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## Epigenetic Silencing of Genes in Human Colon Cancer

**See “A Genome-Wide Search Identifies Epigenetic Silencing of *Somatostatin*, *Tachykinin-1*, and 5 Other Genes in Colon Cancer” by Mori Y, Cai K, Cheng Y, Wang S, Paun B, Hamilton JP, Jin Z, Sato F, Berki AT, Kan T, Ito T, Mantzur C, Abraham JM, Meltzer SJ, on page 797.**

Epigenetics means “in addition to the genetic information encoded in the DNA,” and refers to the study of how environmental factors can change gene function and often organismal phenotype without altering the DNA sequence. One of the first epigenetic modifications to be identified was methylation of DNA, which in mammals, typically targets the nucleotide cytosine when it is 5′ of guanine.<sup>1</sup> Attachment of a methyl group to the 5 position of the pyrimidine ring produces 5-methylcytosine.<sup>2</sup> Like cytosine, 5-methylcytosine has the ability to base pair with guanine in the formation of DNA; however, 5-methylcytosine behaves much differently than cytosine in other respects. Alterations in DNA methylation in the sequences of genes can affect gene transcription and ultimately gene expression.<sup>3</sup> Typically, DNA hypom-

ethylation leads to increased expression and DNA hypermethylation leads to decreased expression of target genes.

Importantly, although epigenetic modifications involving DNA methylation do not involve sequence changes, they are inherited not only mitotically, but also transgenerationally.<sup>4</sup> Thus, although we usually think of heritable diseases as involving mutations in DNA, epigenetic alterations may confer heritable conditions without disruptions in DNA sequence. In addition to DNA methylation, other important epigenetic changes that affect gene expression involve complex modifications of histone proteins and chromatin structure.<sup>5</sup> Taken together, histone modifications and DNA methylation represent fundamental mechanisms for regulating the epigenome. It is believed that DNA methylation in particular plays a key role in epigenetic regulation of gene expression because it is readily accessible and inherently stable.<sup>6</sup>

Alterations in DNA methylation have been associated with a number of human neoplasms and it has now become clear that DNA methylation plays a pathogenic role in the development of cancer. Two general phenomena have been observed. First, hypomethylation with loss of 5-methylcytosine content is associated with expression of proto-oncogene and other factors that contribute to chromosomal instability.<sup>1</sup> Second, DNA hypermethylation causes

silencing of certain genes that function as tumor-suppressor genes, resulting in cancer formation.<sup>7,8</sup> Most 5-methylcytosine is found in CpG dinucleotides concentrated in specific regions of the genome referred to as *CpG islands*. CpG islands are often found in the promoter regions of genes, indicating that they participate in gene regulation. Hypermethylation of CpG islands also appears to be nonrandom. Thus, gene-specific hypermethylation patterns may be useful as diagnostic markers of certain cancers.<sup>9</sup>

Many genes that are sensitive to methylation-dependent silencing are tumor suppressors that regulate cell division, and their loss can lead to uncontrolled cell proliferation. The first tumor-suppressor gene found to be regulated by DNA methylation was *Rb1*, the gene responsible for inherited retinoblastoma; it also plays a role in many sporadic tumors.<sup>10</sup> Subsequently, other tumor-suppressor genes were found to be inactivated by promoter methylation, including *APC*, *p16/CDKN2A*, and *BRCA1* (for a review see Baylin<sup>11</sup>). Despite this compelling pathogenic association, not all hypermethylation events in cancer affect tumor-suppressor genes or other genes associated with cellular proliferation. For example, hypermethylation of *MLH1* in nonpolyposis colon cancer is associated with DNA mismatch repair and contributes to genetic instability.<sup>12</sup> Moreover, changes in DNA methylation can affect genes that are involved with other features of cancer such as tumor invasion and metastasis.<sup>3</sup>

In the current issue of *GASTROENTEROLOGY*, Mori et al<sup>13</sup> used several complimentary techniques to identify genes that are abnormally methylated in colon cancer. Fifty-six colon cancers, 22 noncancerous human colonic mucosae, and 14 human colon cancer cell lines were analyzed. The authors performed microarray analyses to identify genes down-regulated in primary colon cancers compared with normal colonic mucosae. They also identified genes up-regulated in colon cancer cells following demethylation with 5-aza-dC treatment. Fifty-four of the identified genes are potentially involved in the etiology of cancer, and 51 are novel candidate methylation targets in colon cancer.

Nine genes demonstrated methylation in at least one of the 14 colon cancer cell lines. Interestingly, 2 well-known neuroendocrine gastrointestinal peptide hormone genes, namely *somatostatin* (*SST*) and *tachykinin-1* (*TAC1*), were among these 9 genes. The promoter regions of both *SST* and *TAC1* were frequently methylated in colon cancers to a level that was significantly greater than that found in normal colonic mucosae. Moreover, *TAC1* methylation was higher in Duke's stage A/B cancers compared to Duke's stage C/D cancers. *SST* methylation levels were higher in MSI-L cancers than in MSS and MSI-H cancers.

The provocative aspect of these findings is that they provide potential new insights into the pathogenic mechanisms regulating colon cancer. Based on the observations that the expression of *SST* and *TAC1* were significantly lower in colon cancer, one would expect lower levels of their respective gene products (ie, somatostatin and substance P/neurokinin A). Somatostatin is known to suppress tumor growth through 4 distinct mechanisms.<sup>14</sup> First, it is a potent inhibitor of growth factors and hormones that have growth-promoting effects. Second, somatostatin has antiangiogenic effects that reduce the vascular capacity for sustaining neoplastic growth. Third, somatostatin has direct effects on the immune system including monocytes that may limit cell proliferation, and indirect effects on immunoregulatory factors such as tumor necrosis factor that may regulate enzymatic endopeptidase activity to alter the effects of other peptides and growth factors. Finally, somatostatin analogs exert apoptotic effects.<sup>15</sup>

In the gastrointestinal tract, somatostatin is a classic paracrine transmitter, where it is normally released from D cells and acts on adjacent or nearby cells to exert its inhibitory effects. In certain tumors, however, somatostatin operates through an autocrine mechanism to suppress tumor growth. In this manner, somatostatin is both released from and acts on the tumor itself primarily through somatostatin receptors type 2, which are expressed on colon cancers and normal colon epithelia alike.<sup>16</sup> In cancer therapy, somatostatin and smaller, more stable analogs are widely used in treating neuroendocrine tumors where they are particularly effective in reducing hormone secretion. Interestingly, somatostatin analogs may also reduce colon tumor size, and are being investigated as adjuvants to other chemotherapeutic regimens. Because of these biological actions, it is not surprising to find evidence that endogenously produced somatostatin may inhibit cancer formation.

Tachykinin 1 is a precursor for the peptides substance P and neurokinin A, which are best known for their effects on gastrointestinal secretion, motility, and inflammation. Substance P and neurokinin A exert their effects through the neurokinin-1 and -2 receptors, respectively. Substance P can stimulate cell proliferation and inhibit apoptosis, although this latter effect is somewhat controversial.<sup>17</sup> Moreover, substance P potentiates the cytotoxic effects of lymphokine-activated killer T cells against colon cancer cells, and reduces the invasive potential of colon cancer cells.<sup>18</sup> Although less well understood, neurokinin A also possesses some antiproliferative actions. Thus, the discovery of hypermethylation of *TAC1* in colon cancer raises the possibility that its silencing results in reduced expression of substance P and/or neurokinin A. It is interesting to speculate that decreased levels of these peptides facilitate the early stages of carcinogenesis by reducing growth inhibiting signaling or immune surveillance.

Five other genes with potential links to cancer were also identified as undergoing gene promoter methylation in primary colon cancers (*NELL1*, *ENG*, *MAL*, *AKAP12*, and *CAVI*). It is now important to determine not only if these newly identified hypermethylated genes contribute to the genesis of colon cancer, but also if promoter methylation status can lead to cancer detection and prediction of clinical responsiveness to chemotherapeutic treatment regimens.

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