Published in final edited form as:
Curr Colorectal Cancer Rep. 2014 December 1; 10(4): 425–430. doi:10.1007/s11888-014-0245-2.

DNA Methylation and Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is one of the major cancers in the world and second death-causing cancer in the US. CRC development involves genetic and epigenetic alterations. Changes in DNA methylation status are believed to be involved at different stages of CRC. Promoter silencing via DNA methylation and hypomethylation of oncogenes alter genes' expression, and can be used as a tool for the early detection of colonic lesions. DNA methylation use as diagnostic and prognostic marker has been described for many cancers including CRC. CpG Islands Methylator Phenotype (CIMP) is one of the underlying CRC mechanisms. This review aims to define methylation signatures in CRC. The analysis of DNA methylation profile in combination with the pathological diagnosis would be useful in predicting CRC tumors' evolution and their prognostic behavior.

Keywords

DNA methylation; colon cancer

Introduction

Colorectal cancer (CRC) arises from the accumulation of genetic and epigenetic alterations. DNA methylation is one of the most important epigenetic events that is thought to occur during the early stages of such oncogenic transformation [1]. Early detection of colonic lesions is the most effective approach to reduce CRC incidence and mortality [2]. Therefore, molecular studies aimed at the identification of CRC-specific methylation markers may provide useful insight for a better understanding of CRC progression [3,4].

DNA methylation of a number of genes and their significance in CRC has been reported. [5,6] The hypermethylation of several gene promoters including *APC, p16INK4a, TIMP3* in CRC has been reported [5,6,7]. The aberrant methylation of CpG islands within gene

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Conflict of Interest
Hassan Ashktorab and Hassan Brim declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

promoters and/or first exonic/intronic regions is a recognized epigenetic event that leads to transcriptional silencing. Regardless of the biological consequences of methylation-induced silencing of tumor suppressor genes, this epigenetic alteration constitutes a molecular signature that can serve as a promising biomarker for early detection [5,6,8,9]. The ability to detect aberrant DNA methylation from a wide range of biological specimens highlights its robustness and excellent potential to detect markers of clinical utility in a non-invasive manner. In addition, DNA methylation patterns can be applied for the molecular classification of neoplasms as well as for the prediction of therapeutic response and prognosis.[10] Finally, epigenetic therapy, as such, has been shown to be effective in treating hematologic malignancies and might be useful for treating solid organ cancers as well [8,11,12,13,14]. As our understanding of the role of epigenetic alterations in colon carcinogenesis improves, epigenetic-based therapies might be developed. In addition, the identification of epigenetically deregulated signaling pathways may provide means for selecting patients who will be particularly responsive to targeted therapies [10,15,16,17,18,19,20].

Quantitative approaches to identify DNA methylation differences between normal and cancer tissues could lead to the identification of a panel of highly specific methylated markers for the early detection of cancer. A systematic and comprehensive assessment of aberrantly methylated genes in CRC has the potential not only to improve our understanding of the molecular biology of epigenetically driven CRCs, but also the identification of methylated genes that might influence patient's' clinical management. In this review, we discuss, DNA methylation of CRC-associated genes and their clinical and pathological implications.

DNA methylome studies: advantages and limitations

As a result of the human genome sequence and the development of high throughput technologies such as microarray and next generation sequencing, methylation studies have become more comprehensive with the potential of defining global genomic signatures and the environment in which methylation takes place.

We analyzed the global methylation profiles of cancer, adenoma and normal colon tissues using a microarray IH27 that contain ~1.5 million CpG sites encompassing more than 21,500 CpG Islands, spanning more than 17,000 genes and non-coding regions. This analysis revealed a close clustering of the cancer samples while the adenoma samples clustered with normals. This finding is of great relevance as it unequivocally establish the fact that methylation in the carcinogenesis process is gradual which is why adenomas clustered with normal methylome profiles. It is noteworthy that not only methylation is gradual, but genes that were methylated at early stages of the carcinogenic might get demethylated at later stages. Indeed, Skowronski et al. have demonstrated that ischemic conditions that become prevalent at cancer stages deregulates DNA methyltarnsferases and demethylate p16, an early target of methylation [21]. This finding imposes a spatial and temporal characterization of the samples to be analyzed as methylation levels vary in different anatomical sections of the colon. Moreover, our findings with this methylation microarray analysis have led to the clustering of a single adenoma sample with the cancer

samples. The pathological analysis of this specimen revealed that it is of villous histology that points to high carcinogenic potential [22]. This finding reflects the usefulness of this technology in samples analysis and pathogenic behavior prediction. Microarray data analysis for detecting important methylation targets remain, however, a challenge as thousands of CpG sites show statistically significant differences when samples of different histologies were analyzed. While individual CpG sites are analyzed through the microarray technology, which is an advantage as different CpG sites methylation have different weights on gene expression, the huge number of CpG sites of significance becomes a challenge in data analysis. With the recent advent of IH450 microarray, that contains 450K CpG sites, this challenge is becoming colossal.

We have also used Reduced Representation Bisulfite Sequencing (RRBS) technology [5,6] combined with Next Generation Sequencing (NGS) for global methylation analysis in colonic specimens spanning from normal mucosa to adenocarcinoma. RRBS technique enriches and isolates CpG rich DNA fragments making sequencing focused on primary methylation targets. This analysis along with the microarray studies allows a better view of the global picture of DNA methylation. Sequencing data from the samples that underwent genome-wide RRBS analysis revealed several DNA methylation aberration including the Long Interspersed Elements (LINE) in the genome and reflects a decrease in the methylation levels of these sequences from normal to cancer samples. This finding was in line with recent studies that reported the involvement of such sequences in gene expression regulation and more specifically in some oncogenes upregulation. Genomic instability and loss of imprinting genes like IGF2 (insulin-like growth factor 2) may be both initiated by DNA hypomethylation [23,24]. Global hypomethylation may influence tumor progression by making chromosomes more susceptible to breakage and cause disruption of normal gene structure and function, leading to reactivating previously silenced retrotransposons [25,26]. A typical example of global hypomethylation is the LINE-1 repeat sequence. LINE-1 is a typical repeat sequence of which the hypomethylation has been shown to independently prognosticate poor CRC survival and predict poor response to 5-FU (5-fluorouracil) chemotherapy [27,28,29].

For methylated targets, after sequence analysis and alignment to reference sequences, 355 CpG sites were found to be specifically methylated in 14 promoter regions in 13 genes (*ACTB*, *ATXN7L1* (ampilicon 2, *BMP3*, *CDH5*, *EID3*, *GAS7*, *GNAS*, *GPR75*, *HNRNPF*, *NDRG4*, Sept9, *SPAG5-AS1*, and *TNFAIP2*) in African American CRC patients [5,6]. Fifty CpG sites that showed the highest degree of differential methylation were located within six genes, namely: *ATXN7L1*, *BMP3*, *EID3*, *GAS7*, *GPR75*, and *TNFAIP2*.

EID3, which was confirmed as a potential methylation marker, acts as a repressor of nuclear receptor-dependent transcription possibly by interfering with CREBBP-dependent co-activation [30]. This gene may function as a co-inhibitor of other CREBBP/EP300-dependent transcription factors. Its methylation/silencing likely unleashes the expression of several genes that promote cell proliferation and division. Ingenuity pathway analysis (IPA) positioned this gene within a network of Sept proteins, of which Sept9, a CRC stool marker, is a member [31]. The association of *EID3* with such a well-established marker further confirms the validity of the RRBS/NGS data.

More interestingly, the other validated genes, *BMP3* and *GAS7*, were located in a network of pathways that involve TGF-β1 and insulin. While *GPR75* was not located in this same network, probably because of lack of related functional data, other GPR proteins were within the TGF-β1network. The co-positioning of these methylated markers in the same pathway along with insulin and TGF-β1 is of relevance as it puts colon neoplasia in the larger context of diabetes associated molecules such as insulin and TGF-β1. It is noteworthy that *BMP3* methylation in stools has been found to be associated with CRC in IBD patients [32].

We reported *EID3, BMP3, GAS7,* and *GPR75* hypermethylation in African American patients with CRC and there are no other reports except for *GAS7,* which was reported in a recent Korean publication [6]. *GAS7* hypermethylation has recently been shown in CRC compared with normal mucosa in Korean population [15]. In another study of breast cancer, it has been shown that *GAS7* and *BMP3* harbor major epigenetic differences that affect gene expression and play a role in the induction and maintenance of different phenotypes [33]. No epigenetic data is available for *GPR75* or *EID3* in any population. Therefore, their functional analysis needs to be further dissected. These markers need to be validated in other populations and their correlations to other known pathways need to be analyzed.

Comprehensive review of methylation targets in different patients' populations

From our methylation studies and literature review, we identified 47 genes that are used to identify CIMP phenotype in CRC and were defined here as new proposed CRC CIMP gene-panel (Table 1). The criteria we used to select these markers were based on multiple citations found in the literature, which identified tumor suppressor genes using quantitative and qualitative methylation techniques. We identified and added 16 genes (*APC, EVL, CD109, PTEN, TWIST1, DCC, PTPRD, SFRP1, ICAM5, RASSF1A, EYA4, 30ST2, LAMA1, KCNQ5, ADHEF1,* and *TFPI2*) that consistently showed promoter hypermethylation in CRC (Table 1). In our review of the CIMP genes, *APC* and *RASSF1A* were the most cited markers. A difference in methylation assays is highlighted in the Kim et al. [15] study. In one study, *APC*, was reported to be 15% methylated while other studies reported 92.6%, 42%, 47%, 27%, and 24% methylation in various forms of CRC [34]. In addition to having the most citations, studies about CRC methylation in *RASSF1A* and *APC* include a total of 9 different ethnic populations, and both genes combined a range of methylation percentages that was wide (15–93%). *3OST2* was reported to be > 50% methylated in CRC in three studies, 2 of which used more than 100 patients samples and three different ethnic groups. *EVL* was also cited to be > 50% methylated in CRC in 4 studies, 3 of which evaluated White American cohorts. Importantly, *EVL* was shown to be > 65% methylated in African Americans, suggesting that it may play a role in the more aggressive forms of CRC associated with African Americans. *CD109* and *ICAM5* have the fewest citations in our review; however, the roles of genes that regulate extracellular matrix (ECM) are a hot topic in the field as they affect tumors' invasiveness potential. Future studies are likely to identify similar genes involved in CRC. *PTEN*, another important cancer gene, was only found to display low methylation in CRC in two studies (6% and 19%). Loss of *PTEN* is well

documented in other cancers such as prostate. *TWIST1* gene has been studied in three independent ethnic groups and is one of the more recently identified genes that is silenced by promoter methylation in CRC. *DCC* is an excellent candidate to be included in future CIMP panels as it is highly methylated in CRC (82.7%, 80%, 56%, and 44%) in several studies. *SFRP* was shown to be methylated in colon adenoma and was cited as displaying > 90% methylation in CRC in 3 independent studies.[35,36,37]. *EYA4*, one of the most recently identified genes, was found by four independent assays (MSP, methylation array, mass spectrometry, and pyrosequencing) to be hypermethylated in CRC. This exhaustive review of methylated genes in the path to CRC requires further temporal dissection of these markers to assign stage specific methylation that might reflect pathological status specially if they were to be used as biomarkers in blood or stools for CRC risk assessment.

Applications of DNA methylation as biomarkers in Colorectal Cancer

A subset of CRCs have a specific phenotype termed: CIMP (CpG island methylator phenotype) with a high proportion of methylated genes promoters [5,6]. About 30%–40% of proximal CRCs and 3%–12% of distal CRCs are characterized as CIMP [38]. Primary CRCs are divided into three distinct subclasses: CIMP1, CIMP2, and CIMP negative based on epigenetic and clinical profiles. CIPM1 has a good prognosis, whereas CIMP2 is associated with poor prognosis [39]. CIMP status of cancers has been assessed as a predictive marker for 5-FU responsiveness [40]. DNA hypermethylation causes tumor suppressor genes such as P16, VHL (von Hippel-Lindau tumor suppressor), and MLH1 silencing in CRC. Most sporadic MSI colon tumors are CIMP positive and are usually located in the proximal site of the colon (up to 40%) [41,42,43]. DNA mismatch repair (MMR) gene MLH1 inactivated by promoter methylation, resulting in high-level MSI in some sporadic CRCs, is the driving force of genetic instability[44].

Several DNA methylation markers have been proposed as useful early biomarkers for CRC detection. The detection of aberrant methylation of Vimentin in fecal DNA was introduced in CRC diagnosis with a sensitivity and specificity of 88% and 87%, respectively [45,46]. Hypermethylation of GATA4, a transcription factor (GATA binding protein 4) has been identified as a novel biomarker for CRC detection with a sensitivity of 51–71% and a specificity of 84–93% [47]. NDRG4 is a candidate tumor suppressor gene in CRC whose expression is frequently inactivated by promoter methylation and is a potential biomarker for detection of CRC in stool samples [48]. Blood-based tests for CRC detection could have the potential for better applications. The methylation of SEPT9 (septin 9), encoding a GTPase involved in dysfunctional cytoskeletal organization, was detected in CRC patients with an overall sensitivity of 90% and specificity of 88% [49]. This marker’s methylation is not influenced by patients' age, sex, and tumor location. SEPT9 is particularly attractive for biomarker applicability, especially when combined with clinical symptoms such as abdominal pain and/or blood in stools. Promoter methylation of CHFR (checkpoint with forkhead and ring finger domains) was found to be associated with survival and was considered to be an independent predictor for tumor recurrence. IGFBP3 (insulin-like growth factor binding protein 3) and CD109 DNA methylation associated with worse survival for stage II CRC [50].

Conclusion

It is now widely recognized that, in addition to genetic mutations, epigenetic mechanisms especially aberrant DNA methylation are involved in virtually every step of cancer development and progression. Traditional methods cannot sufficiently predict the prognosis of single cancer cases. Clinicians may not be able to accurately decide which patient will be at high risk for recurrence and benefit from chemotherapy. Therefore, it is essential to search for novel biomarkers improving prognosis, that would help clinicians in the decision-making process through a molecularly informed clinical management of patients. High-throughput technologies, such as methylation microarrays and next generation sequencing, have helped advance our understanding of epigenetic events at to the genomic level. Herein, we reviewed the current literature along with our own CRC DNA methylation studies data. Although many specific genes were found to be valuable as biomarkers for cancer detection, more potential epigenetic biomarkers will be found as a result of the wide application of new sequencing platforms with high speed, depth, and accuracy. Epigenetic signatures, including a panel of methylated genes or specific miRNAs profiles, will show the potential in the early diagnosis (screening) and prognosis (therapy response) prediction of CRC patients.

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Shen L, Kantarjian H, Guo Y, Lin E, Shan J, et al. DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. J Clin Oncol. 2010; 28:605–613. [PubMed: 20038729]
2. Suehiro Y, Wong CW, Chirieac LR, Kondo Y, Shen L, et al. Epigenetic-genetic interactions in the APC/WNT, RAS/RAF, and P53 pathways in colorectal carcinoma. Clin Cancer Res. 2008; 14:2560–2569. [PubMed: 18451217]
3. Mokarram P, Kumar K, Brim H, Naghibalhossaini F, Saberi-firoozi M, et al. Distinct high-profile methylated genes in colorectal cancer. PLoS One. 2009; 4:e7012. [PubMed: 19750230]
4. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet. 2006; 38:787–793. [PubMed: 16804544]
5. Ashktorab H, Daremipouran M, Goel A, Varma S, Leavitt R, et al. DNA methylome profiling identifies novel methylated genes in African American patients with colorectal neoplasia. Epigenetics. 2014; 9
6. Ashktorab H, Rahi H, Wansley D, Varma S, Shokrani B, et al. Toward a comprehensive and systematic methylome signature in colorectal cancers. Epigenetics. 2013; 8
7. Gonzalo V, Lozano JJ, Munoz J, Balaguer F, Pellise M, et al. Aberrant gene promoter methylation associated with sporadic multiple colorectal cancer. PLoS One. 2010; 5:e8777. [PubMed: 20098741]
8. Zhang Y, Li Q, Chen H. DNA methylation and histone modifications of Wnt genes by genistein during colon cancer development. Carcinogenesis. 2013; 34:1756–1763. [PubMed: 23598468]
9. Lao VV, Grady WM. Epigenetics and colorectal cancer. Nat Rev Gastroenterol Hepatol. 2011; 8:686–700. [PubMed: 22009203] Along with references 5 and 6, these papers review CRC epigenetics and have additional important information for the readers from experts in the field.

10. Easwaran H, Baylin SB. Epigenetic abnormalities in cancer find a "home on the range". Cancer Cell. 2013; 23:1–3. [PubMed: 23328477]
11. Kikuchi J, Takashina T, Kinoshita I, Kikuchi E, Shimizu Y, et al. Epigenetic therapy with 3-deazaneplanocin A, an inhibitor of the histone methyltransferase EZH2, inhibits growth of non-small cell lung cancer cells. Lung Cancer. 2012; 78:138–143. [PubMed: 22925699]
12. Rius M, Lyko F. Epigenetic cancer therapy: rationales, targets and drugs. Oncogene. 2012; 31:4257–4265. [PubMed: 22179827]
13. Shen H, Laird PW. In epigenetic therapy, less is more. Cell Stem Cell. 2012; 10:353–354. [PubMed: 22482500]
14. Yeo W, Chung HC, Chan SL, Wang LZ, Lim R, et al. Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. J Clin Oncol. 2012; 30:3361–3367. [PubMed: 22915658]
15. Kim YH, Lee HC, Kim SY, Yeom YI, Ryu KJ, et al. Epigenomic analysis of aberrantly methylated genes in colorectal cancer identifies genes commonly affected by epigenetic alterations. Ann Surg Oncol. 2011; 18:2338–2347. [PubMed: 21298349]
16. Pandiyan K, You JS, Yang X, Dai C, Zhou XJ, et al. Functional DNA demethylation is accompanied by chromatin accessibility. Nucleic Acids Res. 2013; 41:3973–3985. [PubMed: 23408854]
17. Ashktorab, h; Hassan, Hassanzadeh Namin; Andrew, Sanderson; Rehana, Begum; Adeyinka, O. Laiyemo, et al. Colorectal adenoma and cancer prevalence in Hispanics. Gastroenterology. 2012; 140:312.
18. Ashktorab H, Nouraie M, Hosseinkhah F, Lee E, Rotimi C, et al. A 50-year review of colorectal cancer in African Americans: implications for prevention and treatment. Dig Dis Sci. 2009; 54:1985–1990. [PubMed: 19554449]
19. Laiyemo AO, Doubeni C, Brim H, Ashktorab H, Schoen RE, et al. Short- and long-term risk of colorectal adenoma recurrence among whites and blacks. Gastrointest Endosc. 2013; 77:447–454. [PubMed: 23337636]
20. Nouraie M, Hosseinkhah F, Brim H, Zamanifekri B, Smoot DT, et al. Clinicopathological features of colon polyps from African-Americans. Dig Dis Sci. 2010; 55:1442–1449. [PubMed: 20225129]
21. Skowronski K, Dubey S, Rodenhiser D, Coomber B. Ischemia dysregulates DNA methyltransferases and p16INK4a methylation in human colorectal cancer cells. Epigenetics. 2010; 5:547–556. [PubMed: 20543577]
22. Ashktorab H, Paydar M, Namin HH, Sanderson A, Begum R, et al. Prevalence of colorectal neoplasia among young african americans and Hispanic americans. Dig Dis Sci. 2014; 59:446–450. [PubMed: 24193352]
23. Cruz-Correa M, Cui H, Giardiello FM, Powe NR, Hylind L, et al. Loss of imprinting of insulin growth factor II gene: a potential heritable biomarker for colon neoplasia predisposition. Gastroenterology. 2004; 126:964–970. [PubMed: 15057734]
24. Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, et al. Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. Cancer Res. 2002; 62:6442–6446. [PubMed: 12438232]
25. Wong JJ, Hawkins NJ, Ward RL. Colorectal cancer: a model for epigenetic tumorigenesis. Gut. 2007; 56:140–148. [PubMed: 16840508]
26. Suter CM, Martin DI, Ward RL. Hypomethylation of L1 retrotransposons in colorectal cancer and adjacent normal tissue. Int J Colorectal Dis. 2004; 19:95–101. [PubMed: 14534800]
27. Ashktorab H, Smoot DT, Farzanmehr H, Fidelia-Lambert M, Momen B, et al. Clinicopathological features and microsatellite instability (MSI) in colorectal cancers from African Americans. Int J Cancer. 2005; 116:914–919. [PubMed: 15856472]
28. Kawakami K, Matsunoki A, Kaneko M, Saito K, Watanabe G, et al. Long interspersed nuclear element-1 hypomethylation is a potential biomarker for the prediction of response to oral fluoropyrimidines in microsatellite stable and CpG island methylator phenotype-negative colorectal cancer. Cancer Sci. 2011; 102:166–174. [PubMed: 21087350]

29. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Chan AT, et al. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. J Natl Cancer Inst. 2008; 100:1734–1738. [PubMed: 19033568]
30. Bavner A, Matthews J, Sanyal S, Gustafsson JA, Treuter E. EID3 is a novel EID family member and an inhibitor of CBP-dependent co-activation. Nucleic Acids Res. 2005; 33:3561–3569. [PubMed: 15987788]
31. Church TR, Wandell M, Lofton-Day C, Mongin SJ, Burger M, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. Gut. 2013
32. Kisiel JB, Yab TC, Nazer Hussain FT, Taylor WR, Garrity-Park MM, et al. Stool DNA testing for the detection of colorectal neoplasia in patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2013; 37:546–554. [PubMed: 23347191]
33. Ronneberg JA, Fleischer T, Solvang HK, Nordgard SH, Edvardsen H, et al. Methylation profiling with a panel of cancer related genes: association with estrogen receptor, TP53 mutation status and expression subtypes in sporadic breast cancer. Mol Oncol. 2011; 5:61–76. [PubMed: 21212030]
34. Kim MS, Lee J, Sidransky D. DNA methylation markers in colorectal cancer. Cancer Metastasis Rev. 2010; 29:181–206. [PubMed: 20135198]
35. Lind GE, Danielsen SA, Ahlquist T, Merok MA, Andresen K, et al. Identification of an epigenetic biomarker panel with high sensitivity and specificity for colorectal cancer and adenomas. Mol Cancer. 2011; 10:85. [PubMed: 21777459]
36. Simmer F, Brinkman AB, Assenov Y, Matarese F, Kaan A, et al. Comparative genome-wide DNA methylation analysis of colorectal tumor and matched normal tissues. Epigenetics. 2012; 7:1355–1367. [PubMed: 23079744]
37. Zhang W, Bauer M, Croner RS, Pelz JO, Lodygin D, et al. DNA stool test for colorectal cancer: hypermethylation of the secreted frizzled-related protein-1 gene. Dis Colon Rectum. 2007; 50:1618–1626. discussion 1626–1617. [PubMed: 17762966]
38. Migliore L, Migheli F, Spisni R, Coppede F. Genetics, cytogenetics, and epigenetics of colorectal cancer. J Biomed Biotechnol. 2011; 2011:792362. [PubMed: 21490705]
39. Shen L, Toyota M, Kondo Y, Lin E, Zhang L, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. Proc Natl Acad Sci U S A. 2007; 104:18654–18659. [PubMed: 18003927]
40. Ide T, Kitajima Y, Ohtaka K, Mitsuno M, Nakafusa Y, et al. Expression of the hMLH1 gene is a possible predictor for the clinical response to 5-fluorouracil after a surgical resection in colorectal cancer. Oncol Rep. 2008; 19:1571–1576. [PubMed: 18497967]
41. Cunningham JM, Christensen ER, Tester DJ, Kim CY, Roche PC, et al. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res. 1998; 58:3455–3460. [PubMed: 9699680]
42. Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, et al. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci U S A. 1994; 91:9700–9704. [PubMed: 7937876]
43. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, et al. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci U S A. 1999; 96:8681–8686. [PubMed: 10411935]
44. Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci U S A. 1998; 95:6870–6875. [PubMed: 9618505]
45. Chen WD, Han ZJ, Skoletsky J, Olson J, Sah J, et al. Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. J Natl Cancer Inst. 2005; 97:1124–1132. [PubMed: 16077070]
46. Itzkowitz SH, Jandorf L, Brand R, Rabeneck L, Schroy PC 3rd, et al. Improved fecal DNA test for colorectal cancer screening. Clin Gastroenterol Hepatol. 2007; 5:111–117. [PubMed: 17161655]
47. Hellebrekers DM, Lentjes MH, van den Bosch SM, Melotte V, Wouters KA, et al. GATA4 and GATA5 are potential tumor suppressors and biomarkers in colorectal cancer. Clin Cancer Res. 2009; 15:3990–3997. [PubMed: 19509152]

48. Melotte V, Lentjes MH, van den Bosch SM, Hellebrekers DM, de Hoon JP, et al. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. J Natl Cancer Inst. 2009; 101:916–927. [PubMed: 19535783]

49. Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado LV, et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Med. 2011; 9:133. [PubMed: 22168215]

50. Yi JM, Dhir M, Van Neste L, Downing SR, Jeschke J, et al. Genomic and epigenomic integration identifies a prognostic signature in colon cancer. Clin Cancer Res. 2011; 17:1535–1545. [PubMed: 21278247]

Table 1

DNA methylation and CIMP-panel Genes in CRC

MINT1	CRABP1	p14	HAND1	APC	RASSF1A
MINT2	NEUROG1	RUNX3	RASSF2	30ST2	EYA4
MINT12	RIZ1	SOCS1	STOX2	EVL	SFRP1
MINT17	TIMP3	WRN	THBD	CD109	ICAM5
MINT25	NDRG4	LOX	UCHL1	PTEN	LAMA1
MINT27	CHFR	ADAMTS1	FBN2	TWIST1	TFPI2
MINT31	HIC1	DUSP26	MGMT	DCC	
p16	IGF2	EDIL3	KCNQ5	PTPRD	
MLH1	IGFBP3	ELMO1	CACNA1G	ADHFE1	

Adapted from [5].