Perspective

Cancer Prevention Research

One-Hit Effects and Cancer

Perspective on Bellacosa et al., p. 48

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Abstract

This perspective on Bellacosa et al. (beginning on p. 48 in this issue of the journal) discusses the important biology of microscopically normal tissues in carriers of germ-line *BRCA1* or *BRCA2* mutations. The work of Bellacosa et al. is an important step toward discerning which pathways may be altered when one *BRCA* allele is inactivated. *Cancer Prev Res; 3(1); 12–5. ©2010 AACR.*

Breast cancer is the most common lethal cancer experienced by women in developed countries, affecting one in eight women during her lifetime. U.S. estimates for 2009 include 192,370 new diagnoses of and ~40,170 deaths from breast cancer (1). Although many factors are implicated in the initial steps of breast cancer development, a significant family history is the most important factor after gender and age. Despite the discovery of multiple breast cancer susceptibility tumor suppressor genes, such as *BRCA1*, *BRCA2*, *PTEN*, *TP53*, *CDH1*, *CHEK2*, and *PALB2*, only ~15% of the number of cases in familial clusters of breast cancer can be explained by strongly or moderately penetrant single-locus susceptibility genes. Therefore, large numbers of familial cases of breast cancer remain unexplained, presenting a challenge for practitioners attempting to counsel family history patients in cancer genetics clinics.

An estimated 5% to 10% of all breast cancers arise in the setting of inherited mutations in *BRCA1* or *BRCA2* and are presumed to be the result of these factors (2). Mutations in either of these genes occur in <50% of families with evidence of possible autosomal transmission of inherited susceptibility (3). Discovered nearly 15 years ago, germ-line mutations in the *BRCA1* and *BRCA2* genes in women (4–6) confer a breast cancer risk that is 10- to 20-fold higher than the risk of women without these mutations. *BRCA1* and *BRCA2* mutation carriers also have an increased risk for ovarian cancer and, to a mild degree, for gastrointestinal, prostate, pancreatic, and male breast cancers. At age 70, the risks of breast and ovarian cancer are 57% and 40%, respectively, for *BRCA1* mutation carriers compared with 49% and 18%, respectively, for *BRCA2* mutation carriers (7). *BRCA1* and *BRCA2* play an important role in double-strand break repair through the homologous recombination pathway and are considered to be tumor suppressor genes.

The first description of oncogenic alterations to tumor suppressor genes came more than 40 years ago from Alfred Knudson, who based the description on epidemiologic studies of retinoblastoma. Knudson postulated that multiple damaging events in DNA (called "hits") are necessary to cause retinoblastoma. Children diagnosed with inherited retinoblastoma carry the first oncogenic event as an inherited or *de novo* germ-line mutation in *RB*, called the "first" hit. The second DNA injury could affect any somatic cell and would be acquired. If this second "hit" affected the wild-type allele of the locus, it could rapidly lead to cancer because both copies of the normal *RB* tumor suppressor gene would be lost. In contrast, nonhereditary retinoblastoma is poised to arise when two somatic *RB* mutations occur in the same cell of susceptible tissue (8).

The model based on these observations of Knudson is called the "two-hit hypothesis"; it illustrates how inherited and somatic mutations might collaborate in carcinogenesis. According to the two-hit hypothesis, individuals carrying a germ-line mutation in one copy of either *BRCA* gene would require just one additional mutation in the same gene in an ovarian or breast cell for tumor initiation. A major question raised by the simple two-hit model is whether a somatic hit, or mutation, to *BRCA1* or *BRCA2* is more likely to occur in cells harboring a first-hit germ-line mutation (versus nonmutant *BRCA* cells) because these cells have subtle changes in DNA structure or other features making their DNA more vulnerable, although such tissues are phenotypically "normal" under light microscopy.

In this issue of the journal, Bellacosa et al. (including Knudson; ref. 9) have analyzed the gene expression changes within normal epithelial breast and ovarian cells from individuals harboring the "one-hit" event of a germ-line mutation in *BRCA1* or *BRCA2*, comparing these expression patterns with those of cells derived from nonmutation carriers. This elegant work comprises several important findings and implications.

First, phenotypically normal epithelial breast and ovarian cells from individuals harboring a germ-line mutation in *BRCA1* or *BRCA2* expressed an altered mRNA repertoire compared with that of normal cells from individuals without mutations. Different gene expression patterns

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were observed between *BRCA1* and *BRCA2* mutant cells and between breast and ovary cells. For example, breast cells with one mutated *BRCA1* allele have several highly upregulated genes in the secretoglobin family, such as mammaglobin (upregulated up to 3-fold) and lipophilin B and C (upregulated up to 12-fold) compared with *BRCA1* wild-type cells. In contrast, in breast cells with one mutated *BRCA2* allele, insulin-like growth factor binding protein 5 was upregulated 10-fold (compared with *BRCA2* wild-type cells). In mutant *BRCA1* ovarian cells, the cyclin B1/cdc2 complex was downregulated 5-fold, whereas mutant *BRCA2* ovarian cells had upregulated cyclooxygenase-1, compared with wild-type cells. These findings suggest that cancer progression may follow divergent pathways in these tissues and does so even before the second hit occurs. These differences suggest that tissue-specific expression changes may contribute, in part, to differences between *BRCA1* and *BRCA2* mutations with regard to their frequency and penetrance in cancer of the breast or ovaries. Furthermore, the presence of some first-hit effects in cancers associated with germ-line *BRCA* mutations suggests that these effects themselves can contribute to carcinogenesis. These issues also may relate to differences in penetrance between different germ-line *BRCA1* mutations or different *BRCA2* mutations.

Second, this work is important because it reaffirms the concept that some cancer markers are differentially expressed and may thus be important clues to the specific pathways involved in each specific cancer. The published data on the relationship between the secretoglobin family and breast cancer have raised controversy. Watson et al. (10) were first to report high expression levels of mammaglobin in breast cancer patients and to postulate that mammaglobin is a potential serum marker for breast cancer. Further work by Bernstein et al. (11) was concordant with the report of Watson et al. A recent report by Sjodin et al. (12), however, does not confirm this finding for either mammaglobin or lipophilin B. They showed that breast cancer tissues had lower expression levels of mammaglobin and lipophilin B in comparison with nonneoplastic breast tissues. Moreover, they reported high variability in the expression of mammaglobin and lipophilin B among the nonneoplastic samples, suggesting the need for caution in evaluating these molecular expression levels in tumors. Therefore, it seems that further investigation is warranted to clarify how these differing results might be reconciled or explained before the genes in question are considered further as serum markers.

Third and perhaps most important, the differentially expressed genes represent an intriguing first step toward identifying early molecular changes that could be useful targets for chemoprevention. The next step could be using these genes as eligibility markers in well-controlled trials. Furthermore, this work has special appeal because it may lead to novel "druggable" chemoprevention targets applicable to individuals at the highest risk for breast and ovarian cancer; such targets would be one of the most important advances in this field since *BRCA1* and *BRCA2* were cloned.

Bellacosa et al. also found that some of the genes enriched in mutant *BRCA1* one-hit cells are expressed in stem and progenitor breast cells. This finding is consistent with recent results of our group, showing that *BRCA1* is an important regulator of breast stem and progenitor cell differentiation (13). It also extends recent studies of Lim et al. (14), indicating that breast tissue obtained from *BRCA1* mutation carriers is enriched in luminal progenitor cells. These findings foreshadowed the present results of Bellacosa et al., showing that gene expression profiles of breast tissue from mutant *BRCA1* carriers are enriched for genes reported to be significantly expressed in stem cells (15).

It is important to highlight the concept of "BRCAness" tumors, which are sporadic tumors that share certain phenotypic, molecular, and cellular markers with familial mutant *BRCA* cancers (16). The identification of the BRCAness phenomenon has paved the way for testing drugs originally targeting hereditary cases in clinical trials in patients with sporadic BRCAness tumors, who also have a poor prognosis. If the one-hit effects discussed here were to be identified in BRCAness or other sporadic breast cancers, it might influence their clinical management.

The results of Bellacosa et al. (9) further affirm the concept arising within other hereditary syndromes that heterozygosity for a mutant tumor suppressor gene may alter the expression profile of phenotypically normal epithelial cells in a tissue-specific manner. Other hereditary cancer syndromes with reported one-hit effects include familial adenomatous polyposis involving heterozygosity for *APC* germ-line mutations (17–20), tuberous sclerosis gene family, and von Hippel-Lindau disease (21). The common conclusion of all these studies is that heterozygosity for a mutant tumor suppressor gene alters the expression profile of normal colonic and other epithelial cells. These studies are expected to help us in understanding the process of hereditary tumorigenesis and, it is hoped, in further integrating our understanding of sporadic malignant transformation and tumor progression.

An important concern over the work of Bellacosa et al. is that establishing normal epithelial breast and ovarian cells in culture requires a major extrinsic manipulation, giving rise to possible artifacts of gene expression. Before this limitation can be set aside, it will be critical to confirm that the differences between *BRCA1* and *BRCA2* mutant cells and control cells in culture are already present *in situ*. Otherwise, the possibility that the differences in expression in culture are due to greater ease in immortalizing *BRCA1*- and *BRCA2*-haplodeficient than non–*BRCA1*- and non–*BRCA2*-haplodeficient cells will remain a nagging concern.

Although microarray technology, as used by Bellacosa et al., is widely accepted for assessing global gene expression under differing biological conditions or at different time points or in representing tumor behavior, its limitations still must be taken into account when contemplating the next steps of its use in patients. Gene expression profiling derived from Affymetrix microarrays shows the amount of each mRNA species present, but very often, depending on the gene structure and regulation, these mRNA levels do

not correlate with levels of active, properly localized proteins or with quantities of the metabolites they produce. The major use of microarray technology remains as a stimulus toward detailed biological experiments or biological model systems designed to discern the cellular function of altered gene expression patterns. Microarray results first must be validated by complementary methods including real-time reverse transcription-PCR, which is the most commonly used and was used by Bellacosa et al. for this purpose. Another higher-level concern is that an original analysis and validation by the same group will introduce intrinsic biases that lead to the conscious or unconscious selection of the best-performing pair of training validation data and analytic mode (22); such bias is frequent.

All global genome studies are plagued by the scourge of the effect of the "square root of *n*," namely, that the number of statistically significant associations that could occur by chance alone will equal the square root of the number of genes (*n*) being analyzed in two samples (23). Bellacosa et al. included only 6 biological replicates per group (6 *BRCA1* mutant, 6 *BRCA2* mutant, and 6 controls), amounting to 18 samples each from breast and ovarian epithelial cells. Pawitan et al. (24) formally analyzed the relationship between the false discovery rate and sample size in a realistic situation involving the 200 (of 2,000) genes most differentially expressed (2-fold) between two groups of five patients each. The false discovery rate was 91%, indicating that a staggering 182 of the 200 selected genes were actually false positives. Therefore, 56 patients per group would have been needed to reduce the proportion of false positives to the usual 5% level. Cases of small-fold differences in expression or small numbers of truly differentially expressed genes require a much larger sample size (versus that used here) to avoid hindering the identification of truly relevant genes.

Individuals harboring a germ-line mutation in a known breast cancer susceptibility gene stand to benefit greatly from chemopreventive drugs and lifestyle strategies aimed at cancer risk reduction. Compounds that interfere with the early stages of cancer formation are especially appropriate for mutation carriers. Indeed, the evidence indicates that risk reduction strategies focused on individuals harboring germ-line mutations are effective (25, 26). For instance, tamoxifen reduced the incidence of breast cancer by 62% among *BRCA2* mutation carriers, although the same effect was not observed in *BRCA1* mutation carriers (27). In addition, a recent case-control study including 3,223 *BRCA1* and *BRCA2* mutation carriers showed a significant reduction in the risk of ovarian carcinoma with the use of hormone contraception in both *BRCA1* and *BRCA2* mutation carriers (28).

The work by Bellacosa et al. (9) points to three major new directions with the potential to advance cancer prevention. First, the molecular effects associated with the first hit (germ-line mutation) can help in understanding the carcinogenic process. Second, the provocative data suggesting different one-hit effects in different tissues from the same individual point to the importance of studying normal tissues of various sites in individuals with germ-line syndromes. Third, molecular identification of one-hit effects may identify new markers of cancer risk and targets for cancer prevention.

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No potential conflicts of interest were disclosed.

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