EDITORIALS

DNA Methylation, Field Effects, and Colorectal Cancer

Edward Giovannucci, Shuji Ogino

The concept of field effect was first introduced by Slaughter et al. *(1)* in 1953, when they studied the presence of histologically abnormal tissue surrounding oral squamous cell carcinoma. This concept, also called field defect or field cancerization, was proposed to explain the development of multiple primary tumors in the same organ and locally recurrent cancer. Field effects are considered to underlie the multicentricity of cancer in many, if not all, patients who have multiple tumors in the same organ but no apparent familial predisposition to those tumors. In the multistep carcinogenesis model proposed by Fearon and Vogelstein *(2)*, genetic alterations occur in a stepwise fashion such that a clone that has growth advantage proliferates, acquires more genetic alterations, and undergoes another selection for survival and growth, eventually resulting in cancer. According to this model, precancerous cells that are in proximity to cancer cells should have at least some, but not all, of the genetic alterations that are present in the fully developed cancer. In support of this model are observations of genetic alterations in fields of precancerous cells in a variety of organs, including lung *(3)*, esophagus *(4)*, vulva *(5)*, cervix *(6)*, and urinary tract *(7)*. Alterations in DNA methylation patterns may also potentially contribute to field effects *(8)*. Aberrant DNA methylation has been demonstrated in a variety of tissues, including in esophageal mucosa in Barrett's esophagus *(8)*, in colonic mucosa affected by ulcerative colitis *(9)*, in normal-appearing bladder mucosa *(10)*, in normal-appearing gastric mucosa *(11)*, and in normal-appearing bronchus in lung cancer resection specimens *(12)*.

In this issue of the Journal, Shen et al. *(13)* describe methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) gene promoter in normal-appearing colorectal mucosa adjacent to colorectal cancer. The authors demonstrated that normal-appearing mucosa located 1 cm away from a colorectal cancer with MGMT promoter methylation was more likely to also have hypermethylation and possibly a higher degree of methylation than normal-appearing colorectal mucosa located 10 cm away from the same colorectal cancer, thus providing evidence for field effects due to DNA methylation in normal-appearing colonic mucosa. These data also indicate that MGMT promoter methylation might occur early in multistep carcinogenesis, even before the emergence of morphologic changes in colorectal mucosa. The biologic consequence of loss of MGMT expression through hypermethylation is that the MGMT protein removes mutagenic adducts from the O^6-guanine base residue in DNA. When left unrepaired, O^6-methylguanine is read by DNA polymerase as adenine, resulting in a G-to-A transition *(14,15)*. MGMT gene silencing by promoter hypermethylation occurs in 20%–40% of colorectal cancers *(16)* and has been associated with an increase in G-to-A mutations in the KRAS and TP53 genes in colorectal tumors *(17,18)*.

The study by Shen et al. *(13)* raises several important issues and potential implications. The authors suggest that the discovery of a marker of a field effect may be useful for early detection and risk assessment in colon cancer. Although this assertion may eventually prove to be true, several issues must be considered. First, it is unclear whether MGMT promoter methylation in apparently normal mucosa is associated with an increased risk of colorectal cancer. Although it is reasonable to conjecture that a neoplastic lesion is more likely to arise within the field characterized with MGMT promoter methylation than in tissue in which cells do not have MGMT promoter hypermethylation, it is also plausible that there might be other as yet unidentified types of field effects—either methylation related or not—that might confer similar or even higher risks of colorectal cancer than the MGMT promoter methylation field defect. In addition, questions remain about whether promoter methylation in other relevant genes, such as MLH1 and CDKN2A (the gene encoding p16), also occurs early in colorectal carcinogenesis, possibly as field effects, and whether methylation field effects involving these genes might confer increased risks for colorectal cancer development. Because MLH1 gene silencing leads to colorectal cancer characterized by microsatellite instability (MSI) *(19)*, MLH1 silencing is likely to be a relatively early event in carcinogenesis. From a practical point of view, it is unclear how much of the large bowel would have to be sampled to sufficiently, if not completely, rule out a localized field effect in individuals without a neoplastic lesion (adenoma or cancer). In individuals with a neoplastic lesion, it is unclear if further information about a field effect would alter the most optimal colonoscopic surveillance schedules or if cancer risks for a particular segment of the large bowel would change according to its distance from a neoplastic lesion with methylation in a particular gene of interest. For example, to support the implementation of differential screening strategies, one would have to show unequivocal and striking differences in risks for a metachronous lesion between individuals who have an advanced adenoma with an identified field effect and individuals who have an adenoma without an identified field effect. Adenoma or cancer patients who do not have abnormal MGMT promoter hypermethylation must have developed cancer through an alternative pathway, and possibly could have other field effects or a high susceptibility to neoplasia through other mechanisms.

Affiliations of authors: Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA (EG); Department of Medicine, Channing Laboratory, Brigham and Women's Hospital/Harvard Medical School, Boston, MA (EG); Cancer Epidemiology Program, Dana-Farber/Harvard Cancer Center, Boston, MA (EG, SO); Department of Pathology, Brigham and Women's Hospital, Boston, MA (SO); Department of Medical Oncology, Dana-Farber Cancer Institute/Harvard Medical School, Boston, MA (SO).

Correspondence to: Edward Giovannucci, MD, ScD, Harvard School of Public Health, Bldg. II, Rm. 331A, 665 Huntington Ave., Boston, MA 02115 (e-mail: egiovann@hsph.harvard.edu).

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The MGMT promoter field effect identified by Shen et al. *(13)* may be a useful intermediate marker in etiologic studies. For example, biochemical reactions involved in one-carbon metabolism are critical for nucleotide biosynthesis and for DNA methylation reactions, and abnormalities in either of nucleotide biosynthesis or DNA methylation could be carcinogenic *(20)*. Deficiencies in nutrients that influence one-carbon metabolism (e.g., folate, vitamins B6 and B12, and methionine) and excess intake of alcohol (which disturbs one-carbon metabolism) have been associated with an increased colorectal cancer risk; in addition, specific genetic polymorphisms in related genes such as MTHFR (the gene for methylenetetrahydrofolate reductase) may modify the association between these nutrients and cancer risk *(21)*. Intake of other nutrients, such as choline, also influences one-carbon metabolism but has not yet been studied in relation to the risk of colorectal neoplasia. Although it is not known if these nutrients are specifically related to alterations in MGMT promoter methylation, they are likely to influence global DNA methylation in colorectal tissue *(22)*. It is thus interesting that individuals who have diets deficient in folate appear to have a higher risk of adenomas or cancers that bear KRAS mutations than individuals who have diets with sufficient levels of these nutrients *(23–25)*. However, it is unclear whether G-to-A transitions in the KRAS gene are overrepresented in these neoplasms. As discussed above, loss of MGMT function is predicted to increase the frequency of G-to-A transition mutations.

The presence of field effects in DNA methylation also raises an important concern about potential chemopreventive approaches and study designs. Could a chemopreventive agent, such as folate, have a different effect in tissue without field effects in DNA methylation than in tissue with field effects in DNA methylation? In animal models, adequate dietary folate early in carcinogenesis may help suppress colorectal tumor formation, but excess folate during late stages of carcinogenesis could potentially enhance carcinogenesis *(26)*. Similarly, in some epidemiologic studies, folate appears to be a protective agent only early in colorectal carcinogenesis *(27)*. In most colorectal neoplasia chemoprevention intervention studies, the study population is composed of individuals who have a colorectal adenoma or an advanced adenoma and who are then monitored for the development of new or metachronous adenomas after treatment. On the basis of the findings of Shen et al. *(13)*, we can surmise that some of these individuals may already have a field effect that involves MGMT promoter methylation. Thus, the findings in such chemoprevention studies may not necessarily reflect the influence of the chemopreventive agent in individuals with normal mucosa (i.e., mucosa without field effects in DNA methylation).

Finally, the findings of Shen et al. *(13)* raise questions about whether reversal of DNA methylation in precancerous cells may prevent the development of new primary cancers in the same organ. Nutrients related to one-carbon metabolism have been shown to influence genomic- and locus-specific hypo- and hypermethylation, and suboptimal diets may be a predisposing factor to abnormalities in DNA methylation *(22)*. However, whether specific dietary alterations can correct DNA methylation field effects, such as that in MGMT promoter methylation, remains unknown. The reversal of DNA methylation abnormalities with drugs is also a possibility, but for this approach to be clinically useful, the drug-related toxicity would have to be very low because colonoscopic surveillance is generally an excellent method to reduce mortality from new lesions in individuals previously diagnosed with adenoma or curable cancer.

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