Colorectal cancer (CRC) is characterized by accumulating genetic and epigenetic alterations affecting oncogenes and tumor suppressor genes involved in critical pathways of cancer initiation and progression. Epigenetic alterations include DNA methylation, histone modifications, nucleosomal remodeling, chromatin looping, and noncoding RNAs and occur early and frequently in CR carcinogenesis (1). Global DNA hypomethylation, the depletion of overall 5-methylcytosine content, is the first epigenetic alteration that was recognized in CRC, occurring gradually, age-dependent, and early in CR carcinogenesis (2, 3). It occurs predominantly at CpG dinucleotides in repetitive sequences, retroviral elements, imprinted loci, and CpG islands and shores (4, 5). Observational and experimental studies show that hypomethylation is associated with aging (3), chromosomal instability, and microsatellite stability and that it induces early-stage CRC and inhibits late-stage CRC. Although observed early in carcinogenesis, hypomethylation is also associated with tumor progression.

Global hypomethylation is accompanied by hypermethylation of specific promoter CpG islands of tumor suppressor and DNA repair genes, which is associated with repressive chromatin alterations and transcriptional silencing. Although all CRCs are characterized by the presence of hypermethylation, a specific subgroup, the CpG island methylation phenotype (CIMP), displays more extensive promoter CpG island methylation of specific loci (6, 7). CIMP CRCs are associated with older age, female sex, proximal location in the colon (right-sided), mucinous differentiation, smoking, and the specific genetic alteration microsatellite instability mediated by hypermethylation of MLH1. CIMP tumors also more commonly have BRAF mutations and are hypothesized to arise specifically through the serrated pathway involving hyperplastic polyps and serrated adenomas. Although hypo- and hypermethylation are considered fundamental epigenetic alterations in CRC, and the affected pathways and the downstream consequences for CR carcinogenesis continue to be identified, the causes of aberrant methylation in CRC (and other malignancies) are still unclear. Since folate is a source of the methyl group added to DNA-creating 5-methylcytosine, the potential of folate exposure to alter DNA methylation is both logical and the subject of many previous studies.

The work reported by Wallace et al. in this issue of the journal (beginning on page 1552) provides an opportunity to examine the epigenetics of CR carcinogenesis through the prism of folic acid effects (8). Wallace et al. examined DNA methylation in the promoter region of 2 genes in colonoscopic biopsies of colonic mucosa collected at a predetermined time point from subjects in a prospective randomized chemoprevention trial of aspirin and folic acid for reducing colon cancer (9). The primary finding of this analysis is a small, but statistically significant, increase in
DNA methylation at CpG sites in the 5’ region of the genes estrogen receptor alpha (ERα) and secreted frizzled-related protein 1 (SFRP1) associated with higher levels of folate in red blood cells (RBC vs. low levels); methylation did not differ in relation to other measures of folate exposure (e.g., plasma folate or supplemental intake). Should we have expected that folate taken in the trial to reduce colon cancer risk would increase promoter-region methylation, a process that may play a role in the development of this disease? Probably not, but the mechanisms through which folate can alter DNA methylation are complicated, and the investigators of the well-designed parent prevention trial certainly did not hope for the results they saw: no reduction in polyp formation and an actual increase in more-advanced colon lesions with the folate intervention (9). The trial results raise questions about the mechanism of action of folate in colon carcinogenesis and at first seem not to fit the protective role of folate intake suggested by many observational studies (discussed later). They also raise concerns for the safety of folate supplementation in patients at risk for colon cancer.

As well stated in the title of the editorial accompanying the primary publication of the prevention trial of folate, “Timing is everything” (10). The issue of timing provides one explanation for the discordance between the effects of folate early in the development of tumors, where the protective effects may reduce the incidence of tumors, and effects later in the development of neoplasms, where folate can stimulate growth and thus increase tumor formation (10). Rather than drawing the simplistic conclusion that early and later neoplasms are just different, however, we should focus on how these somewhat surprising results in both the chemoprevention trial (9) and the subsequent analysis of DNA methylation (8) may help us to understand the mechanisms that might lead to tumor formation in the colon.

Life style and diet play a crucial role in CRC etiology and are currently being investigated for their role in causing aberrant DNA methylation (11). The B-vitamin folate is present mainly in green leafy vegetables, fruits, dairy products, meat, bread, and potatoes, and its role in CR carcinogenesis is interesting since it provides 1-carbon groups for cellular metabolism, especially DNA biosynthesis, repair, and methylation. Contradictory results have come, however, from observational, in vitro, and in vivo experimental studies of the role of folate, DNA methylation, and CRC mainly because of differences in study designs, populations, and endpoints and in folate intakes/levels (12). In rat and mouse models, for example, folate deficiency was associated with increased formation of colon neoplasms (13), whereas high folate levels suppressed intestinal carcinogenesis (14, 15). On the other hand, an inhibitory effect of folate deficiency and a promoting effect of folate supplementation were observed in animals with established neoplasms (15). Human trials of supplemental folate show that it variously has a protective role (16), null associations (17), or associated higher risks (9, 18) with regard to CRC development. The observation of a significant trend toward increasing CRC incidence coincident with the fortification of grain products with folic acid in the United States and Canada (19) is intriguing. These data all point to a possible dual role of folate in CR carcinogenesis, that is, protecting against neoplasia in normal CR mucosa but enhancing the growth of existing premalignant lesions.

Wallace et al. (8) explore the role of changes in the DNA-methylation level of ERα and SFRP1 in normal-appearing colon biopsies in the context of a chemoprevention trial (9) of folic acid (and aspirin). These genes are good choices for detecting variation since they are frequently methylated in colon cancer and in early premalignant colon epithelial cells. It remains unknown whether the changes seen at the 8 specific CpG sites examined in these genes reflect changes at other loci, but it is a reasonable assumption. The large number of biopsies, the quantitative nature of the pyrosequencing, and the detailed clinical information available for this study allowed the authors to examine numerous factors that might influence methylation levels at ERα and SFRP1. Many other influences that potentially alter colon cancer risk, including body mass index, smoking, alcohol intake, and race, were not associated or consistently associated with differences in DNA methylation at these loci. In contrast, other parameters, notably age, confirm previous findings of an association with increases in DNA methylation. Given the examination within this study of numerous dietary intakes (alcohol, fat, carbohydrate, total dietary fiber, protein, and dietary folate) for potential associations with differences in methylation, one should interpret the significant differences for this single measurement (folate in RBCs) with caution, considering multiple testing concerns. The primary focus of the trial, however, was on whether aspirin or folic acid treatment altered the development of colon neoplasia, and the finding that neither treatment was associated with differences in gene methylation is disappointing. This lack of association involved a straight overall comparison between the group randomized to receive folic acid or aspirin and its respective placebo group; a small but statistically significant difference, however, occurred in the level of methylation at these loci in association with RBC folate level.

What does this study tell us about the influence of folate on DNA methylation and on the genesis or prevention of colon carcinoma? First, it provides insight into the complexities of folate exposure, where dietary intake is difficult to quantify, and even supplementation, which would seem to be the simplest way to assure differences among individuals, may not be a good measure of exposure. This difficulty in assessing folate exposure may be due in part to genetic diversity among individuals, with known genetic variation in enzymes involved in 1-carbon metabolism, such as methylenetetrahydrofolate reductase (MTHFR). Indeed, there is good evidence to support an interaction of genetic variation of MTHFR, particularly MTHFR 677,
with differences in promoter-region DNA hypermethylation in colon (20–22) and other tumors (23, 24) and with differences in global hypomethylation in lymphocytes, a potential surrogate for exposure (25, 26). Some studies of interactions between variants of MTHFR and DNA methylation have integrated folate intake (21) or levels (22), suggesting that genetic variation may have modulated the interaction between folate exposure and DNA methylation seen by Wallace et al. or in previous studies (27). There is great complexity, however, in these interactions. For example, investigators of the European Prospective Investigation into Cancer and Nutrition (EPIC; ref. 28) recently reported no association of plasma folate levels with CRC risk in the entire EPIC cohort but did report variation in plasma folate according to MTHFR 677 variation and evidence for increased CRC risk at the lowest levels of folate in patients with the MTHFR 677T phenotype (28). And so although Wallace et al. report no significant association of MTHFR polymorphisms with methylation of either ERα and SFRP1, they do not indicate that they assessed the effect of MTHFR on folate level, which might connect these variables (8). The EPIC finding of an interaction between variation in MTHFR, low folate, and CRC risk (28) provides one potential explanation for the failure of dietary folic acid to alter DNA methylation in the cohort studied by Wallace et al., that is, that folate supplementation may be differentially effective (either protective or harmful) in patients with different rates of 1-carbon metabolism, but perhaps more important, that much of the difference in risk according to variation in folate is seen at the lowest levels of or exposure to folate (28).

Therefore, it is interesting that the subset of patients Wallace et al. chose for analysis of DNA methylation had statistically lower intake, lower plasma levels, and lower RBC levels of folate than did the entire cohort (8). Although no reason was provided for this selection, which was described as a “convenient sample of 1,000,” it might have allowed the authors to observe variation in DNA methylation that would not have been seen, or perhaps would have been even more subtle, in patients with higher folate. Most people in the general population of developed countries may have sufficient folate such that slight dietary folate variation or folic acid supplementation might not alter patterns of DNA methylation and tumor risk. At low folate exposure determined by intake (27) or measured in plasma (28) or RBCs (8), however, DNA methylation may be more significantly altered and may be most important for colon cancer development.

Do the small changes in DNA methylation detected at the specific loci reported by Wallace et al. have any relevance for colon cancer predisposition? This question arises because the authors observed no significant association between the level of ERα or SFRP1 methylation and risk of adenoma or hyperplastic polyps. Perhaps the study was underpowered for this endpoint, but another explanation is that ERα and SFRP1 are not likely to be the sole, or even the most important, epigenetic silencing events in colon cancer initiation and progression. They may provide a sensitive marker, however, of a process that is ongoing throughout regions of the colon and at multiple loci, which together are associated with the development of CRC. The differences in methylation among these colonic biopsies seem to be very small, with the mean methylation at ERα varying from 10.3% (in the lowest RBC folate quintile) to 11.3% (highest quintile) and at SFRP1 varying from 21.2% (lowest quintile) to 23.0% (highest quintile). Although statistically significant trends, these variations are small in comparison with age-associated differences (increases in methylation of 1.7% per decade for ERα and 2.9% per decade for SFRP1). Thus, increases in methylation occurring during the 3-year intervention period may have approached the variation reported for differing RBC folate levels, although baseline biopsies were not examined to directly answer this question. Of perhaps greater importance is the variation according to biopsy site, where mean methylation varies from 9.6% to 12.1% for ERα and 20.8% to 23.3% for SFRP1, with the higher level of methylation observed in rectal or left-sided biopsies. These larger differences place the relatively small variation according to RBC folate in perspective and raise concerns of confounding since the multivariate analysis (Table 5 of Wallace et al.) adjusted for age and other factors but not for biopsy location. The difference in location is of interest and perhaps surprising since, whereas DNA methylation is common to tumors arising in the right and left sides of the colon, methylation is generally more frequent in tumors arising on the right side, which are associated with CIMP (discussed earlier). Therefore, a higher level of methylation in left-side mucosa than right-sided mucosa is not expected.

In conclusion, the study of Wallace et al. sheds new light on the epigenetics of CR carcinogenesis and, in the end, provides some additional support for the role of folate in colon cancer by showing an association between RBC folate and gene-specific promoter-region methylation in colonic tissues. This role is not simple, however. We will need a better understanding of several aspects of the complex biology of colon cancer including the multiple mechanisms through which folate could alter the genome, the interaction between the timing of folate exposure, the level of folate intake, and modification of folate levels by genes including MTHFR, and potentially different folate effects by in parts of the colon before we will fully understand how folate affects CR carcinogenesis.

Disclosure of Potential Conflicts of Interest

J.G. Herman is a consultant to MDxHealth. Dr. van Engeland declared no potential conflicts of interest.

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Cancer Prevention Research

Viewing the Epigenetics of Colorectal Cancer through the Window of Folic Acid Effects

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