Cancer Prevention Research

Perspective

Perspective on Wong et al., p. 23

Time to Think Outside the (Genetic) Box

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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. *Cancer Prev Res; 4(1); 6–8. ©2011 AACR.*

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 (5%) 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

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with *BRCA1*-associated cancers. Many investigators consider triple negativity [negative for the estrogen receptor (ER), progesterone receptor (PR), and HER2 overexpression or amplification] to be the best available definition for 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 pattern; 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*/*EPCAM*) 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 epigenotype 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*, *MLH1*, 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 are more sensitive to certain types of chemotherapy drugs (e.g., PARP1 inhibitors; refs. 26, 27); therefore, individuals with constitutional *BRCA1* methylation could be candidates for chemoprevention trials using such approaches (if the drugs are safe and a high risk for cancer is confirmed). A similar concept could be investigated for potential application to patients with *MLH1* methylation. Separate from this, epigenetic defects are largely biochemical phenomena, raising the prospect of directed epigenetic intervention (16). Indeed, DNA methyltransferase inhibitors can reverse tumor-suppressor gene methylation *in vivo*, and, as discussed earlier, dietary interventions have the potential to affect DNA methylation in the long term. These approaches should certainly be considered for the prevention of cancer in individuals at a high risk based on constitutional epigenetic defects. Thanks to the work of Wong et al. and others (some of whom are discussed here), we can begin to think outside the box of genetic cancer predispositions in exploring new, epigenetic-based avenues for advancing cancer risk assessment and prevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Weitzel JN, Lagos VI, Cullinane CA, Gambol PJ, Culver JO, Blazer KR, et al. Limited family structure and BRCA gene mutation status in single cases of breast cancer. JAMA 2007;297:2587–95.
2. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. Science 2007;318:1108–13.
3. Wong E, Southey M, Fox S, Brown MA, Dowty JG, Jenkins MA, et al. Constitutional methylation of the *BRCA1* promoter is specifically associated with *BRCA1* mutation–associated pathology in early-onset breast cancer. Cancer Prev Res 2011;4:23–33.
4. Hesson LB, Hitchins MP, Ward RL. Epimutations and cancer predisposition: importance and mechanisms. Curr Opin Genet Dev 2010; 20:290–8.
5. Kwon JS, Gutierrez-Barrera AM, Young D, Sun CC, Daniels MS, Lu KH, et al. Expanding the criteria for BRCA mutation testing in breast cancer survivors. J Clin Oncol 2010;28:4214–20.
6. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol 2008;26:5783–8.
7. Hampel H, de la Chapelle A. The Search for unaffected individuals with lynch syndrome: do the ends justify the means? Cancer Prev Res 2011;4:1–5.
8. Wei M, Xu J, Dignam J, Nanda R, Sveen L, Fackenthal J, et al. Estrogen receptor alpha, BRCA1, and FANCF promoter methylation occur in distinct subsets of sporadic breast cancers. Breast Cancer Res Treat 2008;111:113–20.
9. Lee JS, Fackler MJ, Lee JH, Choi C, Park MH, Yoon JH, et al. Basal-like breast cancer displays distinct patterns of promoter methylation. Cancer Biol Ther 2010;9:1017–24.
10. Vargas AC, Silva LD, Lakhani SR. The contribution of breast cancer pathology to statistical models to predict mutation risk in BRCA carriers. Fam Cancer 2010;9:545–53.
11. Tung N, Wang Y, Collins LC, Kaplan J, Li H, Gelman R, et al. Estrogen receptor positive breast cancers in BRCA1 mutation carriers: clinical risk factors and pathologic features. Breast Cancer Res 2010;12:R12.
12. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160–7.
13. Hitchins MP, Ward RL. Favoritism in DNA methylation. Cancer Prev Res 2009;2:847–9.
14. Ligtenberg MJ, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. Nat Genet 2009;41:112–7.
15. Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet 1994;7:536–40.
16. Issa JP. Cancer prevention: epigenetics steps up to the plate. Cancer Prev Res 2008;1:219–22.
17. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 2003;23:5293-300.
18. Ebbing M, Bonaa KH, Nygard O, Arnesen E, Ueland PM, Nordrehaug JE, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B12. JAMA 2009;302:2119–26.
19. Wallace K, Grau MV, Levine AJ, Shen L, Hamdan R, Chen X, et al. Association between folate levels and CpG island hypermethylation in normal colorectal mucosa. Cancer Prev Res 2010;3:1552–64.
20. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A 2007;104:13056–61.
21. Katari S, Turan N, Bibikova M, Erinle O, Chalian R, Foster M, et al. DNA methylation and gene expression differences in children conceived *in vitro* or *in vivo*. Hum Mol Genet 2009;18:3769–78.
22. Candiloro IL, Dobrovic A. Detection of MGMT promoter methylation in normal individuals is strongly associated with the T allele of the rs16906252 MGMT promoter single nucleotide polymorphism. Cancer Prev Res 2009;2:862–7.
23. Estecio MR, Issa JP. Tackling the methylome: recent methodological advances in genome-wide methylation profiling. Genome Med 2009;1:106.
24. Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 2003;299:1753–5.
25. Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet 2008; 40:741–50.
26. Tassone P, Tagliaferri P, Perricelli A, Blotta S, Quaresima B, Martelli ML, et al. BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells. Br J Cancer 2003;88:1285–91.
27. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 2009;361:123–34.

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