

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.

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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

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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 cause transcriptional silencing although it appear 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 vogue, 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 aetiologically 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 *CXCL12*, 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 *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).

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 *CDKN2A* (p16). However, a recent meta-analysis documented that the hypermethylation of *CDKN2A* is not an independent prognostic factor in CRC (46). Although Ling *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 *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 *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 *et al.* show intermediate-methylation epigenotype with *KRAS*-mutation (+) correlated with worse prognosis (43) and Barault *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 (57-60).

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

1. Pancione M, Remo A, Colantuoni V. Genetic and epigenetic events generate multiple pathways in colorectal cancer progression. *Patholog Res Int* 2012;1-11.
2. Haggard FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. *Clin Colon Rectal Surg* 2009;22:191-97.
3. Slattery ML, Curtin K, Sweeney C, Levin TR, Potter J, Wolff RK, et al. Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. *Int J Cancer* 2007 ;120:656-63.
4. Chan AT, Giovannucci EL. Primary prevention of colorectal cancer. *Gastroenterology* 2010 ;138:2029-43.
5. Worthley DL, Leggett BA. Colorectal cancer: molecular features and clinical opportunities. *Clin Biochem Rev* 2010; 31:31-38.
6. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; 138:2073-87.
7. Wu C, Bekaii-Saab T. CpG Island Methylation, Microsatellite Instability, and BRAF Mutations and Their Clinical Application in the Treatment of Colon Cancer. *Chemother Res Pract* 2012; 2012:359041.
8. Samowitz WS, Curtin K, Wolff RK, Tripp SR, Caan BJ, Slattery ML. Microsatellite instability and survival in rectal cancer. *Cancer Causes Control* 2009; 20:1763-68.
9. Pino MS, Chung DC. Microsatellite instability in the management of colorectal cancer. *Expert Rev Gastroenterol Hepatol* 2011; 5:385-99.
10. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroenterology* 2010; 138:2059-72.
11. Grady WM. Genomic instability and colon cancer. *Cancer Metastasis Rev* 2004; 23:11-27.
12. Rajagopalan H, Lengauer C. Aneuploidy and cancer. *Nature* 2004; 432:338-41.
13. Smith G, Carey FA, Beattie J, Wilkie MJ, Lightfoot TJ, Coxhead J. Mutations in APC, Kirsten-ras and p53 - alternative genetic pathways to colorectal cancer. *Proc Natl Acad Sci USA* 2002; 99:9433-38.
14. Fodde R, Kuipers J, Rosenberg C, Smits R, Kielman M, Gaspar C, et al. Mutations in the APC tumour suppressor gene cause chromosomal instability. *Nat Cell Biol* 2001; 3:433-8.
15. Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. *Br J Surg* 2002; 89:845-60.
16. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A* 1999; 96: 8681-8686.
17. Lao VV, Grady WM. Epigenetics and colorectal cancer. *Nat Rev Gastroenterol Hepatol* 2011; 8:686-700.
18. Hughes LA, Khalid-de Bakker CA, Smits KM, van den Brandt PA, Jonkers D, Ahuja N, et al. The CpG island methylator phenotype in colorectal cancer: progress and problems. *Biochim Biophys Acta* 2012; 1825:77-85.
19. Esteller M, Herman JG. Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. *J Pathol* 2002; 196: 1-7.
20. Kaneda A, Yagi K. Two groups of DNA methylation markers to classify colorectal cancer into three epigenotypes. *Cancer Sci* 2011; 102:18-24.
21. Toyota M, Issa JP. CpG island methylator phenotypes in aging and cancer. *Semin Cancer Biol* 1999; 9:349-57.
22. Feinberg AP. The epigenetics of cancer etiology. *Semin Cancer Biol* 2004; 14:427-32.
23. de Vogel S, Wouters KA, Gottschalk RW, van Schooten FJ, de Goeij AF, de Bruijne AP, et al. Genetic variants of methyl metabolizing enzymes and epigenetic regulators: associations with promoter CpG island

- hypermethylation in colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2009 ;18:3086-96.
24. van Rijnsoever M, Grieu F, Elsaleh H, Joseph D, Iacopetta B. Characterisation of colorectal cancers showing hypermethylation at multiple CpG islands. *Gut* 2002; 51:797-802.
25. Ahuja N, Issa JP. Aging, methylation and cancer. *Histol Histopathol* 2000; 15:835-842.
26. Saito Y, Jones PA. Epigenetic activation of tumor suppressor microRNAs in human cancer cells. *Cell Cycle* 2006; 5:2220-22.
27. Doi A, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet* 2009; 41:1350-53.
28. Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, et al. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet* 2009 ;41:178-86.
29. Issa JP. CpG island methylator phenotype in cancer. *Nat Rev Cancer* 2004; 4:988-93.
30. Ang PW, Loh M, Liem N, Lim PL, Grieu F, Vaithilingam A, et al. Comprehensive profiling of DNA methylation in colorectal cancer reveals subgroups with distinct clinicopathological and molecular features. *BMC Cancer* 2010; 10:227.
31. Shen L, Toyota M, Kondo Y, Lin E, Zhang L, Guo Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. *Proc Natl Acad Sci U S A* 2007; 104:18654-59.
32. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* 2006; 38:787-93.
33. Ahuja N, Mohan AL, Li Q, Stolker JM, Herman JG, Hamilton SR, Baylin SB, et al. Association between CpG island methylation and microsatellite instability in colorectal cancer. *Cancer Res* 1997; 57:3370-74.
34. Hawkins N, Norrie M, Cheong K, Mokany E, Ku SL, Meagher A, et al. CpG island methylation in sporadic colorectal cancers and its relationship to microsatellite instability. *Gastroenterology* 2002; 122: 1376-87.
35. Ward RL, Cheong K, Ku SL, Meagher A, O'Connor T, Hawkins NJ. Adverse prognostic effect of methylation in colorectal cancer is reversed by microsatellite instability. *J Clin Oncol* 2003; 21: 3729-36.
36. Shen L, Catalano PJ, Benson AB, 3rd, O'Dwyer P, Hamilton SR, Issa JP. Association between DNA methylation and shortened survival in patients with advanced colorectal cancer treated with 5-fluorouracil based chemotherapy. *Clin Cancer Res* 2007; 13:6093-98.
37. Barault L, Charon-Barra C, Jooste V, de la Vega MF, Martin L, Roignot P, et al. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. *Cancer Res* 2008; 68:8541-46.
38. Ogino S, Kawasaki T, Kirkner GJ, Loda M, Fuchs CS. CpG island methylator phenotype-low (CIMP-low) in colorectal cancer: possible associations with male sex and KRAS mutations. *J Mol Diagn* 2006; 8:582-88.
39. Ogino S, Cantor M, Kawasaki T, Brahmandam M, Kirkner GJ, Weisenberger DJ, et al. CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies. *Gut* 2006; 55:1000-1006.
40. Ogino S, Meyerhardt JA, Kawasaki T, Clark JW, Ryan DP, Kulke MH, et al. CpG island methylation, response to combination chemotherapy, and patient survival in advanced microsatellite stable colorectal carcinoma. *Virchows Arch* 2007; 450:529-37.
41. Ogino S, Kawasaki T, Kirkner GJ, Kraft P, Loda M, Fuchs CS. Evaluation of markers for CpG island methylator phenotype (CIMP) in colorectal cancer by a large population-based sample. *J Mol Diagn* 2007; 9:305-14.
42. Lee S, Cho NY, Yoo EJ, Kim JH, Kang GH. CpG island methylator phenotype in colorectal cancers: comparison of the new and classic CpG island methylator phenotype marker panels. *Arch Pathol Lab Med* 2008; 132:1657-65.
43. Yagi K, Akagi K, Hayashi H, Nagae G, Tsuji S, Isagawa T, et al. Three DNA Methylation epigenotypes in human colorectal cancer. *Clin Cancer Res* 2010; 16:21-33.
44. Deng G, Kakar S, Tanaka H, Matsuzaki K, Miura S, Sleisenger MH, et al. Proximal and distal colorectal cancers show distinct gene-specific methylation profiles and clinical and molecular characteristics. *Eur J Cancer* 2008; 44:1290-301.
45. Miller S, Steele S. Novel molecular screening approaches in colorectal cancer. *J Surg Oncol* 2012; 105:459-67.
46. Shima K, Nosho K, Baba Y, Cantor M, Meyerhardt JA, Giovannucci EL, et al. Prognostic significance of CDKN2A (p16) promoter methylation and loss of expression in 902 colorectal cancers: Cohort study and literature review. *Int J Cancer* 2011; 128:1080-94.

47. Liang JT, Chang KJ, Chen JC, Lee CC, Cheng YM, Hsu HC, et al. Hypermethylation of the p16 gene in sporadic T3N0M0 stage colorectal cancers: association with DNA replication error and shorter survival. *Oncology* 1999; 57:149-56.
48. Lee S, Cho NY, Choi M, Yoo EJ, Kim JH, Kang GH. Clinicopathological features of CpG island methylator phenotype-positive colorectal cancer and its adverse prognosis in relation to KRAS/BRAF mutation. *Pathol Int* 2008; 58:104-13.
49. Kim JH, Shin SH, Kwon HJ, Cho NY, Kang GH. Prognostic implications of CpG island hypermethylator phenotype in colorectal cancers. *Virchows Arch* 2009; 455: 485-94.
50. Sanchez JA, Krumroy L, Plummer S, Aung P, Merkulova A, Skacel M, et al. Genetic and epigenetic classifications define clinical phenotypes and determine patient outcomes in colorectal cancer. *Br J Surg* 2009; 96: 1196-204.
51. Kakar S, Deng G, Sahai V, Matsuzaki K, Tanaka H, Miura S, et al. Clinicopathologic characteristics, CpG island methylator phenotype, and BRAF mutations in microsatellite-stable colorectal cancers without chromosomal instability. *Arch Pathol Lab Med* 2008; 132: 958-64.
52. Rodriguez J, Frigola J, Vendrell E, Risques RA, Fraga MF, Morales C, et al. Chromosomal instability correlates with genome-wide DNA demethylation in human primary colorectal cancers. *Cancer Res* 2006; 66: 8462-68.
53. Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroenterology* 2005; 129: 837-45.
54. Ferracin M, Gafà R, Miotto E, Veronese A, Pultrone C, Sabbioni S, et al. The methylator phenotype in microsatellite stable colorectal cancers is characterized by a distinct gene expression profile. *J Pathol* 2008; 214: 594-602.
55. Belinsky SA, Palmisano WA, Gilliland FD, Crooks LA, Divine KK, Winters SA, et al. Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. *Cancer Res* 2002; 62: 2370-77.
56. Samowitz WS, Albertsen H, Sweeney C, Herrick J, Caan BJ, Anderson KE, et al. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. *J Natl Cancer Inst* 2006; 98: 1731-38.
57. Issa JP, Ahuja N, Toyota M, Bronner MP, Brentnall TA. Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res* 2001; 61: 3573-77.
58. Jawad N, Direkze N, Leedham SJ. Inflammatory bowel disease and colon cancer. *Recent Results Cancer Res* 2011; 185: 99-115.
59. Iaru AV, Cheng Y, Agarwal R, Yang J, David S, Abraham JM, et al. Unique patterns of CpG island methylation in inflammatory bowel disease-associated colorectal cancers. *Inflamm Bowel Dis* 2012; 18: 641-48.
60. Sanchez JA, DeJulius KL, Bronner M, Church JM, Kalady MF. Relative role of methylator and tumor suppressor pathways in ulcerative colitis-associated colon cancer. *Inflamm Bowel Dis* 2011; 17: 1966-70.
61. Bouzourene H, Hutter P, Losi L, Martin P, Benhattar J. Selection of patients with germline MLH1 mutated Lynch syndrome by determination of MLH1 methylation and BRAF mutation. *Fam Cancer* 2010; 9: 167-72.
62. Khamas A, Ishikawa T, Mogushi K, Iida S, Ishiguro M, Tanaka H, et al. Genome-wide screening for methylation-silenced genes in colorectal cancer. *Int J Oncol* 2012; 41: 490-96.
63. Jover R, Nguyen TP, Pérez-Carbonell L, Zapater P, Payá A, Alenda C, et al. 5-Fluorouracil adjuvant chemotherapy does not increase survival in patients with CpG island methylator phenotype colorectal cancer. *Gastroenterology* 2011; 140: 1174-81.
64. Ahn JB, Chung WB, Maeda O, Shin SJ, Kim HS, Chung HC, et al. DNA methylation predicts recurrence from resected stage III proximal colon cancer. *Cancer* 2011; 117: 1847-54.
65. Van Rijnsoever M, Elsaleh H, Joseph D, McCaul K, Iacopetta B. CpG island methylator phenotype is an independent predictor of survival benefit from 5-fluorouracil in stage III colorectal cancer. *Clin Cancer Res* 2003; 9: 2898-903.
66. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. *Gut* 2009; 58: 90-96.