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DNA methylation biomarkers in colorectal cancer: A review of diagnostic, prognostic, and predictive applications

Mohammadreza Karbalaee Hashemiyan [1, 2], **Mohsen Hemmati-Dinarvand** [1,2 *]

[1] Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
[2] Department of Clinical Biochemistry, Kashan University of Medical Sciences, Kashan, Iran

[*] **Corresponding author:** Mohsen Hemmati-Dinarvand. Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran. **Email:** Mohsen_h43@yahoo.com

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Abstract

Colorectal cancer (CRC) remains a significant global health challenge, largely due to its high mortality when diagnosed at advanced stages. Conventional diagnostic approaches, including colonoscopy and fecal-based assays, are limited by variable sensitivity and suboptimal patient adherence, highlighting the pressing need for non-invasive, robust biomarkers for early detection, prognostic assessment, and therapeutic monitoring. Epigenetic modifications, particularly DNA methylation alterations, have emerged as promising candidates in this regard. This review examines the role of DNA methylation in CRC pathogenesis, emphasizing hypermethylation of tumor suppressor genes and global hypomethylation as central mechanisms driving tumor initiation and progression. We discuss extensively validated methylation biomarkers -including SEPT9, SDC2, MGMT, NDRG4, BMP3, VIM, SFRP, p16, LINE-1, BCAT1, IKZF1, and RASSF1A- and their clinical relevance in CRC screening, prognostication, and recurrence monitoring. Additionally, we address the predictive significance of MLH1 methylation in modulating response to 5-fluorouracil–based chemotherapy, highlighting its potential in guiding personalized treatment strategies. Collectively, these insights underscore the transformative potential of DNA methylation biomarkers in CRC management, facilitating earlier detection, individualized prognostic evaluation, and optimized therapeutic outcomes.

Keywords: Colorectal cancer, DNA methylation, Biomarkers, Prognosis, Diagnosis

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide, accounting for approximately 9% of all cancer deaths, with an estimated 935,173 fatalities in 2020.[1] The five-year survival rate for metastatic CRC remains critically low, at approximately 10%. Although several diagnostic approaches exist, including the fecal immunochemical test (FIT), fecal occult blood test (FOBT), and colonoscopy, each presents notable limitations. Colonoscopy is invasive and requires extensive bowel preparation, while fecal-based assays often demonstrate limited sensitivity for early-stage disease detection. Consequently, there is an urgent need for novel diagnostic strategies and reliable biomarkers that facilitate early CRC detection and allow monitoring of therapeutic responses in patients receiving targeted treatments. Notably, early-stage diagnosis can improve five-year survival rates to nearly 90%.[2, 3]

It is well established that the accumulation of genetic and epigenetic alterations drives cancer initiation and progression.[3,4] Genetics examines changes in the DNA sequence itself, whereas epigenetics studies heritable modifications in gene expression that occur without altering the underlying DNA sequence. While a universally accepted definition of epigenetics is lacking, consensus emphasizes two key aspects: epigenetics refers to heritable changes in gene expression that occur in differentiated cells without modifications in the DNA sequence.[2,3,5] Recent studies have underscored the pivotal role of epigenetic modifications in carcinogenesis.[6] Key epigenetic alterations associated with various cancers include abnormal DNA methylation, dysregulated histone modifications, and altered expression of non-coding RNAs.[7] Among these, aberrant DNA methylation is the most prevalent and widely studied modification.[8]

Due to its stability and early occurrence during

tumorigenesis, DNA methylation has emerged as a promising biomarker for early cancer detection. DNA methylation biomarkers offer multiple advantages: they appear in the early stages of cancer development, are often tissue- or tumor-type specific, and can be assessed across multiple genomic regions via CpG dinucleotide analysis. Recent research has focused on identifying cancer-specific hypermethylated genes in circulating tumor-derived cell-free DNA (cfDNA). cfDNA consists of DNA fragments freely circulating in the bloodstream, released along with nucleic acids, proteins, and extracellular vesicles from tumor cells into blood and other bodily fluids.[9] Over the past decade, tumor-derived cfDNA has gained prominence as a minimally invasive biomarker that can be extracted from blood, urine, stool, and other biological samples. Its detection enables early cancer diagnosis, prediction of therapeutic response, prognosis assessment, and determination of tissue-of-origin [Figure-1].[10] An ideal hypermethylated DNA biomarker should be detectable using minimally invasive approaches, even when originating from sites distant from the primary tumor.

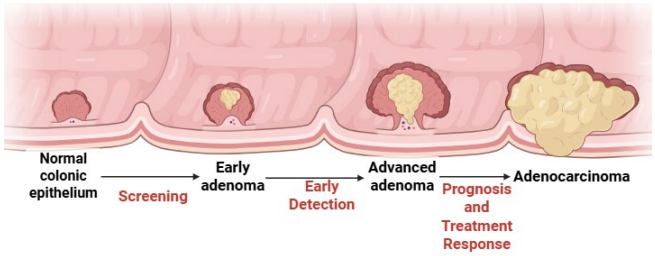

Figure 1. Schematic representation of adenoma-to-adenocarcinoma progression, highlighting DNA methylation biomarkers with diagnostic and prognostic relevance in CRC.

In this review, we first provide an overview of the fundamental principles of DNA methylation in CRC, emphasizing its role in tumorigenesis. We then highlight promising DNA methylation biomarkers with potential applications in CRC screening and early detection, particularly those detectable in body fluids. Additionally, we discuss DNA methylation-based markers that have demonstrated utility in prognostication and in predicting therapeutic response. Finally, we examine accumulating evidence supporting the clinical potential of these epigenetic markers as reliable biomarkers for CRC.

DNA methylation in colorectal cancer

DNA methylation, a critical epigenetic modification involving the addition of a methyl group to the 5' position of cytosine within CpG dinucleotides, plays a central role in gene regulation [Figure-2]. In CRC, aberrant DNA methylation is a hallmark of tumorigenesis. Global hypomethylation, particularly in repetitive DNA sequences and proto-oncogenes, promotes chromosomal instability, thereby activating oncogenic pathways and driving uncontrolled cell proliferation.[11] This genome-wide hypomethylation facilitates chromosomal rearrangements and reactivation of transposable elements, enhancing tumorigenic potential. Conversely, hypermethylation of CpG islands in promoter regions of tumor suppressor genes results in transcriptional silencing, disrupting critical cellular processes such as DNA repair, apoptosis, and cell cycle regulation. Such epigenetic silencing establishes a permissive environment for malignant transformation and tumor progression. For example, hypermethylation-mediated silencing of MLH1, a gene involved in DNA mismatch repair, is strongly associated with microsatellite instability, a common feature in a subset of CRCs.[11] Collectively, global hypomethylation activating oncogenes and site-specific hypermethylation repressing tumor suppressor genes contribute synergistically to CRC initiation and progression.

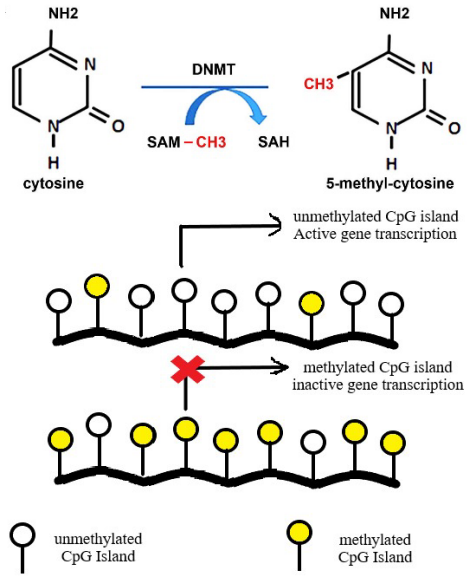

Figure-2. DNMT enzymes catalyze the addition of methyl groups (-CH_3) to cytosine bases within CpG dinucleotides, typically in gene promoter regions. When DNMT binds DNA, it recognizes CpG islands and transfers a methyl group from S-adenosylmethionine (SAM) to cytosine, forming 5-methylcytosine. A) Unmethylated CpG islands in promoter regions facilitate gene transcription. B) Methylated CpG islands inhibit transcription by blocking access of the transcriptional machinery (DNMT: DNA methyltransferase; SAM: S-adenosylmethionine).

Aberrant methylation, including promoter hypermethylation of tumor suppressor genes and global hypomethylation of repetitive genomic regions, drives CRC development by perturbing critical cellular pathways. For instance, hypermethylation of APC and SFRP1, inhibitors of the Wnt/β-catenin pathway, promotes uncontrolled cell proliferation and tumor initiation.[12,13] Concurrently, global hypomethylation, particularly at LINE-1 retrotransposons, enhances chromosomal instability and oncogene activation, correlating with advanced disease stage and poor prognosis.[13,14] Environmental factors, such as high dietary fructose intake, may exacerbate these methylation changes and contribute to observed racial disparities in CRC risk.[12, 15]

The CpG Island Methylator Phenotype (CIMP), defined by widespread promoter hypermethylation, characterizes a subset of CRC tumors frequently associated with microsatellite instability (MSI) and BRAF mutations.[13,15] CIMP-positive tumors exhibit distinct clinical and pathological behaviors, highlighting the influence of methylation on tumor heterogeneity and progression.[14] Importantly, hypermethylation of cfDNA derived from CRC, detectable in blood or stool, has emerged as a promising non-invasive biomarker with potential for high sensitivity and specificity.

Biomarkers for screening and early detection

The growing understanding of epigenetic alterations in colorectal tumorigenesis has created an opportunity for developing sensitive and specific, minimally invasive methylation-based biomarkers for CRC. Consequently, numerous studies have investigated the diagnostic value of these markers.

SEPT9: A crucial cytoskeletal component

SEPT9 interacts with microtubules and actin filaments to regulate cell shape, polarity, intracellular transport, and cytokinesis through scaffold formation. It also stabilizes the plasma membrane and facilitates cytoskeletal dynamics essential for migration and differentiation.[16] Acting as a tumor suppressor, aberrant SEPT9 methylation can disrupt gene regulation, promoting abnormal cell behavior and tumorigenesis. Studies indicate that SEPT9 expression decreases during the progression from adenoma to CRC,[17] with hypermethylation likely representing a late event in tumor development.[18] While plasma mSEPT9 exhibits lower sensitivity for early adenomas, it demonstrates substantial diagnostic value and correlates with CRC stage.[19] Elevated SEPT9 methylation is also associated with reduced disease-free survival following CRC resection.[20] Diagnostic evaluation reports a sensitivity of 75.1% and specificity of 95.1% for CRC detection.[21] Variability in sensitivity and specificity across studies likely reflects differences in detection platforms, population characteristics, and cutoff thresholds. Overall, SEPT9 remains one of the most extensively validated blood-based methylation biomarkers for CRC screening.

ALX4: A homeobox gene regulating cell differentiation and development

Aberrant methylation of ALX4 has been implicated in CRC pathogenesis. Using methylation-specific arbitrarily primed PCR, ALX4 fragments were identified as highly methylated in colorectal adenomas and cancers. Methylation of ALX4 occurs frequently in colon adenomas and primary CRC relative to normal colonic mucosa, with significant association ($P<0.0001$). Hypermethylation of ALX4 has also been observed in liver metastases and adenocarcinomas of the esophagus, stomach, and bile ducts, suggesting a broader role in gastrointestinal malignancies. Serum levels of ALX4 methylation in CRC patients are significantly higher than in non-cancer controls ($P<0.0001$), with a sensitivity of 83.3% and specificity of 70% at a defined cutoff.[22] Its modest specificity suggests that ALX4 may be most effective as part of a multi-gene methylation panel rather than as a standalone diagnostic marker.

SDC2: A membrane-associated protein

SDC2 contributes to cell proliferation, migration, and tissue integrity. While it is normally expressed in mesenchymal cells, SDC2 is absent from epithelial cells of healthy colonic tissue.[23] Aberrant CpG methylation of SDC2 is frequently observed in CRC tumors, highlighting its potential as an early detection biomarker. Methylation levels of SDC2 in stool samples correlate with CRC presence rather than clinical stage.[24,25] Oh et al.,[26] demonstrated elevated SDC2 methylation in primary tumors, adenomatous polyps, and hyperplastic polyps, with no detectable methylation in normal tissues. Notably, SDC2 methylation levels increased with lesion severity, indicating its utility in early CRC detection. Variations in sensitivity and specificity across studies likely reflect differences in sample type (stool vs. plasma) and analytical methodology. These findings support SDC2 as a promising component of multi-target assays for non-invasive CRC screening.

MGMT: A DNA repair enzyme

Silencing of MGMT occurs in approximately 40% of metastatic CRC cases, impairing protein function.[27,28] Shima et al.,[29] evaluated 855 CRC cases using

methylation-specific PCR (MSP) and immunohistochemistry (IHC), revealing 38% promoter methylation and 37% loss of expression. Another study of 70 patients found MGMT hypermethylation in 90% of tumors, whereas no methylation was detected in healthy controls.[30] Differences in reported prevalence likely reflect variation in detection methods, tissue type, and cohort characteristics. While MGMT methylation shows diagnostic potential, its inconsistent frequency limits clinical utility unless standardized protocols are employed.

NDRG4: A regulator of cellular proliferation and stress response

NDRG4 participates in cell proliferation, differentiation, growth, and stress responses, modulating pathways including PI3K/AKT and MAPK/ERK, regulating the cell cycle and apoptosis, and maintaining homeostasis under oxidative stress.[31] CpG island methylation of the NDRG4 promoter has been identified as a CRC biomarker,[32] validated in independent studies.[33] Bagheri et al.,[34] reported 86% sensitivity and 92% specificity for CRC detection, with methylation levels correlating with tumor stage. A meta-analysis further confirmed NDRG4 methylation as a significant early detection marker.[35] Methylated NDRG4 in stool and urine also demonstrates high sensitivity and specificity,[36,37] underscoring its promise as a non-invasive biomarker. Detection rates may vary with tumor stage and sample type, suggesting that combining NDRG4 with other markers could enhance diagnostic accuracy.

BMP3: A tumor suppressor

BMP3 regulates epithelial homeostasis and inhibits excessive proliferation via the SMAD signaling pathway, while promoting differentiation of intestinal epithelial cells.[38] Loh et al., (2008) first demonstrated BMP3 hypermethylation during early polyp formation and CRC progression, with methylation detected in 55% of colorectal tumors.[39,40] Subsequent plasma-based studies showed higher BMP3 methylation frequency in patients with polyps than in healthy controls, with 40% sensitivity and 94% specificity.[41] Panels combining BMP3 with other methylation markers, such as NDRG4, achieved 100% sensitivity and 89% specificity for CRC detection [42]. While BMP3 methylation offers diagnostic potential, limited sensitivity indicates it is most effective as part of a multi-marker panel.

VIM: Involved in cancer invasion and metastasis

Vimentin (VIM), an intermediate filament protein, supports cell shape, organelle positioning, and migration, particularly in mesenchymal cells. It is also implicated in wound healing, signal transduction, and cellular plasticity, playing a role in cancer invasion and metastasis. VIM promoter methylation has been detected in stool DNA from CRC patients,[43,44] with reported sensitivities ranging from 41.1% to 52% and specificities of 85-88%.[36,43,45] Differences in reported values may reflect assay sensitivity and pre-analytical variations in stool processing. Despite these variations, VIM methylation remains a reliable component of stool-based CRC detection panels.

Prognostic biomarkers

Prognostic biomarkers are critical for predicting tumor behavior, treatment response, and disease recurrence in CRC. They provide precise information regarding disease risk and progression, thereby guiding clinical decision-making. Among these, epigenetic modifications - particularly DNA methylation alterations- have emerged as highly informative prognostic indicators. This section compares key DNA methylation biomarkers, highlighting their diagnostic and prognostic significance.

SFRP gene family and Wnt signaling pathway

Aberrant methylation of the SFRP gene family has been identified across multiple human cancers. One principal mechanism driving tumorigenesis is activation of the Wnt signaling pathway resulting from downregulation of SFRP expression.[46] Promoter methylation of SFRP1 and SFRP2 has been associated with increased CRC risk.[46] Kumar et al.,[47] evaluated SFRP1 promoter methylation in 54 CRC patients at stages II–III and reported significant associations with lymph node invasion (P=0.05) and reduced overall survival (OS), suggesting SFRP1 methylation as a potential prognostic biomarker. Additionally, SFRP2 hypermethylation was observed in 66.7% of CRC patients.[47] Across studies, methylation rates vary due to differences in cohort size, tumor stage distribution, and analytical sensitivity. Collectively, SFRP1 and SFRP2 methylation appear to reflect tumor aggressiveness and Wnt pathway dysregulation in CRC.

p16 Tumor Suppressor Gene

The p16 tumor suppressor gene plays a pivotal role in cell cycle regulation. Ye et al.,[48] reported significantly higher p16 methylation in CRC tissues compared with adjacent normal tissue. Li et al.,[49] found that p16 hypermethylation correlated with lymph node metastasis, occurring in 32.3% of CRC cases, whereas another study reported a lower frequency of 15.1%.[50] Variability across studies likely reflects methodological heterogeneity, including differences in methylation-specific PCR protocols, cutoff thresholds, or tissue sampling. Despite

these differences, the consistent association of p16 methylation with advanced disease suggests its utility as a prognostic marker for metastatic progression.

Table-1. Summary of DNA methylation biomarkers in colorectal cancer

Biomarker	Gene Symbol	Role/Function	Application	Sample Type	Sensitivity / Specificity	References
SEPT9	SEPT9	Tumor suppressor; hypermethylation silences expression, associated with late-stage CRC.	Diagnostic	Blood / Plasma	75.1% sensitivity, 95.1% specificity	[18]
SDC2	SDC2	Membrane-associated protein regulating proliferation and migration; frequently hypermethylated in CRC.	Diagnostic	Stool	86% sensitivity, 92% specificity	[23–26]
NDRG4	NDRG4	Regulates cell proliferation, differentiation, and stress response; promoter methylation in CRC.	Diagnostic	Stool, Urine	86% sensitivity, 92% specificity	[31–37]
BMP3	BMP3	Tumor suppressor; hypermethylation occurs in early polyps and CRC progression.	Diagnostic	Stool, Plasma	40% sensitivity, 94% specificity	[38–42]
MGMT	MGMT	DNA repair enzyme; promoter methylation impairs genomic stability.	Diagnostic, Predictive	Tissue, Serum	90% methylation in CRC cases	[27–30]
VIM	VIM	Cytoskeletal protein; promoter methylation detected in stool DNA of CRC patients.	Diagnostic	Stool	52% sensitivity, 88% specificity	[43–45]
LINE-1	LINE-1	Retrotransposon; hypomethylation correlates with genomic instability and tumor progression.	Prognostic	Tissue	Associated with advanced CRC stages	[51–54]
SFRP1 / SFRP2	SFRP1/2	Wnt pathway inhibitors; promoter methylation activates oncogenic signaling.	Prognostic	Tissue	66.7% methylation in CRC (SFRP2)	[46, 47]
p16	CDKN2A	Cell cycle regulator; hypermethylation linked to lymph node metastasis.	Prognostic	Tissue	32.3% methylation in CRC	[48–50]
BCAT1 / IKZF1	BCAT1 / IKZF1	BCAT1: Metabolic enzyme; IKZF1: Transcriptional regulator; aberrantly methylated in CRC.	Prognostic	Plasma	66% sensitivity for CRC detection	[55–60]
RASSF1A	RASSF1A	Tumor suppressor; methylation associated with poor prognosis.	Prognostic	Tissue	Independent prognostic factor	[61–63]
MLH1	MLH1	DNA mismatch repair; promoter methylation causes microsatellite instability (MSI).	Predictive (5-FU response)	Tissue	15% of CRC cases exhibit MSI	[66–74]
WNT5A	WNT5A	Non-canonical Wnt ligand; methylation suppresses EMT and metastasis.	Predictive (5-FU response)	Tissue	32–35% methylation in CRC	[75–77]

LINE-1 Hypomethylation and Genomic Instability

LINE-1 (long interspersed nuclear elements-1) are transposable elements normally heavily methylated in somatic cells, preserving genomic stability by preventing their mobilization.[51] Shadman et al.,[51] examined LINE-1 promoter methylation across CRC stages and found higher methylation in non-advanced adenomas compared to advanced adenomas and adenocarcinomas. A meta-analysis by Bachitta et al.,[52] confirmed the association of LINE-1 hypomethylation with poor prognosis, disease progression, and advanced tumor stage.[53,54] These findings indicate that global DNA hypomethylation, reflected by LINE-1 demethylation, contributes to chromosomal instability and tumor aggressiveness. LINE-

1 methylation status may thus serve as a surrogate marker of genomic integrity and disease severity in CRC.

BCAT1 and IKZF1 as biomarkers for CRC detection

Promoter methylation of IKZF1 has been linked to disrupted cell proliferation and differentiation,[55] while BCAT1 is frequently hypermethylated in CRC.[56] Mitchell et al.,[57] reported that BCAT1 and IKZF1 expression levels in plasma were markedly elevated in CRC patients, whereas healthy controls showed levels of 3.5% and 4.9%, respectively. Another study involving 2,127 samples, including 129 CRC cases, demonstrated an overall sensitivity of 66%, with stage-specific detection rates of 38%, 69%, 73%, and 94% for stages I–IV, respectively.[58] In patients with recurrent CRC, post-surgical positivity for BCAT1/IKZF1 correlated with higher risk of residual disease and recurrence, outperforming conventional CEA testing; positive cases were twice as likely to experience recurrence.[59,60] These findings highlight BCAT1 and IKZF1 as promising blood-based biomarkers for non-invasive CRC detection and recurrence monitoring. Standardization of methylation detection platforms will be essential for routine clinical implementation.

RASSF1A tumor suppressor gene

RASSF1A regulates cell proliferation and apoptosis and is frequently epigenetically silenced in cancers, including CRC.[61] Ni et al.,[62] assessed RASSF1A methylation in patients with laterally spreading tumors (LST), observing levels intermediate between polypoid adenomas and CRC. These data suggest RASSF1A as a potential biomarker for early CRC detection. Sun et al.,[63] reported that RASSF1A methylation independently predicted prognosis in patients receiving oxaliplatin-based chemotherapy. A meta-analysis further identified RASSF1A hypermethylation as a significant risk factor and prognostic indicator.[64] RASSF1A methylation is particularly relevant in stages II and III CRC, and ongoing research may reveal its potential as a therapeutic target to enhance chemotherapy efficacy.

Epigenetic biomarkers, particularly DNA methylation alterations, offer substantial promise for CRC diagnosis, prognosis, and therapeutic guidance. Markers including SFRP, p16, LINE-1, BCAT1, IKZF1, and RASSF1A are associated with tumor progression, metastasis, and patient survival. Assessing their methylation status can improve early CRC detection and aid in predicting treatment response. However, cross-study variability underscores the need for standardized detection methods and large, longitudinal cohorts to confirm prognostic reliability. Future research should focus on validating these biomarkers in large-scale studies and integrating them into clinical practice to enable personalized CRC management.

Predictive biomarkers for treatment response

Chemotherapy resistance in CRC contributes to variable patient responses to 5-fluorouracil (5-FU)-based regimens, resulting in heterogeneous clinical outcomes and prognosis. This variability underscores the need for effective predictive biomarkers to guide therapeutic decision-making. Although numerous biomarkers have been evaluated, only a subset of methylation markers has demonstrated sufficient evidence to support their utility in predicting treatment response.[65] Interpatient differences often reflect molecular heterogeneity, including variations in DNA repair capacity, epigenetic regulation, and tumor microenvironment composition. Consequently, understanding predictive methylation patterns may enable more precise, individualized treatment strategies.

MLH1 methylation as a predictive marker

MLH1, a central component of the DNA mismatch repair (MMR) system, encodes a protein that collaborates with other DNA repair enzymes to recognize and correct replication errors.[66] Aberrant hypermethylation of the MLH1 promoter silences gene expression, compromising genomic stability and contributing to CRC development.[67] Approximately 15% of CRC cases display high microsatellite instability (MSI), largely reflecting MLH1 silencing via promoter methylation.[68] In clinical practice, MLH1 promoter methylation assays are a cost-effective approach to identifying MSI-high tumors in sporadic CRC.[69]

The prognostic and predictive significance of MLH1 methylation has been demonstrated in multiple studies. Fu et al.,[70] evaluated 115 stage II CRC patients, stratifying them by combined CIMP and MLH1 methylation status. Tumors that were CIMP-positive/MLH1-unmethylated exhibited increased aggressiveness and poorer outcomes, with the shortest disease-free survival (DFS) and overall survival (OS) among subgroups. These findings indicate that integrating CIMP status with MLH1 methylation provides a meaningful classification of stage II CRC subtypes. A meta-analysis of 47 studies including 4,296 cases and 2,827 controls confirmed a significant association between MLH1 methylation and CRC risk.[66] Kuan et al.,[71] further demonstrated that patients with advanced CRC and MLH1-methylated tumors experienced higher recurrence rates compared with patients harboring locally invasive, unmethylated tumors.

Beyond prognostic value, MLH1 methylation serves as a

predictive biomarker for 5-FU-based chemotherapy. Multiple clinical reports indicate that MLH1 methylation correlates with treatment response and overall survival.[72,73] In vitro studies suggest that MLH1 downregulation, typically resulting from promoter hypermethylation, reduces cancer stem cell sensitivity to 5-FU,[74] highlighting MLH1's critical role in mediating chemotherapy cytotoxicity. However, predictive associations are not uniformly consistent across studies, potentially due to differences in patient cohorts, MSI testing criteria, and treatment regimens. Standardization of methylation cutoffs and integration with additional MMR markers may enhance MLH1's clinical utility as a predictive biomarker.

WNT5A methylation and treatment response

The Wnt signaling network regulates proliferation, differentiation, and other fundamental cellular processes, with dysregulation constituting a hallmark of CRC.[75] While canonical Wnt/β-catenin hyperactivation -often driven by APC or CTNNB1 mutations- initiates tumorigenesis, non-canonical Wnt ligands, including WNT5A, exert context-dependent effects. Physiologically, WNT5A antagonizes canonical Wnt signaling, but in CRC, elevated WNT5A expression can paradoxically promote epithelial-to-mesenchymal transition (EMT), enhancing tumor cell invasion and metastasis.[76]

Epigenetic silencing of WNT5A via promoter hypermethylation occurs in approximately 32–35% of CRC cases, even during early stages. Clinically, WNT5A hypermethylation is associated with improved progression-free survival and enhanced sensitivity to 5-FU-based chemotherapy.[77] Mechanistically, WNT5A inactivation may suppress EMT and reduce metastatic potential, rendering tumors more responsive to treatment. These observations position WNT5A methylation as a promising predictive biomarker, enabling stratification of patients likely to benefit from 5-FU while identifying those who may require alternative therapies, such as inhibitors targeting non-canonical Wnt pathways. Nonetheless, some studies report conflicting associations between WNT5A methylation and survival outcomes, likely reflecting differences in tumor stage, epigenetic heterogeneity, and treatment protocols. Integrating WNT5A methylation with additional Wnt pathway biomarkers may improve predictive accuracy.

Conclusion

DNA methylation represents a cornerstone of CRC biology, linking molecular pathogenesis to clinical translation. Across diagnostic, prognostic, and predictive domains, methylation-based biomarkers highlight how epigenetic regulation can inform all stages of CRC management.

For screening and early detection, non-invasive assays targeting methylated genes such as SEPT9, SDC2, BMP3, and NDRG4 demonstrate high sensitivity and specificity, often surpassing conventional methods like FIT, underscoring their potential to enable timely intervention.

In prognostication, methylation of genes including SFRP1, p16, LINE-1, BCAT1, IKZF1, and RASSF1A correlates with tumor aggressiveness, metastatic potential, and patient survival, providing tools for precise risk stratification and personalized treatment planning. For instance, LINE-1 hypomethylation indicates aggressive disease, while RASSF1A methylation patterns associate with treatment outcomes.

For predictive applications, MLH1 hypermethylation and WNT5A methylation offer insights into chemotherapy responsiveness, particularly to 5-FU-based regimens. These markers provide a molecular basis for tailoring therapeutic strategies, enhancing patient outcomes through precision oncology approaches.

Despite these advances, challenges remain, including assay variability, limited population validation, and the absence of standardized detection protocols. Future research should focus on integrating liquid biopsy technologies, multi-omics approaches, and large-scale validation studies to overcome these barriers and facilitate consistent clinical implementation.

Overall, comprehensive methylation profiling holds substantial promise for transforming CRC management - from early detection and prognostication to personalized treatment optimization.

Practical points in Biochemistry/Nutrition:

► Blood/stool DNA methylation of genes like SEPT9 and NDRG4 enables non-invasive early CRC detection. Methylation of MLH1 and WNT5A helps predict chemotherapy response for personalized treatment.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

CRC: Colorectal Cancer; FIT: Fecal Immunochemical Test;

FOBT: Fecal Occult Blood Test; cfDNA: Cell-free DNA; CIMP: CpG Island Methylator Phenotype; MSI: Microsatellite Instability; DNMT: DNA Methyltransferase; SAM: S-adenosylmethionine; MSP: Methylification-specific PCR; IHC: Immunohistochemistry; OS: Overall Survival; DFS: Disease-free Survival; 5-FU: 5-Fluorouracil; MMR: Mismatch Repair; EMT: Epithelial-to-Mesenchymal Transition; PI3K/AKT: Phosphoinositide 3-kinase/Protein Kinase B; MAPK/ERK: Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase; MLH1: MutL Homolog 1; APC: Adenomatous Polyposis Coli; SFRP1/2: Secreted Frizzled-Related Protein ½; LINE-1: Long Interspersed Nuclear Element-1; SEPT9: Septin 9; ALX4: Aristaless-like Homeobox 4; SDC2: Syndecan 2; MGMT: O-6-Methylguanine-DNA Methyltransferase; NDRG4: N-myc Downstream Regulated Gene 4; BMP3: Bone Morphogenetic Protein 3; VIM: Vimentin; p16 / CDKN2A: Cyclin-Dependent Kinase Inhibitor 2A; BCAT1: Branched-Chain Amino Acid Transaminase 1; IKZF1: IKAROS Family Zinc Finger 1; RASSF1A: Ras Association Domain Family Member 1A; WNT5A: Wnt Family Member 5A; BRAF: B-Raf proto-oncogene, serine/threonine kinase; CTNNB1: Catenin Beta 1; LST: Laterally Spreading Tumor; CEA: Carcinoembryonic Antigen.

Authors' contributions

MKH reviewed the literature and drafted the manuscript. MHD supervised and revised the manuscript. All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Institutional Review Board approval (code: IR.KaUMS.REC.1396.119) was obtained (April 2023).

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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