrated *FANCF* methylation. Thus, their study suggests that disruption of the FANC-BRCA pathway occurs de novo in ovarian cancers and may contribute to selective sensitivity to platinum salts, which has significant clinical implication.

Fanconi anemia patients are predisposed to many types of cancer, including acute myeloid leukemia, squamous cell carcinoma of the head and neck, gynecological cancers, and esophageal cancer. About 10% of women diagnosed with breast or ovarian cancer each year

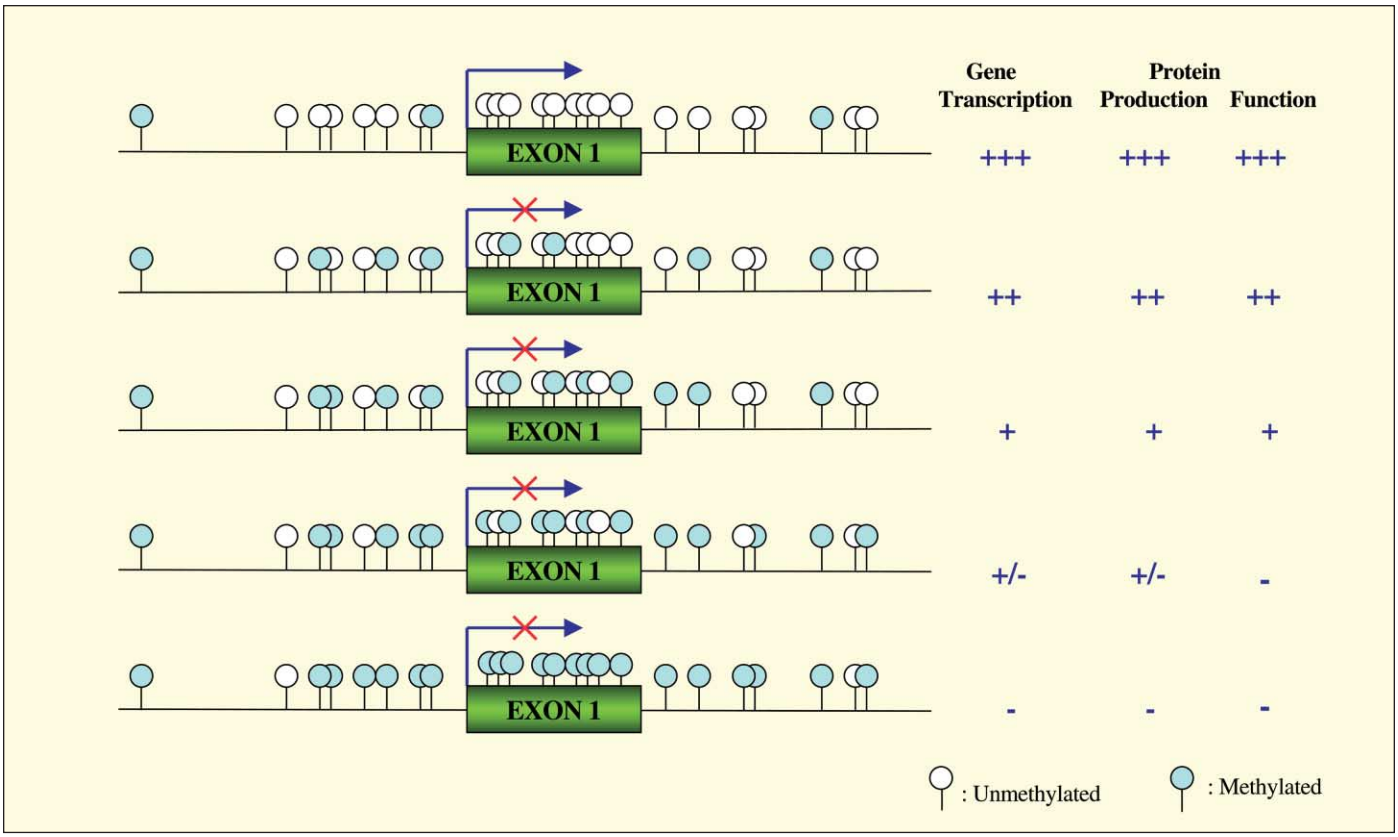

Figure 2. The heterogeneity of DNA methylaiton and gene expression

In cancer, the dynamics of genetically and epigenetically mediated loss of gene function are very different. The results of the progressive and heterogeneous methylation are an increasing degree of transcriptional loss and a variable decrease in protein production in individual cells of tumor.

are estimated to carry highly penetrant, germline mutations in the *BRCA1* or *BRCA2* gene (Antoniou et al., 2003). Somatic mutations of *BRCA1* or *BRCA2* genes are rare, yet epigenetic changes in the form of promoter methylation resulting in transcriptional silencing of the *BRCA1* gene have been demonstrated in about 5%–15% of nonfamilial ovarian cancer cases and 11%–31% of nonfamilial breast cancers (Catteau and Morris, 2002). Interestingly, *BRCA2* is generally hypomethylated and overexpressed in breast and ovarian cancers (Collins et al., 1997). Though the numbers are small, this study suggests that 8% of established ovarian cancer cell lines and about 20% of primary ovarian cancers have hypermethylation at the *FANCF* gene promoter. It is not clear whether methylation was bi-allelic or associated with gross chromosomal deletion or loss of heterozygosity (LOH) in the primary tumors. *FANCF* gene is located on the short arm of chromosome 11, band p15 (11p15), a region containing a number of imprinted genes associated with cancer, which is also frequently lost in ovarian cancer and other tumor types (Lu et al., 1997). In a preliminary study of 75 primary breast tumors, we have observed 13 tumors with *FANCF* promoter methylation. Further exploration of the epigenetic silencing of *FANCF* gene will likely provide insight into the etiology of nonfamilial cancers as has been demonstrated for the *BRCA1* gene.

DNA microarray analyses indicate that breast cancers arising in the setting of germline *BRCA1* mutations have unique gene expression profiles, which are identical to sporadic tumors with methylated *BRCA1* and distinct from other types of breast cancer (van't Veer et al., 2002). The observed similarities between *BRCA1*-mutated and some *BRCA1*-methylated sporadic tumors support a tumor progression model in which early loss of BRCA1 causes defects in chromosome structure, cell division, and viability, so that a *BRCA1*-deficient cell must acquire additional alterations that overcome these problems and presumably force tumor evolution down a limited set of pathways (Venkitaraman, 2002). While it is not clear that methylation of *FANCF* will have similar effects as *BRCA1*, a model of carcinogenesis involving the FA-BRCA pathway can be proposed in which promoter methylation can serve as a "first hit" in the *BRCA1* or *FANCF* genes just as inherited mutation can serve as a "first hit" in the *BRCA1* and *BRCA2* genes (Figure 1B). In cases where the first copy is methylated, the second copy may be inactivated by LOH or methylation.

Taniguchi et al. (2003) also provide further evidence that, as is the case with deleterious point mutations and gross chromosomal deletions, aberrant promoter methylation is associated with a loss of gene function that can provide a

selective advantage to transformed cells. However, in contrast to the clonal evolution associated with genetic mutations, promoter CpG island hypermethylation is a more gradual and progressive process (Jones and Baylin, 2002). The process can vary between individual DNA strands and between cells, creating methylation heterogeneity even in long established cell culture. Because the degree of transcriptional silencing is usually dependent on the density of methylated CpG sites in the island, this methylation heterogeneity can lead to heterogeneous populations of cells with varying levels of gene expression and different properties (Figure 2). This phenomenon could, in part, explain why OAW42 cell line that has 54% of its CpG sites methylated is partially CDDP sensitive while 2008 cell line that is 95% methylated is CDDP hypersensitive. Likewise, while initial methylation of *FANCF* followed by *FANCF* demethylation could be one explanation for the observed CDDP resistance in C13* cell line, methylation heterogeneity might also explain the phenomenon. It is conceivable that CDDP eradicated cells with the highest degree of methylation, leaving an outgrowth of a population of cells that are nonmethylated.

This study raises important questions regarding the use of demethylating agents in the treatment of ovarian cancer. We do not know nearly enough about the different pathways that are subject to epigenetic silencing, hence clinical trials using these agents should proceed cautiously. Nonetheless, we now have sophisticated techniques to detect de novo methylation, and these tools should prove useful as we develop novel strategies for the early detection, prevention, and treatment of ovarian cancer.

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