Abstract

Many patients develop cancers that have clinical features of inherited syndromes (e.g., young age of onset and unique pathology) but lack mutations in the genes characteristic of the disease. In this issue of the journal, Wong et al. report that somatic epigenetic inactivation could explain some such cases in the setting of BRCA1-associated breast cancer. Here, we discuss the implications of this work in terms of the etiology, risk, and potential prevention of cancer.

Identification of high-risk populations is a research priority in cancer prevention. Genetic predisposition to cancer development is an obvious marker for risk, and several interventions have been shown to reduce cancer mortality in this group of patients. It remains puzzling, however, that despite more than 2 decades of research into mutations in the genes most commonly responsible for inherited cancer, such mutations are not found in a large number of cases that share clinical criteria for having inherited cancer (young age at onset, specific pathology, etc.; ref. 1). For many years, this absence of mutations was easy to explain away—our knowledge of cancer genetics was thought to be fragmentary, and these puzzling cases were assumed to carry mutations in genes yet to be identified. However, large-scale sequencing efforts in major human malignancies such as colorectal cancer (2) have not uncovered previously unknown high-frequency mutations. We are left, therefore, with the somewhat unsatisfying hypothesis that rare mutations in multiple genes or a combination of alterations in several genes that act collectively underlie the many cases of inherited cancer with no known cancer-associated mutation.

In this issue of the journal, Wong et al. (3) present data supporting an alternative concept: that is, constitutional epigenetic defects can account for a significant proportion of cancers with the clinical/pathologic features but not the characteristic mutations of inherited cancers. This study was conducted in 255 female breast cancer patients without an identified germline BRCA1 (or BRCA2) mutation and 169 age-matched female controls. The breast cancers in the cases were required to share certain features of inherited BRCA1-associated disease (onset at a young patient age (<40 years), and aggressive pathology). The patients were assessed for 9 tumor pathology features deemed to be characteristic of cancers in BRCA1 mutation carriers and then were divided into 3 groups, as follows: 5 or more characteristic features (group 1), 4 features (group 2), and 3 or fewer features (group 3). Thirty-one percent of the patients with the most BRCA1-like tumors (group 1) had detectable BRCA1 methylation in peripheral blood-derived DNA, and the cancers themselves had high levels of BRCA1 methylation. The frequency of peripheral blood BRCA1 methylation was much lower (3%) in patients whose cancers had fewer BRCA1 features (group 3), and the non–BRCA1-like cancers themselves had low levels of BRCA1 methylation. There was an overall decreasing gradient of BRCA1 methylation from group 1 to group 3, and this methylation even correlated with the number of BRCA1-like pathology features within group 1. The relatively high proportion of alleles affected in the peripheral blood of patients in group 1, and the presence of this methylation in a few control women without cancer makes it unlikely that the BRCA1 methylation was due to circulating tumor cells. Instead, BRCA1 now joins MLH1 and MSH2 in a short list of cancer predisposition genes that can be methylated rather than mutated in the tissues of some patients whose tumors have features of inherited cancer (4). It is quite interesting that peripheral blood BRCA1 methylation was not found in relatives (even those with a breast cancer history) of study patients with peripheral blood BRCA1 methylation but was found in up to 3.6% of controls. Future studies should address whether women with this methylation are indeed at an elevated risk of cancer development. It will also be important (though difficult) to verify that normal cells carrying methylated alleles indeed have lower expression of BRCA1.

The approach of Wong et al. to the definition of BRCA1-associated breast cancers is novel. An accurate definition remains a major issue, and this research would be significantly limited by an ultimate failure to correctly classify sporadic breast cancers sharing essential characteristics...
negativity correlated significantly with these cancers (5). Wong et al. did find that ER and PR negativity correlated significantly with BRCA1 methylation and that adding ER and PR negativity did not increase their model’s power for predicting BRCA1 methylation. Parallel work in Lynch syndrome has found an epigenetic link with non-germline Lynch syndrome-like tumors (6, 7). Ongoing work is attempting to identify “phenocopies” of BRCA1-associated breast cancers in hopes of distinguishing tumors with intact BRCA1 genes but therapeutic vulnerabilities [e.g., to poly(ADP-ribose) polymerase-1 (PARP1) inhibitors] similar to those of tumors with mutated BRCA1. The potential role of BRCA1 methylation in non-BRCA1 (mutation)-associated breast carcinomas has been difficult to clearly characterize; despite the identification of methylated BRCA1 in larger subsets of basal-like and familial non-BRCA1 (mutation)-associated than in hereditary BRCA1 breast tumor cells (8, 9). Defining the characteristics of BRCA1-associated tumors has been challenging (10); perhaps, the novel classification system used by Wong et al. will help to shed light on this confusion and permit a clearer focus on the role of epigenetic mechanisms. It will be important for future studies to integrate pathology (including the newly discovered predictive weight of high mitotic index and circumscribed growth patterns; ref. 3) with triple-negativity testing and other potential molecular markers (11, 12) to explore the relationship of BRCA1 methylation in peripheral blood to BRCA1-associated breast cancer.

The causes of “constitutional” methylation remain unknown (13). In the case of MSH2, methylation was traced to a genetic defect in an adjacent gene (TACSTD1/EPICAM) that leads to transcription through the MSH2 promoter and eventual DNA methylation–associated silencing of this allele (14). This case is thus an example of a true, albeit indirect, genetic predisposition to cancer due to methylation. In the case of methylated BRCA1 described here, however, no associated mutation has been identified, and several features suggest a primary epigenetic defect (4). The DNA methylation is often incomplete and mosaic, and there seems to be no or very infrequent transmission of the methylated alleles, which is consistent with the reprogramming of the epigenome that occurs early in development. It is likely, therefore, that the observed methylation of BRCA1 is due to a developmental defect, or is acquired postnatally. Indeed, acquired promoter methylation is a feature of aging in epithelial tissues (15), and constitutional methylation may result from similar epigenetic errors during stem cell replication. If this cause and effect is confirmed, it has important implications for cancer causation and prevention at the population level (16). For example, we know from animal studies that maternal diet can have a substantial effect on the genotype of offspring (17). One of the key dietary factors in this regard is folate, a vitamin that is given in large quantities to pregnant women (to reduce the risk of neural tube defects) and as supplemental content within the U.S. food supply as well. Folate supplementation has come under fire recently, with some studies raising safety concerns by showing an association with an increased incidence of and/or mortality from various cancers (18). Furthermore, laboratory research has linked high levels of folate with accelerated age-related DNA methylation (19), leading one to wonder whether diet could have had an influence on the constitutional methylation as reported by Wong et al. (3). Indeed, folate (and diet in general) may be just the tip of an iceberg of epigenetic modulators. Exposure to environmental chemicals can affect DNA methylation in pregnant mice (20), and in vitro fertilization was associated with incomplete epigenetic reprogramming at some loci in children (21), raising the fear that each of these events may lead to constitutional defects such as that reported by Wong et al. in this issue of the journal.

The prevalence of constitutional methylation of BRCA1, MLL1, or MSH2 DNA in patients with cancer is low. At first glance, this low prevalence might suggest that the problem is relatively minor. However, there are several issues that need to be addressed before the real magnitude of constitutional methylation of cancer susceptibility genes is known. The first problem is technical. DNA methylation at low levels (below a 5% absolute level of methylation) has been difficult to measure reliably. Sensitive techniques are fraught with false-positive results, and quantitative techniques have a 5% detection threshold. Wong et al. used 2 separate and complementary techniques to detect low levels of methylation, something that is not yet routinely done. A similar approach in another study revealed constitutional methylation of the DNA repair gene MGMT in 8.9% of patients (22), suggesting that the real frequency of epigenetic defects could be higher than previously estimated. Next-generation sequencing now allows much more precise quantitative DNA methylation measurements (23), and studies with this modern technology will be needed to ascertain the population prevalence of aberrant DNA methylation in apparently normal tissues. The second problem involves target tissues. Aberrant DNA methylation can be substantially tissue-specific, and studies of white blood cells could underestimate the problem posed by this specificity. Readily accessible epithelial tissues (e.g., buccal smears and nipple aspirates) may provide a better source of tissues for constitutional methylation studies. Finally, a sole focus on DNA methylation will almost certainly lead to an underestimation of the role of epigenetic alterations in cancer development. For example, relaxation of insulin-like growth factor 2 imprinting is a constitutional epigenetic defect associated with cancer risk (24) but without reproducible alterations in DNA methylation. Other mechanisms of epigenetic regulation, such as the trimethylation of histone H3 lysine 27 associated with polycomb group protein, are also involved in gene silencing in cancer, often independently of DNA methylation (25). Therefore, the true contribution of developmental or acquired epigenetic defects to cancer will only be known when sensitive
methods to profile multiple epigenetic abnormalities are developed.

It goes without saying that the concept of an epigenetic predisposition to cancer has important implications for cancer prevention. This concept works at the levels both of the specific gene affected and the mechanisms of gene deregulation. In specific systems, BRCA1-deficient cells of the specific gene affected and the mechanisms of gene cancer prevention. This concept works at the levels both predisposition to cancer has important implications for developed.

methods to profile multiple epigenetic abnormalities are developed.

It goes without saying that the concept of an epigenetic predisposition to cancer has important implications for cancer prevention. This concept works at the levels both of the specific gene affected and the mechanisms of gene deregulation. In specific systems, BRCA1-deficient cells of the specific gene affected and the mechanisms of gene cancer prevention. This concept works at the levels both predisposition to cancer has important implications for developed.

....

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
Time to Think Outside the (Genetic) Box

Jean-Pierre J. Issa and Judy E. Garber


Updated version
Access the most recent version of this article at:
http://cancerpreventionresearch.aacrjournals.org/content/4/1/6

Supplementary Material
Access the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2010/12/30/4.1.6.DC1

Cited articles
This article cites 27 articles, 14 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/4/1/6.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerpreventionresearch.aacrjournals.org/content/4/1/6.full#related-urls

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.