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Epigenetic Events in Gastrointestinal Cancer

Florin M. Selaru, MD^{1,3}, Stefan David, MD^{1,3}, Stephen J. Meltzer, MD^{1,2}, and James P. Hamilton, MD¹

¹ Division of Gastroenterology and Hepatology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

² Sidney Kimmel Comprehensive Cancer Center, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Abstract

Epigenetics refers to heritable changes in gene expression that are, unlike mutations, not attributable to alterations in DNA sequence. Two predominant epigenetic mechanisms are DNA methylation and histone modification. Epigenetic regulation of gene expression has emerged as a fundamental pathway in the pathogenesis of numerous malignancies, including cancers of the digestive system—in fact, many exciting discoveries in epigenetics have come out of the study of cancers of the gastrointestinal tract and hepatobiliary tree. Epigenetic modifications of DNA in cancer and precancerous lesions offer the promise of novel biomarkers for early cancer detection, prediction, prognosis, and response to treatment. Furthermore, reversal of epigenetic changes represents a potential target of novel therapeutic strategies and medication design. In the future, innovative diagnostic tests and treatment regimens will likely be based on epigenetic mechanisms and be incorporated into the gastroenterologist's practice.

DNA methylation

DNA methylation refers to the addition or subtraction of a methyl group to a cytosine residue in a sequence of DNA. This methylation is controlled by DNA methyltransferase enzymes. Global (i.e., genome-wide) decreases in methylation, or *hypomethylation*, are most functionally relevant when they occur in coding regions of genes, leading to alternative versions or levels of mRNA. It is theorized that hypomethylation contributes to carcinogenesis by favoring mitotic recombination, leading to deletions, translocations, and chromosomal rearrangements. The addition of methyl groups, or *hypermethylation*, is much more gene specific. Regions of the genome that are rich in the sequence of a cytosine preceding a guanine (CpG dinucleotide) are known as CpG islands. In particular, CpG islands exist in the promoter regions of approximately half of all genes. Hypermethylation of CpG islands in the promoter region of a gene results in its transcriptional silencing and loss of protein expression (Figure 1). Thus, hypermethylation of tumor suppressor genes is now recognized as a means of silencing alternative to mutation or allelic loss in the development of cancer.

Correspondence: James P. Hamilton, Division of Gastroenterology and Hepatology, Department of Medicine, Johns Hopkins University School of Medicine, Ross 918, 720 Rutland Avenue, Baltimore, Maryland 21205, USA. jpahamilton@jhmi.edu.

³These authors contributed equally to this work.

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Hypermethylation of genes involved in the cell cycle, DNA repair, angiogenesis, metabolism of carcinogens, apoptosis, and cell–cell interaction has been implicated in carcinogenesis. Methylation can also inhibit the transcription of microRNA, resulting in tumorigenesis. It should be noted that hypermethylation also occurs as a normal physiologic process—for example, during inactivation of the second X chromosome (Barr body) in females. Why aberrant methylation occurs is not fully understood. It is recognized that certain genes are methylated in an age-related fashion, while others are methylated in a cancer-specific pattern. Indeed, one carcinogenetic pathway of particular relevance to gastroenterology is the CpG island methylator phenotype (CIMP). CIMP⁺ cancers have distinct clinical, pathologic, and genetic features (see below). Whether carcinogens, diet (e.g., folate), or other environmental factors contribute to methylation remains to be elucidated and is an area of active research.

Histone modification

Histones are the protein components of chromatin, the structure around which DNA is wound (Figure 1). Histones may undergo several types of post-translational modification, including methylation, acetylation, phosphorylation, and ubiquitination. These modifications can affect interactions between DNA and histones, leading to alterations in gene transcription, DNA repair, DNA replication, and even the organization of chromosomes. In general, histone acetylation is associated with transcriptional activation. Thus, deacetylation is implicated in the silencing of tumor suppressor genes in carcinogenesis. Indeed, histone deacetylase inhibitors are in early-phase clinical trials for the treatment of several cancers, with promising results.

Esophageal cancer

Epigenetic modifications are important in the pathogenesis of both esophageal squamous-cell carcinoma and esophageal adenocarcinoma. Esophageal squamous-cell carcinoma, the most frequent esophageal neoplasm worldwide, is characterized by aberrant hypermethylation of several functionally relevant sets of genes: *p16–CDKN2A*, *p14–ARF*, and *p15–CDKN2B* (cell cycle regulators); *RASSF1A* (a mediator of cell cycle arrest); *CDH1*, *CDH13*, and *CLDN3* (cell adhesion molecules); *hMLH1*, *MSH2*, and *MGMT* (DNA repair genes); *APC*, *WIF1*, *SFRP1*, and *SFRP2* (signaling modulators); and *RARB*, *RUNX3*, *CDX2*, *HTLF*, and *TIG1* (transcriptional regulators). Promoter hypermethylation is also of critical importance in the development of esophageal adenocarcinoma. Moreover, methylation of several key genes can also be detected in Barrett's esophagus, highlighting the application of epigenetic alterations as biomarkers of neoplastic transformation.

The most relevant genes targeted by hypermethylation in esophageal adenocarcinoma are *CDKN2A* and *RPRM* (cell cycle arrest); *DAPK* (apoptosis); *CDH1* (adhesion); *TIMP3* (extracellular matrix degradation); *MGMT* (purine metabolism); and *APC*, *SFRP1*, *SFRP2*, *WIF1*, *AKAP12*, *RUNX3*, *SOCS1*, and *SOCS3* (signaling and transcriptional regulators). Detection of panels of hypermethylated genes has also been shown to predict response to chemotherapy and radiation in both types of esophageal cancer, as well as to predict neoplastic progression in Barrett's esophagus. Amplification of the gene encoding histone demethylase is another epigenetic phenomenon reported in esophageal cancer.

Gastric cancer

Multiple reports have been published regarding gene hypermethylation in both intestinal- and diffuse-type gastric cancer (GC) (for example, *APC*, *CDH1*, *p15*, *p16*, *p14*, *CHFR*, and *RUNX3*). Interestingly, the methylation profile differs between the intestinal and diffuse types of GC, providing molecular evidence that these two cancer subtypes are pathogenetically distinct. For example, *CDH1* is hypermethylated more frequently in diffuse-type than in

intestinal-type GC. The *p16* gene is hypermethylated largely in intestinal-type GC, whereas *p14* hypermethylation occurs predominantly in diffuse-type GC. Hypermethylation may also be used to determine prognosis in GC. For example, patients with *CDH1* and *MGMT* hypermethylation in GC had earlier recurrences of cancer after surgery than did patients without hypermethylation of these genes. In one study, using five methylation markers (*CDH1*, *DAPK*, *GSTP1*, *p15*, and *p16*), the sensitivity of detection of GC approached 90%. Thus, methylation profiles appear to be potentially useful tools as biomarkers for GC.

Colon cancer

Multiple epigenetic events contribute to colorectal carcinogenesis. Global genomic hypomethylation was first described in colon cancer more than 20 years ago. Gene-specific CpG island hypermethylation is also prevalent in colorectal cancer (CRC), of which examples include *hMLH1*, *p16*, *p14*, *RARB*, *SFRP*, and *WRN*. Hypermethylation of microRNA 124a is also reported to be associated with CRC. Similarly, hypermethylation has been detected in colorectal adenomas and aberrant crypt foci, suggesting that methylation is an early event in the pathogenesis of CRC. In addition, methylation-induced transcriptional inactivation appears to be the driving molecular mechanism underlying the hyperplastic polyp–serrated adenoma–adenocarcinoma sequence. The CpG island methylator phenotype (CIMP) has been described as an alternative pathway of CRC pathogenesis. CIMP⁺ cancers exhibit hypermethylation of multiple genes, as well as microsatellite instability. Interestingly, CIMP⁺ cancers tend to be located in the right colon, are more common in women or older patients, and have mucinous features on histologic examination. Finally, mutations in histone modifier genes and reduced levels of monoacetylated and trimethylated histones have been described in CRC.

Liver cancer

Hepatocellular carcinoma (HCC) has manifold etiologies, and epigenetic regulation appears to play a critical role in its pathogenesis. Both global hypomethylation and gene-specific hypermethylation have been extensively reported. Some examples of genes silenced by hypermethylation in HCC are *SOCS1*, *RASSF1A*, *GSTP1*, and *p16*. Hypermethylation of these and other genes has also been seen in preneoplastic cirrhosis and dysplastic nodules. This finding may ultimately be useful as a prognostic biomarker to define therapy. The CIMP⁺ phenotype is prevalent in a subset of HCCs. In addition, relative to HCCs in patients with nonviral hepatitis, HCCs in patients with viral hepatitis manifest a unique set of genes inactivated by hypermethylation.

Cholangiocarcinoma

The hypermethylation and subsequent inactivation of several genes is associated with cholangiocarcinoma. Genes found to be hypermethylated include inhibitors of the cell cycle as well as other tumor suppressor genes (*p16*, *p14*, *14-3-3 sigma*, *RASSF1A*), genes involved in adhesion and metastasis (*CDH1*), and DNA repair genes (*MLH1*, *GSTP1*). Whether or not these epigenetic events can be used to aid in the diagnosis of cholangiocarcinoma remains to be elucidated.

Pancreatic cancer

The pathogenesis of pancreatic ductal adenocarcinoma involves hypermethylation of multiple genes: *CDKN1A*, *CDKN1C*, *CCND2*, *RARB*, *RPRM*, and *SPARC* (cell cycle regulation); *BNIP3* and *WWOX* (apoptosis); *MLH1* (DNA mismatch repair); and *TFPI2*, *SOCS1*, *DUSP6*, *HHIP*, *RUNX3*, *MDF1*, *FOXO1*, *SFRP4*, and *SFRP5* (cell signaling and transcriptional regulation). Aberrant hypermethylation of *SFRP1*, *RPRM*, and *LHX1* has been described in pancreatic cancer precursor lesions, known as PanINs (pancreatic intraepithelial neoplasias),

of varying degrees, suggesting that aberrant CpG island methylation is an early event in this disease.

Hypermethylation of several cancer-related genes in pancreatic juice obtained from cancer patients offers the potential for a new diagnostic modality.

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Figure 1. Epigenetic modification of DNA. A chromosome with a strand of DNA uncoiling is shown. The top half of the figure depicts how DNA winds around the histone. Alterations to the histone affect the way DNA is transcribed. The bottom half illustrates the addition of methyl groups to CpG islands. This process can inhibit the transcription of genes.