Cancer Prevention Research

Perspective

Perspective on Wallace et al., p. 1552

Linking Epidemiology to Epigenomics—Where Are We Today?

Cornelia M. Ulrich[1,3,5] and William M. Grady[2,4]

Abstract

Cancer is the consequence of genetic and epigenetic alterations. Genetic mutations likely result in part from exposure to environmental carcinogens, giving rise to a large field of cancer-prevention study of these carcinogens and ways to develop strategies to avoid them. Our understanding of regulatory epigenetic mechanisms associated with DNA methylation, histone modifications, and microRNA production is increasing rapidly. The involvement of these processes in carcinogenesis raises the possibility that environmental exposures may promote or prevent cancer through affecting the epigenome. Modifying the epigenome to prevent cancer is particularly intriguing because epigenetic alterations are potentially reversible, unlike gene mutations, and because certain dietary factors, such as the B-vitamin folate, may affect genes' DNA methylation status (as reported by Wallace et al., beginning on page 1552 in this issue of the journal). Rapidly improving techniques for assessing epigenetic alterations promise to yield important insights for cancer prevention. *Cancer Prev Res; 3(12); 1505–8. ©2010 AACR.*

Introduction

The importance of genetic alterations to tumor development and progression was established many decades ago. More recently, epigenetic aberrations have been identified as similarly important players in cancer development and progression (1). Our understanding of the many regulatory epigenetic mechanisms associated with DNA methylation, histone modifications, and microRNA production has increased at an astounding pace, and many of these processes have been found to be altered in carcinogenesis (1, 2). Nevertheless, our understanding of the processes mediating cancer-related epigenetic alterations is in many respects still in its infancy.

DNA methylation of cytosines in CpG dinucleotides located in CpG islands is an important regulatory mechanism for gene transcription. Epigenomic changes (which are detected in genome-wide assessments of epigenetic alterations in genomic DNA) in cancers can involve both global DNA hypomethylation, which is present at non-CpG island sites and often at repeat or satellite regions of DNA (perhaps silencing ancient viral gene promoters), and concurrent CpG-island DNA hypermethylation (commonly associated with promoters of tumor-suppressor and other genes). In addition, some tumors are characterized by the CpG island methylator phenotype (CIMP), a molecular subtype characterized by extensive hypermethylation and gene silencing (3, 4). *BRAF* mutations are strongly associated with the CIMP of colorectal cancer. Yet, we have learned little to date about the overall processes that trigger epigenetic disarray in cancer.

Is there a potential role for health behaviors in influencing epigenetic processes and thus for preventing cancer by manipulating the cancer epigenome? Studies among young and older (genetically identical) twin sets suggest that lifestyle factors are responsible for at least some of the epigenetic variability between individuals (5). Geographic differences in worldwide tumor methylation patterns further support the notion that different environmental exposures have varying influences on the epigenetic state of loci in the genome (6). Epidemiologic studies of predictors of abnormal DNA methylation have identified, not surprisingly, age as the major risk factor for gene-promoter hypermethylation (7, 8). They also have pointed quite consistently to smoking as a predictor of methylated genes in cancers, perhaps with an emphasis on smoking in youth, when a high specific susceptibility to environmentally induced alterations of the epigenome may occur (9–11). Heavy metals and pollutants also have been reported in association with certain abnormal methylation patterns (12). Generally these studies have been quite limited, however, because they involved only hypermethylation at a few specific genes (e.g., p.16) rather than a comprehensive and representative assessment of methylation patterns.

Epigenetic signatures are reset during embryogenesis, when the epigenetic state of DNA is particularly modifiable

Authors' Affiliations: [1]Public Health Sciences Division and [2]Clinical Research Division, Fred Hutchinson Cancer Research Center; [3]Department of Epidemiology and [4]Division of Gastroenterology, University of Washington Medical School, University of Washington, Seattle, Washington; and [5]German Cancer Research Center and National Center for Tumor Diseases, Heidelberg, Germany

Corresponding Author: Cornelia M. Ulrich, National Center for Tumor Diseases Heidelberg (NCT), Division of Preventive Oncology, Im Neuenheimer Feld 460, D-69120 Heidelberg, Germany. Phone: +49-6221-56-5528; Fax: +49-6221-56-5231; E-mail: neli.ulrich@nct-heidelberg.de

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or pliable through the effects of environmental factors. Elegant work by Waterland et al. has shown this pliancy by showing that the epigenetic state of transposable elements, which are sequences of DNA that can physically move to different sites in the genome through a "cut and paste" process, can be modified by early nutritional exposures, particularly to folate, in mouse embryos (13, 14). Exposing pregnant females or neonates to folate and other 1-carbon donors, such as, vitamin B12 or choline, can change the methylation state of metastable epialleles (e.g., the A^{vy} allele of the Agouti gene or the Axin gene promoter) and thus dramatically alter the phenotype of newborn mice. Folate exposure (in the mothers or neonates) can change the coat color of mice with the A^{vy} allele by increasing the CpG methylation of the 5′promoter region of the A^{vy} allele from brown to yellow, and folate given to mothers can influence whether the tail is kinked or unkinked in mice with the *Axin* gene promoter by inducing increased methylation of the *Axin* gene promoter. These findings suggest that dietary supplementation has the potential to have unintended harmful consequences (in addition to hoped-for beneficial effects) through altering the epigenetic state of genes.

Why would folate in particular have the potential to influence epigenetic processes? Folate is critical for the synthesis of *S*-adenosylmethionine, the universal donor of methyl groups including for the enzymatic methylation of CpG sites in DNA through the DNA methyltransferases, which are a class of enzymes [including DNA (cytosine-5-methyltransferase 1 (DNMT1), DNMT3a, and DNMT3b] that methylate cytosines in CpG dinucleotides (15). Thus, the role of folate and other nutrients such as, vitamin B12 related to the functioning of folate-mediated 1-carbon metabolism has been studied extensively for their role in modifying the epigenetic state of sites in DNA (15). Moreover, DNA hypomethylation has been linked to low intakes of folate in animal models and several human studies (16–18), and conversely, folic-acid supplementation has been associated with increased global DNA methylation in leukocytes and colonic mucosa among patients with colorectal adenomas (19). Genetically reduced activity of 5,10-methylene-tetrahydrofolate causes decreased methylation capacity, and studies in humans support such an association, particularly for individuals with a low-folate diet (15, 20–22). Gene–environment interactions between DNA methyltransferase polymorphisms and 1-carbon status have been reported in the causation of colorectal adenomas (23). Rodent studies provide direct evidence that early-life dietary supplementation by 1-carbon nutrients can alter DNA methylation patterns in promoter regions, resulting in long-term changes in gene transcription (13, 14, 24). The role of folate status in determining promoter methylation and associated gene silencing in humans is less evident, and studies to date have often been small and rather inconsistent. There is some evidence that CIMP is associated with polymorphisms in 1-carbon metabolism (25), suggesting that long-term altered patterns of this metabolic pathway may be necessary to promote tumors with specific methylation characteristics.

The work reported by Wallace et al. in this issue of the journal (26) provides another important step toward elucidating the influence of folate and other factors on the epigenetic state of the genome and the risk for cancer. They investigated the methylation status of estrogen receptor alpha (*ERα*) and secreted frizzled-related protein 1 (*SFRP1*) in the healthy mucosa of a subset of participants in a large randomized controlled trial of folic acid (the synthetic form of folate), initially designed to test the prevention of the recurrence of colorectal adenomas. These 2 genes were chosen because they tend to be commonly epigenetically silenced in carcinomas and adjacent mucosa. These investigators used the large and well-characterized trial data set to explore CpG island methylation in the colon mucosa in the context of demographic, lifestyle, dietary, and genetic factors as part of a cross-sectional study. A key finding of this work is that there were few predictors of cancer-associated epigenetic alterations. Only increasing age, non-African American race, and folate levels in red blood cells (RBC) emerged as statistically significant predictors. Perhaps this finding attests to the overall stability of DNA methylation patterns at specific locations, or perhaps it suggests that we need yet finer tools to measure DNA methylation. The study also suggests that methylation patterns differ by large-bowel region, with methylation at CpG islands of *ERα* and *SFRP1* more likely in the rectum, which is consistent with several previous studies. The observation of greatest interest is the positive association between promoter hypermethylation and RBC folate, a biomarker of long-term folate status. The linear increase in methylation with higher RBC folate levels corresponds to an approximately 10-year acceleration in age-related methylation in the top quartile of RBC folate versus the bottom quartile. This finding is particularly important in light of concerns over recent high levels of folate ingestion in the United States (27, 28). These levels have been rising during the past decade, in large part because of the increasing use of folic acid–containing supplements including multivitamins and "functional food" items that include folic acid and because of folic-acid fortification mandated in 1998 as a means for raising folate levels in the general population but especially in pregnant women to prevent neural tube defects, which is a devastating class of birth defects (28).

Despite a large body of data showing anticancer effects of folate, recent studies have suggested a more complex effect of folate on cancer initiation and progression. An increasing body of evidence suggests that folate plays a dual role in carcinogenesis, both preventing early lesions and potentially promoting tumors once preneoplastic or neoplastic lesions have developed (28–31). Indeed, the trial population studied by Wallace et al. showed an increased risk of advanced or multiple colorectal adenomas associated with 6–8 years of using folic-acid supplements (29). It has been hypothesized that this increase may be due to undetected precursor lesions in the colon of patients with prior adenomas, suggesting that subclinical tumors or

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microadenomas could grow more rapidly with folic-acid supplementation perhaps because of increased availability of nucleotides (28, 32, 33). This trial also has reported a 2.6-fold increased risk of prostate cancer in the folic-acid arm, further raising concerns about folic-acid fostering the growth of premalignant or malignant lesions (34). Although the potential tumor growth–promoting effects of folic acid are most likely attributable to folate's impact on nucleotide synthesis, the work by Wallace et al. (26) suggests that folic acid also may have a long-term impact on DNA methylation patterns, with unknown consequences. Of note, this study did not report any effects in the treatment arm (folic-acid supplementation) on DNA methylation patterns, only cross-sectional associations; this discrepancy in data raises questions of whether higher doses or longer-term administration of folate may be necessary to produce any effects on DNA methylation, or whether the cross-sectional association between RBC folate and promoter methylation is simply a chance finding or due to other, confounding variables.

The study by Wallace et al. (26) provides additional information about the correlation between folate and DNA methylation but raises as many questions as it answers. The modest correlation that the investigators found between RBC folate and the DNA methylation state of *ERα* and *SFRP1* raises many questions about environmental influences on the methylation state of the epigenome. It is not clear if environmental factors primarily influence the methylation state of genes during early development or what duration and concentration of exposure is needed to induce a change in the epigenetic state of a gene in adults, if indeed they can affect the DNA of adult humans. Furthermore, it is not known if some organs in the body may be more susceptible than others to environmental factors that can alter the epigenome. Also, there is a strong interplay between chromatin structure, which is regulated by histone–DNA interactions, and methylation. It is not known whether alterations in the methylation status of DNA loci require exposure to factors that alter the chromatin structure as well as the DNA methylation state.

Clearly, there is much to be done in the field of epigenetic epidemiology. With the increasing availability of high-throughput and refined tools to measure DNA methylation, we are entering a phase where we can attempt to discern predictors in a comprehensive and contextually meaningful manner (35). Future studies can focus on capturing overall CpG island patterns, rather than the patterns of just a few selected genes. Predictors of both hypermethylation at CpG island sites and of general hypomethylation should be investigated. In addition, studies of epigenetic characteristics other than aberrant DNA methylation (e.g., histone modification states and microRNA expression) are just beginning and may provide important insight into the impact of health behaviors on the regulation of gene expression. The general lack of availability of target tissue, where the changes that really matter in cancer development occur, has imposed an important limitation on the field of epigenetic epidemiology; studies to date generally have had to rely on lymphocytes. The use of colon tissue by Wallace et al. (26) is a major strength of their work. It will be critical to resolve what aspects from studies in lymphocytes are relevant and apply these findings to studies utilizing the many large biorepositories containing lymphocytes from existing epidemiologic study populations. Newly established epidemiologic studies also are addressing the problem of tissue availability by collecting tissues, from buccal scrapes to adipose biopsies, and important discoveries about the epidemiology of cancer are expected from this work. As we progress toward using better assays, novel tools for tissue collection, and creative sample-collecting modalities, research on epidemiologic predictors of cancer-associated epigenetic alterations is bound to provide important clues to the puzzle of cancer-associated epigenetic dysregulation over an individual's lifetime and may help identify new cancer prevention strategies.

Disclosure of Potential Conflicts of Interest

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