The CpG island methylator phenotype (CIMP) in colorectal cancer

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ABSTRACT
It is clear that colorectal cancer (CRC) develops through multiple genetic and epigenetic pathways. These pathways may be determined on the basis of three molecular features: (i) mutations in DNA mismatch repair genes, leading to a DNA microsatellite instability (MSI) phenotype, (ii) mutations in APC and other genes that activate Wnt pathway, characterized by chromosomal instability (CIN) phenotype, and (iii) global genome hypermethylation, resulting in switch off of tumor suppressor genes, indicated as CpG island methylator phenotype (CIMP). Each of these pathways is characterized by specific pathological features, mechanisms of carcinogenesis and process of tumor development. The molecular aspects of these pathways have been used clinically in the diagnosis, screening and management of patients with colorectal cancer. In this review we especially describe various aspects of CIMP, one of the important and rather recently discovered pathways that lead to colorectal cancer.

Keywords: Colorectal Cancer, CIMP, clinical implication.

Introduction
The development of CRC finds its base in genetic and epigenetic aberrancies that are gained during life (environmental, lifestyle), inherited, or both (1). Epidemiologic studies have determined a number of risk factors for CRC including age, family history of colon cancer or inflammatory bowel disease, cigarette smoking, diet, race, obesity, physical inactivity, and intake of alcohol (2). These risk factors cause genetic and epigenetic changes in colorectal epithelial cells that, together with genetic makeup that was inherited, may result in the development of a tumor (1, 3-4). In general, 3 pathways are distinguished in CRC that are characterized by three distinct pathways of genomic instability: (i) mutations in DNA mismatch repair genes, leading to a DNA microsatellite instability (MSI) phenotype, (ii) mutations in APC and other genes that activate Wnt pathway, characterized by chromosomal instability (CIN) phenotype, and (iii) global genome hypermethylation, resulting in switch off of tumor suppressor genes, indicated as CpG island methylator phenotype (CIMP) (5).

Microsatellite instability (MSI) is responsible for approximately 15-20 % of all CRC cases. MSI tumorigenesis is driven by the inactivation of mismatch repair genes, which play a key role in promulgating genetic stability by repairing DNA
replication errors, inhibiting recombination between non-identical DNA sequences and interfering in responses to DNA damage. Sporadic MSI tumors are generally affected by promoter hypermethylation of the mismatch repair gene MLH1 resulting in the inactivation of this gene (6). The familial form of MSI CRC is hereditary non-polyposis CRC (HNPCC, or Lynch syndrome), which is caused by germline mutations in the mismatch repair genes MLH1, PMS2, MSH6, or MSH2, and accounts for about 3-5% of all CRC cases (5-6). Compared to MSS (microsatellite stability), MSI tumors are more often located in the proximal colon, poorly differentiated, and of a mucinous, or signet ring, histological type. Another prevalent finding in MSI CRC is the usual abundant presence of tumor-infiltrating T cells. MSI tumors have often been associated with a better patient prognosis compared with MSS tumors (7-9).

The chromosomal instability (CIN) is observed in 70%-85% of CRCs and is often described in terms of MSS CRCs (5). However, it should be noted that MSS is not tantamount to chromosomal instability, since some tumors harbor only one of these two traits. It is commonly accepted that the majority of MSS tumors follow the CIN pathway of tumorigenesis.

The accurate cause of chromosomal instability is not known, but it has been suggested to be a consequence of abnormalities in the mitotic checkpoint, centrosome number and function, telomere function, DNA damage response, or loss of heterozygosity (LOH, generally found in chromosome 1, 5, 8, 17, and 18), that lead to such large genomic aberrations (10-12). A number of key events related with the development of CIN CRC have been recognized, including mutations in tumor suppressor genes (TSGs) and oncogenes such as APC, TP53, KRAS, CTNNB1, and PIK3CA, and LOH in chromosome 18q (containing the tumor suppressor genes SMAD2, SMAD4, and DCC) (10-15).

In 1999, as another pathway of tumorigenesis, the CpG Island Methylator Phenotype (CIMP) was explained by Toyota et al. (16). CpG island methylator phenotype (CIMP) is a subset of colorectal cancers that happen through an epigenetic instability pathway and that are characterized by vast hypermethylation of promoter CpG island sites, resulting in the inactivation of several tumor suppressor genes or other tumor-related genes (17).

Role of Epigenetics in normal cells and cancer cells

Epigenetic regulation of gene expression is a general key mechanism that is operative in normal tissues and has an important role in the preservation of genomic stability, embryonic development, and tissue differentiation (17). CpG (cytosine preceding guanine) islands are regions within the genome that are common in promoter sites rich in CpG dinucleotides. More than 50% of human genes have been found to be regulated in this way, by promoters including CpG islands. Several CpG dinucleotides, which are methylated in normal cells, are unmethylated in cancer (16-18). In cancer cells, CpG islands may also be aberrantly hypermethylated, causing inappropriate silencing of gene expression. Aberrant genomic methylation is thought to result in tumorigenesis by deregulating gene expression of key genes (18-20). This phenotype has been reported in several tumor types, including gastric, lung, liver, ovarian, glioblastomas, endometrial, breast, leukemias and CRC (18).

DNA methylation is an enzymatic process that adds a methyl group to the 5-position of cytosine by DNA methyltransferases (DNMT) to produce 5-methylcytosine. Usually, the favorite substrate for DNMT is a CG dinucleotide sequence, which has therefore been termed CpG (17). CpG islands are short stretches of CpG rich regions that are often correlated with the promoter region of genes (21).
Methylation of CpG islands within the promoter region causes transcriptional silencing although it appears that decreased gene expression is only typical of a subgroup of methylated genes in colorectal cancer. Methylation that happens in CpG sites outside of promoter site, called gene body methylation, may cause transcriptional activation (22-24).

Such studies revealed that in colorectal cancer, there appears to be two types of methylation that are associated with cancer progression: type A (for age-related) methylation, and type C (for cancer-specific) methylation. Initially, type A methylation arises as a function of age in normal colorectal epithelial cells. By affecting genes that regulate the growth and/or differentiation of these cells, such methylation may result in a predisposition state that precedes tumor formation in the colon (21, 25). First, altered methylation of ESR1, IGF2, and TUSC3 was observed to happen in histologically normal colon epithelium in an age-dependent manner, and then other genes were also shown to prohibit age-dependent methylation (25). Almost 50% of the genes that have age-related methylation are the same genes as those occupied in the pathogenesis of colon cancer; suggesting a role for these genes in the increased cancer ability that is correlated with aging. Fascinating, aberrant hypermethylation of specific gene promoters and global loss of methylation were seen during the aging process, suggesting that the same mechanism(s) involved for age-related and cancer-related DNA methylation. The cause of age-related altered methylation is unknown. Age-related hypermethylation happens as a consequence of development of local predisposing factors in DNA that influence regions such as SP1 binding sites or tandem B1 (22-26). Type C methylation, by contrast, was found exclusively in a subset of cancers, which display a CpG island methylator phenotype (CIMP) (21).

Many studies have expanded the idea of CpG islands to “CpG island shores,” which are also abnormally methylated in cancer. CpG island shores are regions of DNA with a low density of CpG dinucleotides that are up to 2 Kb upstream of a CpG island. The methylation of CpG island shores is correlated with transcriptional inactivation and expression of splice variants. The methylation of these CpG Island shores appears to be tissue specific, and seems to be changed in colorectal cancer. Nonetheless, the role of altered methylation of CpG island shores in the development of cancer is still contentious (27-28).

**The CpG Island Methylator Phenotype (CIMP)**

DNA hypermethylation in CpG-rich promoters is now recognized as a subgroup of CRCs. However, the pathophysiology of hypermethylation remains ambiguous. Cancers can be classified according to their degree of methylation, and those cancers with high degrees of methylation (the CpG island methylator phenotype, or CIMP) represent a clinically and etiologically distinct group that is characterized by ‘epigenetic instability’. Furthermore, CIMP-associated cancers seem to have a distinct epidemiology, a distinct histology, distinct precursor lesions and distinct molecular features (29-31). The intuition of CIMP led to the proposal of a tumorigenic pathway of CRC driven by promoter hypermethylation and hence epigenetic, rather than genetic, inactivation of tumor suppressor genes. Many genes (though probably not all) that have been identified to be affected in CIMP have important functions in the cell, (e.g. CDKN2A, the gene coding for the tumor suppressor p16), whereas others have unknown functions (17). For instance, aberrant methylation of CXLC12, a chemokine ligand, in human colorectal cancer can foster metastatic property of colon cancer cell lines (17). In addition, most CIMP CRCs are characterized by promoter CpG island methylation of the mismatch repair gene, MLH1, resulting in its transcriptional inactivation (32-33).
Different classification in CIMP reporting

The accurate description of CIMP has not been equal among studies. Actually, which specific methylated loci should be used to define CIMP is the main challenge in studying and evaluation of CIMP tumors. The majority of studies have commonly contained the classic panel: hMLH1, p16, MINT1, MINT2, and MINT31 (34-37) and in addition to these 5 loci, this panel may be further developed to contain: CACNA1G, CRABP1, IGF2, NEUROG1, RUNX3, SOCS1, HIC1, IGFBP3, and WRN (38-42). Given that the loci marker panel and criteria for CIMP vary, we should take a caution, when analyzing clinical outcome results from CIMP studies.

Weisenberger et al. classified CRC into CIMP-negative and CIMP-positive cancers using MethyLight technology (32). They showed a great correlation of CIMP cancers with BRAF mutations. Subsequently, in an interesting paper, Shen et al. have suggested the division of CIMP colon cancers into three different groups CIMP1, CIMP2 and CIMP-negative (31). Genetically, these three groups correspond to very distinct profiles. Based upon a study of 97 primary CRC cases, CIMP1 tumors are often MSI tumors (80%), and have BRAF mutations (53%), but CIMP2 tumors have KRAS mutations (92%), but rarely are MSI or have BRAF or TP53 mutations (31).

In another paper, Ogino et al. quantified DNA methylation in five CIMP-specific gene promoters [CACNA1G, CDKN2A (p16), CRABP1, MLH1, and NEUROG1] and defined CRC with 1/5 to 3/5 methylated promoters as CIMP-low, with 4/5 or 5/5 methylated promoters as CIMP-high and with 0/5 methylated promoters as CIMP-0 tumors. The study suggested that; CIMP-low CRC is associated with male sex and KRAS mutations (38). Barault et al. defined three different subgroups of Methylation (No-CIMP, CIMP-low and CIMP-high) according to evaluation of methylation status for five markers (hMLH1, p16, MINT1, MINT2, and MINT31). They concluded that methylation is an independent prognostic factor in MSS CRC (37).

In another study, a total of 202 CpG sites were found to be differentially methylated between tumor and normal tissue (30). Methylation data from these sites revealed the existence of three CRC subgroups referred to as CIMP-low (CIMP-L, 21% of cases), CIMP-mid (CIMP-M, 14%) and CIMP-high (CIMP-H, 65%). In comparison to CIMP-L tumors, CIMP-H tumors were more often located in the proximal colon and showed more frequent mutation of KRAS and BRAF (30).

Ogino et al. suggested using eight markers (CACNA1G, p16CDKN2A, CRABP1, IGF2, hMLH1, NEUROG1, RUNX3, and SOCS1) to classification CRC subgroups if 1 to 5 out of 8 markers methylated known as CIMP-low, when none of each markers methylated means CIMP-0, and 6 to 8 out of 8 markers have promoters methylated are - CIMP-high (41).

In a recent study, Japanese scientists selected 44 new methylation markers, among 1,311 candidate silencing genes, on a genome-wide scale. They classified CRC clustered into high-, intermediate and low methylation epigenotypes (43).

Clinicopathological characteristics of CIMP Subtypes

In interesting paper by Deng et al., methylation status of 31 proximal and 43 distal colorectal tumors was identified using 14 marker genes (44). These data showed that proximal and distal CRC have distinct gene-specific methylation profiles.

Pathological characterization of CIMP tumors are the high rate of mutations (KRAS or BRAF), wild type p53, proximal colon location, mucinous histological type, higher age at diagnosis, poor differentiation, and higher occurrence in female gender and older patients (29).

It is worthwhile to note that, several of the clinico-pathological characteristics correlated with
CIMP-high are also related to MSI. However, the associations between CIMP-high and a right-sided location, mucinous histological type, and \textit{BRAF} mutation were confirmed in analyses of MSI and MSS tumors separately, indicating that these traits are associated to CIMP-high independently of MSI screening status (7, 18, 29-35).

\textbf{CIMP and CRC Patient Prognosis and survival}

Though the ratio of CRC patients who survive their illness has increased with oncologic therapy and surgery, CRC mortality is still high. A patient prognosis is estimated in clinical practice based mainly on tumor stage. Although much effort has been put into identifying novel prognostic biomarkers, few have been utilized in clinical practice (45).

In recent years much regard has been focused on DNA hypermethylation of specific genes involved to development of CRC to predict CRC patient outcome. In general, it seems that the aberrant DNA methylation of genes is predominantly occurred in the early stages of colon cancer formation and less involved to progression events (36).

One of the key studied genes is \textit{CDKN2A} (p16). However, a recent meta-analysis documented that the hypermethylation of \textit{CDKN2A} is not an independent prognostic factor in CRC (46). Although Ling \textit{et al.} suggested that the presence of p16 hypermethylation predicts shorter survival in T3N0M0 stage (47). Results have been reported for an abundance of other genes, but for none has a prognostic role been established. Although the results are not decisive, many studies have found a poor prognosis in MSS CIMP+ CRC patients (35-37, 48-50). Kakar \textit{et al.} suggested that CIMP does not appear to play a key role in CRC without MSI and CIN (51-53), although the microarray analysis revealed that CIMP tumors represent a distinct molecular class within MSS CRC (54).

Ward \textit{et al.} documented that DNA Methylation is associated with a worse outcome in CRC, but this adverse prognostic influence is lost in those methylated tumors showing MSI (35).

Patients with CIMP-high CRC showed a trend of decreased cancer-specific survival compared with CIMP-negative, which was not statistically significant in many studies (35-37). However, in the MSS tumor subgroup, CIMP-high was associated with a very poor prognosis, which was statistically significant (37, 42). On the other hand, patients with CIMP-low CRC had a worse prognosis compared with CIMP-negative (43).

The importance of a CIMP-intermediate subgroup in CRC patient survival is unclear. Few studies have presented results for survival of CIMP-intermediate tumor patients separately. Recently Yagi \textit{et al.} show intermediate-methylation epigenotype with KRAS-mutation (+) correlated with worse prognosis (43) and Barault \textit{et al.} found a worse prognosis in patients with CIMP-intermediate CRC (37). Most other studies have reported non-significant trends of a worse prognosis in CIMP-intermediate compared to CIMP-negative (35-36, 38-40). CpG island methylation may predict poor survival in metastatic MSS CRC treated with chemotherapy (40) and show shortened survival in advanced CRC treated with 5- fluorouracil (36).

Many studies have found the association between CIMP status and other important epidemiological and molecular factor (55-60). Interestingly, there has been an association reported between smoking and CIMP. In smokers, there is increased CpG methylation at the bronchial epithelium (55). In colon cancer, cigarette smoking is associated with CIMP tumors and has a significant relationship to the number of cigarettes smoked (56).

Also, the increased risk for colon cancer in inflammatory bowel disease (IBD) is hypothesized to have a link with DNA Methylation (57). However, studies have not shown an increased
incidence of CIMP in IBD-associated CRC as compared to sporadic cancers (57-50).

Issa et al. studied methylation patterns of five genes in the normal and dysplastic mucosa of patients with ulcerative colitis (UC), a condition associated with a marked increased risk of colon cancer (57). The result of this study are consistent with the hypothesis that age-related methylation marks (and may lead to) the field defect that reflects acquired predisposition to colorectal neoplasia. This paper suggest that chronic inflammation is associated with high levels of methylation, perhaps as a result of increased cell turnover, and that UC can be viewed as resulting in premature aging of colorectal epithelial cells (57). Studies show that aging, rather than inflammation per se, promotes CIMP (+) CRCs in IBD patients (59-60).

**CIMP as a biomarker for early detection and response to therapy**

Epigenetic biomarkers can be used for prognosis, prediction and diagnosis of CRC. With regard to the use of methylated genes as biomarkers specifically for CRC, the most advanced uses are as DNA-based colon cancer screening assays (16). Aberrantly methylated genes affect a number of genes in colon cancer, such as CDKN2A/p16, MGMT, THBS1, TIMP3, CDKN2A (p14ARF) and MLH1, some of which are found early in the polyp-cancer sequence making them useful as early detection markers(16). Methylated MLH1 is currently being used as a marker for sporadic as in front of hereditary (i.e. Lynch syndrome) MSI cancers. The most of sporadic MSI tumors have methylated MLH1, whereas it is rare in Lynch syndrome tumors. So, methylated MLH1 status can be used to detection patients who should be considered for genetic testing for Lynch syndrome (61). Khamas et al. used genome-wide screening for methylation-silenced genes and suggested that THSD 1 gene may play a important role in the developing of CRC and can be applied in clinical use as a biomarker (62).

The interactions between CIMP status and response to chemotherapy have been investigated, but the results have been contradictory (63-65). Despite the predictive role of CIMP is controversial, it has been hypothesized that DNA methylation is a useful biomarker of recurrence in resected stage III proximal but not distal CRC (64). Many studies suggested that, the presence of CIMP has predicted benefit of 5FU-based treatment in stages II/III colon cancer (36, 65); however, there is also evidence showing a trend for resistance to chemotherapy in CIMP tumors (63).

In the majority of the studies that have analyzed patients who did not receive chemotherapy treatment, tumors identified as MSS, and CIMP have a worse survival outcome (35, 37, 40, 42, 48). In contrast, two studies report better outcomes with CIMP tumors (63, 66). The conflicting data may be due to the different criteria used across the studies to define CIMP status, or that CIMP tumors are heterogeneous and need to be further classified.

**Summary**

The development of colon adenomas to adenocarcinomas is believed to be driven by genetic alterations, such as mutations in TP53, but may also be a result of epigenetic alterations. Epigenetic dysregulation is central to cancer development and progression. This dysregulation includes hypomethylation, leading to oncogene activation and chromosomal instability; and hypermethylation, leading to tumor suppressor gene silencing and chromatin modification, acting directly, and cooperatively with methylation changes, to modify gene expression. Although it has been more than a decade since CIMP was first described in CRC, its molecular basis and prognostic value has not been without
controversy, and the cause of CIMP remains unknown yet. Recent studies have showed a shorter survival in CIMP+ patients, although the results have not always been statistically significant. The evidence for a poor prognosis in MSS CIMP+ has been more persuasive. However, some studies have showed conflicting results. In conclusion, personalized medicine has become a significant part of the modern management of CRC cancer. Additionally, it is essential to have a consensus on a standardized panel of loci to define CIMP, similar to the standardized panel utilized to identify MSI.

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References


