BRCA-1 in Sporadic Epithelial Ovarian Cancer: Lessons Learned from the Genetics of Hereditary Disease

□□*Commentary on Quinn et al., p. 7413*

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Individuals with familial breast or ovarian cancer typically inherit a germ-line mutation in either the *BRCA-1* or *BRCA-2* genes, whose protein products are involved in the repair of double-stranded DNA breaks through homologous recombination (1). Tumors that arise in the setting of a BRCA-1 or BRCA-2 germ-line mutation usually show loss of the wild-type allele (loss of heterozygosity). The remaining mutated gene encodes for a protein that is defective in DNA repair, leading to a state of enhanced sensitivity to DNA-damaging agents, such as the platinum analogues. It is thought that this heightened sensitivity to drugs such as cisplatin or carboplatin partly explains the more favorable prognosis of patients with hereditary ovarian cancer when compared with patients who develop this tumor in the absence of a genetic predisposition (2–4).

Genes involved in familial syndromes, such as *Li-Fraumeni* (*p53*), *von Hippel Lindau* (*VHL*), and *retinoblastoma* (*RB*), are often mutated in the sporadic versions of their respective tumors (e.g., breast cancer, renal cell cancer, and retinoblastoma). In these examples, the genetic syndrome serves as a predictive model for the role of a given gene in the development of sporadic disease. Despite the powerful influence that germ-line BRCA-1 or BRCA-2 mutation has on the development of familial ovarian and breast cancers, however, somatic mutation of these genes is almost never found in sporadic tumors. This observation suggests that loss of either BRCA-1 or BRCA-2 function is not required for malignant transformation in sporadic disease, or that loss of function is indeed required, but that it is occurring through mechanisms other than mutation (5).

Several lines of evidence now suggest that the latter possibility may be correct, especially as it relates to the BRCA-1 gene product. The majority of patients (65-90%) with sporadic ovarian cancer have decreased tumor expression of BRCA-1 protein when compared with benign entities such as cystadenomas (6, 7). In addition, many such patients are also found to have low levels of tumor BRCA-1 mRNA expression, often associated with loss of heterozygosity for the BRCA-1 locus (6, 8). This is an important observation because loss of heterozygosity suggests the presence of only one remaining allele that may be inactivated or functioning at a reduced level. In this regard, approximately 15% to 31% of sporadic cases show epigenetic hypermethylation of the BRCA-1 promoter, leading to decreased transcription and, ultimately, decreased protein expression (9). Taken together, these observations suggest that a subset of patients with sporadic disease may be functionally BRCA-1 deficient, in the absence of a BRCA-1 mutation, in some cases due to epigenetic silencing of the remaining gene promoter.

In this issue of *Clinical Cancer Research*, Quinn-O'Brien and co-workers assess the functional and clinical significance of the BRCA-1 pathway in patients with sporadic ovarian cancer (10). The investigators found that reduction of wild-type BRCA-1 mRNA and protein levels in ovarian cancer cell lines induces a state of enhanced platinum sensitivity. This observation suggests that cancer cells deficient in BRCA-1 are unable to effectively repair platinum-induced DNA damage, consistent with the known role of this protein in mediating DNA repair through homologous recombination (1). Conversely, suppression of BRCA-1 mRNA was associated with marked *in vitro* resistance to the taxanes, which are also useful agents in the management of patients with advanced ovarian cancer. This observation supports the idea that BRCA-1 is a regulator of the mitotic spindle assembly and of the G_2-M checkpoint, although the exact mechanism by which BRCA-1 facilitates taxane-induced cytotoxicity remains unclear (11). Furthermore, in 70 patients with sporadic ovarian cancer, the researchers found that low to intermediate levels of BRCA-1 mRNA were associated with improved survival after platinum-based therapy ($P = 0.0017$), whereas those with high levels experienced a nonsignificant trend toward improved survival following taxane-based therapy ($P = 0.12$). Based on these data, Quinn-O'Brien and collegues speculate that down-regulation of BRCA-1 mRNA expression confers an enhanced degree of platinum sensitivity, which may lead to improved clinical outcome in patients with sporadic ovarian cancer. They further suggest that it may someday be possible to use BRCA-1 mRNA levels to select for patients who are most likely to benefit from either platinum or taxane therapy in this disease.

The present work represents an interesting first step in determining the role of the BRCA-1 pathway in sporadic disease, although it is important to acknowledge several difficulties in interpreting the results of this type of study. One problem involves the analysis of BRCA-1 mRNA as a dichotomous as opposed to a continuous variable. Like most metrics in biology, BRCA-1 mRNA expression represents a continuum from low to high, and arbitrary cutoffs are fraught with the potential for bias. For instance, as shown in Fig. 3A of their article (10), the group of patients with high BRCA-1 mRNA levels is very heterogeneous, with many patients showing relatively low mRNA values (some that are very close to those found in the low-intermediate groups). We are not told how the cutoff was

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chosen between the low-intermediate and the high groups, but this is obviously of critical importance in determining whether we are observing a finding of biological significance or perhaps a statistical artifact related to how the groups were defined. In other words, was the cutoff chosen because it happened to provide the most impressive survival split or was it chosen based on a biological or functional rationale (e.g., a significant change in protein expression between the two groups)? How stable would the survival split be if another arbitrary cutoff was used? Because mRNA levels are being used as surrogates for protein expression, are the researchers convinced that there is good correlation between mRNA and protein expression in their patient cohort? In view of these concerns, it would be important to confirm these observations in a separate set of patient samples that were not used in the original analysis. This type of independent validation would provide confidence that the investigators' dichotomous definition of high versus low-intermediate BRCA-1 mRNA is clinically relevant.

Another challenge in doing these types of studies involves sample selection. The investigators chose 70 patient samples, but we do not know the period during which the samples were collected. In fact, the majority of the patients in this study (66%) received single-agent platinum or platinum in combination with cyclophosphamide, which are older regimens used before the mid-1990s (i.e., before the realization that combination platinum plus taxane therapy was superior). Is it quite possible, therefore, that a significant number of samples in this study were collected over a decade ago? Do older tissue samples have a higher chance of containing degraded RNA that might interfere with accurate quantitation? In this regard, Table 2 of their article shows that patients who received non–taxane-containing chemotherapy (and who therefore may represent an older set of samples) were enriched for low levels of BRCA-1 mRNA compared with their taxane-treated counterparts (10). There is no biological or clinical reason to account for such an imbalance in mRNA levels, thus raising a technical concern over the ability to accurately measure BRCA-1 mRNA in older tissue samples. It is difficult to know how the clinical outcome of this study might be affected by this potential bias.

Assuming that the relationship between BRCA-1 mRNA and survival is confirmed in an independent patient cohort, it is tempting to assume that the mechanism for this association involves heightened sensitivity to platinum analogues. The results of the researchers' cell line studies and the results of their clinical outcome analysis, however, do not prove that the same mechanism is at work in each case. In fact, the investigators do not formally show that low BRCA-1 mRNA levels are predictive of response to platinum in this patient cohort (as defined by standard clinical or serologic response criteria). With overall response rates of 70% to 80% with combination platinum and taxane chemotherapy, a large study would be needed to address this question with sufficient statistical power. The other possibility (which is not mutually exclusive) is that low BRCA-1 mRNA levels are associated with a more indolent natural history of the disease and therefore function as a classic prognostic factor. Many of us are impressed that patients who develop ovarian cancer in the setting of a BRCA-1 or BRCA-2 germ-line mutation can live with recurrent disease for many years, not necessarily because their tumor might remain sensitive to subsequent chemotherapy, but because it can grow very slowly. This distinction is not simply academic because the investigators propose that one day it may be possible to use BRCA-1 mRNA levels to select patients who might derive the most benefit from platinum-based chemotherapy. To do this with confidence, we must be certain that the survival relationship observed in Figs. 3B and 4A (10) is due to a predictive, rather than a prognostic, effect of BRCA-1 mRNA. With respect to using levels of BRCA-1 mRNA to predict which patients might benefit from taxanes, the situation is even more complicated. It must be kept in mind that the researchers have failed to show a statistically significant relationship between clinical outcome and taxane use in patients with high tumor levels of BRCA-1 mRNA (Fig. 4B; ref. 10). We must be careful to avoid overinterpreting a non-significant trend in clinical data even if it satisfies our preconceived notion of how the data should look based on the results of cell line studies.

These issues aside, the observation of Quinn et al. is intriguing because it suggests that a subset of patients with sporadic, non–BRCA-1-mutated disease may have a "BRCA-like" physiology. In addition to measurement of BRCA-1 mRNA, it may also be possible to identify such patients based on their tumor's BRCA-1 protein levels (7) or by defining a BRCA-like gene expression profile using microarray analysis (12). Thus, treatments that are particularly effective for patients with hereditary ovarian cancer may eventually be more broadly applicable to selected patient with BRCA-like, sporadic disease. Indeed, such a treatment may already exist in the form of a small-molecule inhibitor of poly(ADP-ribose) polymerase, which is showing promising activity in clinical trials of patients with recurrent hereditary breast or ovarian cancer (13). The study of Quinn and co-workers gives us hope that someday it may be possible to more accurately select patients who may benefit from such strategies, including those with the sporadic form of the disease. Whether by mutation, epigenetic hypermethylation of the promoter, or through other mechanisms not yet fully defined (14), it seems that many roads are leading us down the BRCA pathway in patients with epithelial ovarian cancer.

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