One-Hit Effects and Cancer

Perspective on Bellacosa et al., p. 48

Maria D. Iniesta, Janet Chien, Max Wicha, and Sofia D. Merajver

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.
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 "BRCA1ness" tumors, which are sporadic tumors that share certain phenotypic, molecular, and cellular markers with familial mutant BRCA cancers (16). The identification of the BRCA1ness phenomenon has paved the way for testing drugs originally targeting hereditary cases in clinical trials in patients with sporadic BRCA1ness tumors, who also have a poor prognosis. If the one-hit effects discussed here were to be identified in BRCA1 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

Burroughs Wellcome Trust, Breast Cancer Research Foundation, and NIH R01-CA77612 (S.D. Merajver) and Alfonso Martin Escudero Foundation Fellowship (M.D. Iniesta).

Received 11/13/09; revised 11/21/09; accepted 11/23/09; published 1/5/10.

References

One-Hit Effects and Cancer

Maria D. Iniesta, Janet Chien, Max Wicha, et al.


Updated version
Access the most recent version of this article at:
http://cancerpreventionresearch.aacrjournals.org/content/3/1/12

Supplementary Material
Access the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2010/01/05/3.1.12.DC1

Cited articles
This article cites 27 articles, 11 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/3/1/12.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.