



Review

The Dual Role of DNA Hypermethylation and Hypomethylation in Colorectal and Gastric Tumorigenesis: Mechanisms and Non-Invasive Biomarker

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Abstract

DNA methylation is a key epigenetic modification catalyzed by DNA methyltransferases (DNMTs) and predominantly occurs at cytosine-phosphate-guanine (CpG) islands, which are often located in gene promoter regions. Hypermethylation of CpG islands within gene promoters can silence tumor suppressor gene expression, thereby disrupting normal cellular functions, including maintenance of genomic stability and regulation of cell growth, and contributing to tumor initiation and progression. In contrast, global hypomethylation may promote genomic instability and oncogene activation. This review discusses the molecular mechanisms underlying DNA methylation and evaluates its functional and clinical significance in colorectal and gastric cancers, with emphasis on its potential application as a noninvasive biomarker for diagnosis.

Keywords: DNA methylation; DNA demethylation; gastrointestinal cancer; clinical biomarker

1. Introduction

DNA methylation is a crucial epigenetic modification that regulates gene expression by adding methyl groups to cytosine bases in Cytosine-Phosphate-Guanine (CpG) islands. In cancer, this process becomes dysregulated: tumor suppressor genes often undergo promoter hypermethylation, leading to their silencing, while global hypomethylation can promote genomic instability [1]. These alterations play a pivotal role in tumor initiation and progression. Gastrointestinal cancers, including gastric and colorectal cancer, frequently exhibit such aberrant methylation patterns. This review systematically explores the molecular mechanisms of DNA methylation and demethylation, focusing on their roles in gene silencing and gastrointestinal tumorigenesis. Furthermore, it highlights the growing clinical significance of DNA methylation as sensitive biomarkers for early detection, prognosis, and monitoring of colorectal and gastric cancers, underscoring their potential in advancing cancer diagnostics and personalized medicine.

2. Mechanism of DNA Methylation and Demethylation

2.1 DNA Methylation and Key Enzymes in DNA Methylation

2.1.1 DNA Methylation

The chemical essence of DNA methylation lies in an enzymatic process catalyzed by DNA methyltransferases (DNMTs), whereby a methyl group (-CH₃) is covalently attached to the fifth carbon (C5) of a cytosine base, resulting

in the formation of 5-methylcytosine (5-mC) [2–4]. This transformation can be summarized by a simplified chemical equation: cytosine + S-adenosylmethionine (SAM) → 5-methylcytosine + S-adenosylhomocysteine (SAH) (Fig. 1 *De novo* methylation) [4–6].

The catalytic mechanism proceeds through three well-defined steps. To begin with, a cysteine residue within the active site of DNMT acts as a potent nucleophile, attacking the C6 position of the cytosine ring to generate an enzyme-substrate covalent intermediate [7–10]. After that, this covalent bond alters the electron distribution within the pyrimidine ring, increasing the nucleophilicity of the C5 position [11], which attacks the electrophilic methyl carbon of SAM. The methyl group is thereby transferred from SAM to the C5 atom of cytosine, forming the core structure of 5-mC [10,12–14]. In the end, a general base within the active site (such as a glutamate residue) abstracts a proton from the C5 position of the nascent 5-mC, cleaving the covalent bond at C6 and releasing the product, 5-mC, into the DNA duplex. Meanwhile, the DNMT enzyme is restored to its original state, ready for another catalytic cycle [10,12].

2.1.2 DNMTs

DNMTs are a critical family of enzymes that primarily catalyze the process of DNA methylation, which involves the transfer of a methyl group from SAM to cytosine bases in DNA, resulting in the formation of 5-mC [15]. This process plays a central role in epigenetic regulation. Based on their distinct functions and mechanisms, DNMTs can be classified into three main categories: maintenance methyl-



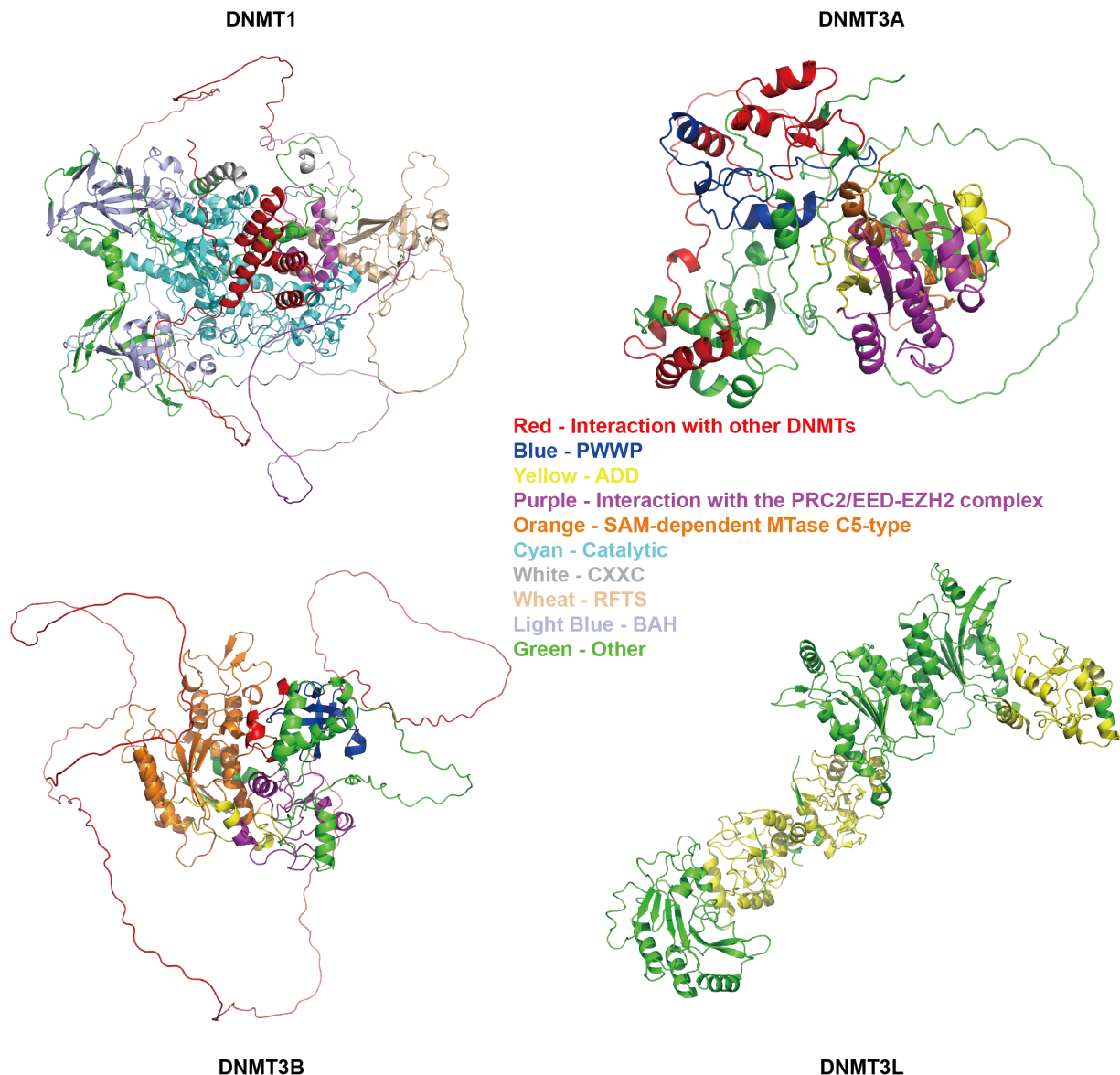


Fig. 1. Structure of DNMTs. PWWP, Pro-Trp-Trp-Pro domain; ADD, ATRX-DNMT3-DNMT3L domain; PRC2, Polycomb Repressive Complex 2; EED, Embryonic Ectoderm Development; EZH2, Enhancer of Zeste Homolog 2; SAM, S-AdenosylMethionine; CXXC, CXXC zinc finger domain; RFTS, Replication Focus Targeting Sequence; BAH, Bromo-Adjacent Homology domain. The image was obtained from AlphaFold (<https://alphafold.com/>) and visualized using PyMOL (<https://www.pymol.org>).

transferases, *de novo* methyltransferases and auxiliary regulatory factors [9,16,17] (Fig. 1). These members work in concert to ensure the accurate execution of DNA methylation in biological processes such as gene expression and genomic stability.

2.1.2.1 Maintenance Methyltransferases – DNMT1. The primary function of DNMT1 is to accurately maintain and transmit DNA methylation patterns to daughter cells during DNA replication, a mechanism that relies on its specific recognition and catalytic activity toward hemi-methylated DNA [18]. After DNA replication, the parental strand retains its original methylation marks, while the newly syn-

thesized daughter strand remains unmethylated, resulting in a transient hemi-methylated state. DNMT1 specifically recognizes these hemi-methylated CpG sites and uses the methylated parental strand as a template to catalyze the methylation of the corresponding cytosine residues on the daughter strand, thereby restoring full methylation. This process constitutes the basis of “cellular memory” in epigenetic inheritance, ensuring the stable transmission of gene expression patterns—such as the silencing or activation of tissue-specific genes—across cell generations, and maintaining the silent state of critical functional genes like tumor suppressor genes [19]. Furthermore, in female somatic

cells, DNMT1 is involved in sustaining the stable silencing of one X chromosome, underscoring its essential role in epigenetic regulation [20].

2.1.2.2 *De Novo* Methyltransferases – DNMT3A/DNMT3B. DNMT3A and DNMT3B are *de novo* DNA methyltransferases responsible for establishing novel DNA methylation patterns at previously unmethylated CpG sites during early embryonic development and germ cell formation [21–23]. Unlike maintenance methyltransferases, they operate independently of pre-existing methylation templates [24]. Their catalytic activity directly methylates unmodified DNA, with targeting specificity guided by their structural domains and interactions with partner proteins [25,26]. The targeted recruitment of DNMT3A/3B to specific genomic loci is facilitated by multiple mechanisms, for example, their Pro-Trp-Trp-Pro (PWWP) domain recognizes H3K36me3—a histone mark enriched in transcriptionally active regions—while specific transcription factors anchor them to several gene promoters [27]. Furthermore, the PWWP domain of DNMT3B is essential for its interaction with the transcription factor ZHX1 (which contains a homeobox motif), and this interaction potentiates DNMT3B-mediated transcriptional repression [28]. Additionally, during gametogenesis, they collaborate with DNMT3L to establish parent-of-origin-specific methylation marks at imprinting control regions (ICRs) [29,30]. Biologically, DNMT3A and DNMT3B play essential roles in embryogenesis and cellular differentiation by establishing methylation landscapes that guide pluripotent stem cell fate and lineage commitment [21,22]. They also contribute to genomic stability by silencing transposable elements and retrotransposons through methylation, thereby preventing mutagenic transposition [31].

2.1.2.3 Auxiliary Regulatory Factors – DNMT3L. DNMT3L is an epigenetic regulatory factor that, although lacking catalytic activity itself, functions as a key auxiliary protein that significantly enhances and modulates the methylation functions of DNMT3A and DNMT3B [29,30]. Mechanistically, DNMT3L forms stable complexes with DNMT3A or DNMT3B, inducing conformational changes that greatly increase their affinity for the methyl donor SAM, thereby effectively promoting catalytic efficiency [29,30]. Biologically, DNMT3L is highly expressed during gametogenesis, where it is essential for establishing correct parent-of-origin-specific methylation patterns at ICRs [29,30].

2.1.3 Structural Insights into the Distinct Functions of DNMTs

According to UniProt (<https://www.uniprot.org/>), we analyzed the structures of DNMTs and presented in Fig. 1, where each color represents a distinct functional domain.

Both DNMT1 and DNMT3A/B contain specific domains that mediate interactions with other DNMTs, enabling them to form complexes and exhibit functional synergy [32]. Furthermore, the C-terminal of these enzymes all harbor a conserved SAM - dependent methyltransferase domain, which constitutes the molecular basis for their DNA methyltransferase activity. Additionally, the presence of domains that facilitate interaction with the Polycomb Repressive Complex 2/Embryonic Ectoderm Development-Enhancer of Zeste Homolog 2 (PRC2/EED-EZH2) complex provides structural support for their recruitment to specific genomic loci and the execution of their catalytic functions [33].

Fundamental differences in domain architecture between DNMT1 and DNMT3A/B dictate their distinct roles in epigenetic regulation. DNMT1 contains unique Replication Focus Targeting Sequence (RFTS) and Bromo-Adjacent Homology (BAH) domains, which collectively confer its characteristic maintenance DNA methylation activity. The RFTS domain mediates autoinhibition by occupying the DNA-binding channel of the catalytic center, which is relieved specifically after DNA replication when Ubiquitin-like with Plant HomeoDomain finger (PHD) and RING Finger Domains 1 (UHRF1) and other proteins recognize the hemimethylated DNA signal [34,35]. This ensures that DNMT1 specifically methylates hemimethylated CpG sites, enabling accurate copying of the parental methylation pattern to the daughter strand. The BAH domain mediates protein-protein interactions, contributing to the stabilization of DNMT1 at the DNA replication fork [36]. Moreover, its CXXC zinc finger (CXXC) domain specifically binds to unmethylated CpG dinucleotides, positioning an auto-inhibitory linker between the DNA and the active site, thereby ensuring that only hemimethylated CpG sites undergo methylation [8,34].

In contrast, DNMT3A and DNMT3B are equipped with distinct targeting modules—the ATRX-DNMT3-DNMT3L (ADD) and PWWP domains—enabling them to perform *de novo* methylation [37,38]. The ADD domain recruits DNMT3A/B to the promoters of genes destined for transcriptional silencing by recognizing the unmethylated state of H3K4me0 [38], while the PWWP domain specifically recognizes H3K36me2/3; particularly in DNMT3B [39]. This dual recognition system allows DNMT3A/B to establish novel DNA methylation patterns in specific functional regions of the genome, such as promoters and gene bodies, based on distinct histone modification signals, thereby precisely establishing the epigenetic landscape during embryonic development and cell differentiation [9,37].

DNMT3L possesses an ADD domain structurally similar with that of DNMT3A/B but lacks intrinsic catalytic activity. It interacts with DNMT3A/B via its ADD domain, stabilizing its conformation and thereby functioning as a crucial auxiliary factor that significantly enhances the *de novo* methyltransferase activity [29,30,37].

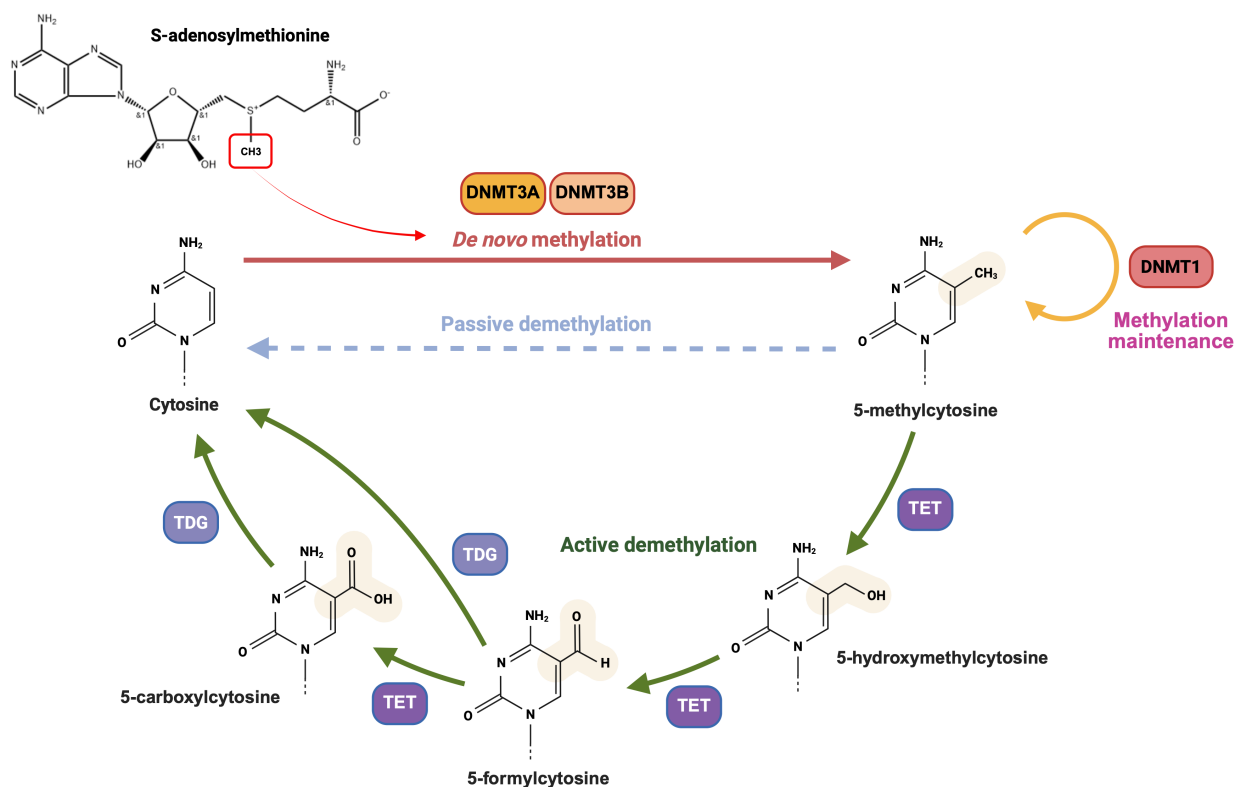


Fig. 2. Chemical mechanism of DNA methylation and demethylation. DNA methylation and demethylation are concurrent dynamic processes. Methylation, catalyzed by DNA methyltransferases, covalently adds a methyl group to form 5-methylcytosine. Demethylation occurs via passive or active mechanisms. Passive demethylation dilutes methylation during DNA replication without chemical removal, while active demethylation enzymatically removes methyl groups via TET - mediated oxidation and TDG mediated - base excision repair throughout the cell cycle. DNMT, DNA methyltransferase; TET, ten-eleven-translocation enzyme; TDG, thymine-DNA glycosylase. Created in BioRender. (2025) <https://BioRender.com/dveowg0>.

2.2 DNA Demethylation and Key Enzymes in DNA Demethylation

2.2.1 DNA Demethylation

DNA methylation and demethylation are dynamically opposing processes that occur concurrently. Demethylation proceeds through two distinct mechanisms: passive and active (Fig. 2 Passive demethylation & Active methylation) [40–42].

Passive demethylation is a cell division-dependent process that leads to the dilution of methylation levels during DNA replication due to incomplete maintenance of methylation patterns, without involving direct chemical removal of the methyl group [16,42]. In contrast, active demethylation enables rapid and specific removal of methyl groups at any stage of the cell cycle. Active demethylation is primarily mediated by the Ten-eleven-translocation (TET) dioxygenase family and can be divided into two sequential biochemical stages: The first stage involves TET-mediated iterative oxidation, during which TET enzymes progressively catalyze the oxidation of 5-mC to generate 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and finally 5-carboxylcytosine (5-caC) [43–46]. The sec-

ond stage entails base excision repair (BER), which consists of recognition/excision and repair/replacement steps by thymine-DNA glycosylase (TDG) [44,45,47].

2.2.2 Key Enzymes in DNA Demethylation

2.2.2.1 TET. The TET enzyme family, including TET1/2/3, plays a key role as dioxygenases in the active DNA demethylation process. Their primary function is to catalyze the stepwise oxidation of 5-mC, successively generating 5-hmC, 5-fC and 5-caC [43,48]. Structurally, both TET1 and TET3 contain a CXXC zinc finger domain that specifically recognizes and binds to unmethylated CpG islands, thereby recruiting these enzymes to specific genomic regions such as gene promoters [48]. Due to a chromosomal inversion, TET2 has lost its CXXC domain (which is now encoded by the *IDAX* gene) and relies on interactions with other proteins for its targeting mechanism [49]. The catalytic function of all TET enzymes depends on the C-terminal dioxygenase domain, which uses Fe^{2+} and α -ketoglutarate (α -KG) as cofactors to efficiently catalyze the multi-step oxidation of 5-mC [48]. Due to their ability to reverse DNA methylation, TET enzymes are regarded as

Table 1. Key enzymes in DNA methylation and demethylation.

Process participation	Enzyme	General description	Key structural domains	Primary biological functions	Citations
Methylation	DNMT1	Maintenance Methylation	RFTS, CXXC, BAH, Catalytic Domains	Propagates methylation patterns during DNA replication, ensuring epigenetic memory and cellular identity inheritance.	[8,9,15,18–20,34–36]
	DNMT3A/3B	<i>De novo</i> Methylation	PWWP, ADD, Catalytic Domains	Establishes novel methylation patterns <i>de novo</i> ; critical for embryogenesis, silencing transposons, and genomic imprinting.	[9,21–31,37–39]
	DNMT3L	Auxiliary Regulatory Factors	Similar with DNMT3A/3B but lacks catalytic domain	Enhances the catalytic activity and specificity of DNMT3A/3B, particularly during germ cell development.	[9,29,30,37]
Demethylation	TET	Active Demethylations Initiation	CXXC (TET 1/3), IDAX (TET2), Dioxygenase Domain	Initiates active DNA demethylation by oxidizing 5-mC to 5-hmC, 5-fC, and 5-caC.	[43–46,48,49]
	TDG	Glycosylase	Uracil-DNA Glycosylase Domain	Completes active demethylation by excising TET oxidation products (5-fC/5-caC), initiating BER to restore an unmodified cytosine.	[44,45,47,50–54]

DNMT, DNA methyltransferases; TET, Ten-eleven-translocation; TDG, thymine-DNA glycosylase; RFTS, Replication Focus Targeting Sequence; CXXC, CXXC zinc finger domain; BAH, Bromo-Adjacent Homology domain; PWWP, Pro-Trp-Trp-Pro domain; ADD, ATRX-DNMT3-DNMT3L domain; 5-mc, 5-methylcytosine; 5-hmc, 5-hydroxymethylcytosine; 5-fc, 5-formylcytosine; 5-caC, 5-carboxylcytosine; BER, base excision repair.

“erasers” or dynamic regulators in epigenetic modulation [43–45] (Table 1, Ref. [8,9,15,18–31,34–39,43–54]).

2.2.2.2 TDG. TDG is a key glycosylase involved in the BER pathway and plays a central role in the final step of active DNA demethylation [45,47]. Specifically, glycosylases such as TDG recognize 5-fC and 5-caC and hydrolyze the N-glycosidic bond between the aberrant base and the deoxyribose sugar, resulting in an apurinic/aprimidinic (AP) site [50]. Subsequently, AP endonuclease I (APE1) cleaves the phosphodiester backbone at the AP site [51]. DNA polymerase (typically Pol β) then incorporates an unmodified cytosine (C) into the gap, using the guanine (G) on the complementary strand as a template. Finally, DNA ligase seals the nick, completing the repair process and restoring an unmethylated cytosine [51–53]. Structurally, TDG belongs to the uracil-DNA glycosylase superfamily. Its active site pocket is specialized in shape and chemical properties to efficiently and specifically accommodate 5-fC and 5-caC, while showing minimal activity toward normal bases such as C and T [50]. Additionally, TDG participates in repairing G - U or G - T mismatches caused by the spontaneous deamination of cytosine or 5-mC, thereby contributing to genomic stability [54]. This characteristic ensures high fidelity in the repair process. Owing to its functions, TDG is regarded as a “quality inspector” of the genome and a “terminal executor” in the demethylation process [44] (Table 1).

3. Mechanism of DNA Methylation in Gene Silencing

CpG islands, which are often located in promoter regions, play a critical role in the initiation of transcription. Methylation of these CpG-rich regions can disrupt the assembly of the transcription initiation complex, leading to failure of transcriptional activation and consequent silencing of the associated gene [55]. Mechanistically, DNA methylation-mediated gene silencing operates through two principal modes: direct physical interference with transcription factor binding, and indirect repression via recruitment of methyl-binding proteins and histone modifiers that promote a transcriptionally inactive chromatin state (Fig. 3) [15].

3.1 Direct Physical “Obstacle”

DNA methylation at CpG islands within gene promoter regions directly interferes with the transcriptional initiation process. The core mechanism involves the methyl group added to the cytosine base, which creates a protruding steric hindrance that physically occupies the major groove of the DNA double helix [56]. This region is precisely where many transcription factors must recognize and bind to their specific DNA sequences. Consequently, the methyl “obstacle” directly impedes the ability of critical transcription factors to bind to their target sites [4]. This failure in binding prevents the successful assembly of the transcription initiation complex, ultimately halting the gene transcription process. In essence, methylation effectively

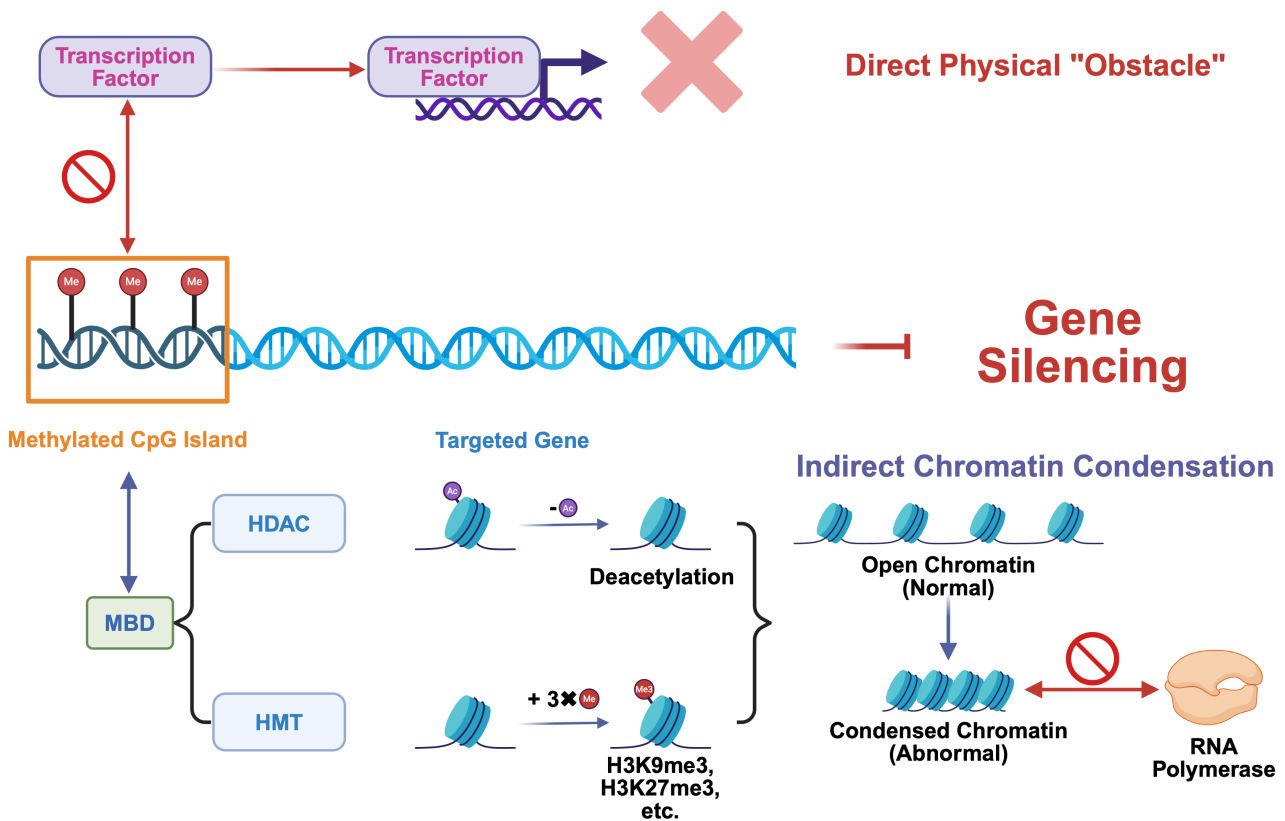


Fig. 3. Mechanism of DNA methylation in gene silencing. Gene silencing via DNA methylation occurs through two primary mechanisms: direct physical blockade of transcription factor access and indirect repression mediated by methyl-binding proteins and histone modifiers, which collectively establish transcriptionally inactive chromatin. CpG island, Cytosine-Phosphate-Guanine island; MBD, methyl-CpG-binding domain protein; HDAC, histone deacetylase; HMT, histone methyltransferase. Created in BioRender. (2025) <https://BioRender.com/o6r8csp>.

blocks the DNA, preventing the transcription factor from engaging and thus directly leading to gene silencing [57].

3.2 Indirect Chromatin Condensation

In addition to direct steric hindrance, DNA methylation exerts a more potent and prevalent gene silencing effect through an indirect mechanism involving the recruitment of repressive complexes and chromatin remodeling. This process initiates when methyl-CpG-binding domain proteins (MBDs), such as MeCP2, recognize and bind to methylated CpG sites, serving as “readers” of the DNA methylation mark [58,59]. These MBD proteins then act as platforms to recruit chromatin-modifying enzymes, including histone deacetylases (HDACs) and histone methyltransferases (HMTs) [60]. HDACs remove acetyl groups from histones, promoting chromatin condensation [61,62], while HMTs catalyze the addition of repressive histone marks [62], such as H3K9me3 [63] and H3K27me3. These modifications collectively facilitate the transition from an open, transcriptionally permissive euchromatin state to a tightly packed, transcriptionally inert heterochromatin structure [63]. As a result, the chromatin becomes inaccessible to

the transcriptional machinery, leading to stable and heritable gene silencing [15].

4. DNA Hypermethylation and Hypomethylation Associated With Poor Gastrointestinal Tumorigenesis

4.1 Hypermethylation

DNA hypermethylation of tumor suppressor genes contributes to the carcinogenesis of solid tumors. For example, hypermethylation of tumor suppressor genes such as TP53 leads to their silencing, which is associated with enhanced tumor malignancy and progression [64]. In colorectal neuroendocrine neoplasms (CRNEN), hypermethylation of TREM1 locus (e.g., at site cg04451353) promotes the infiltration of immunosuppressive cell subsets such as CD14⁺CD16⁻ monocytes, thereby driving tumor progression [65]. In colorectal cancer (CRC), hypermethylation of ZNF671 and ZNF132 is associated with a low Immunoscore and contributes to an immunosuppressive microenvironment by negatively regulating T-cell and macrophage functions [66]. A novel research proves that under chronic Cd exposure, TET2 is inhibited, thus lead-

Table 2. DNA hypermethylation and hypomethylation associated with gastrointestinal tumorigenesis.

Hyper/Hypo-methylation	Cancer type	Key enzyme change	Gene expression change	Mechanism	Citation
Hyper-methylation	CRNEN	/	<i>TREM1</i> Low	Promotes infiltration of immunosuppressive cell subsets, such as CD14 ⁺ CD16 ⁻ monocytes.	[65]
	CRC	/	<i>ZNF671/132</i> Low	Correlates with low Immunoscore and negatively regulates T-cell and macrophage functions.	[66]
		TET2 Inhibited by Cd	<i>HADHB</i> Low	Activates the FAK signaling pathway and ultimately contributes to CRC progression.	[67]
	GC	/	<i>SFRP2</i> Low	Activates downstream WNT signaling pathway aberrantly, facilitating tumor proliferation.	[68]
			<i>RPRM</i> Low	Attenuates p53 signaling pathway, promoting malignant transformation of cells.	
		DNMT1 Overexpression	<i>SOX21</i> Low	Induces overexpression of downstream CKS2, driving gastric cancer progression.	[69]
Hypo-methylation	COAD	/	Lnc00346 High	Upregulates the miR-589-5p/CCL5 axis to enhance tumor progression.	[70]
	CRC	/	<i>MAGEA</i> High	Triggers aberrant expression of cancer-testis antigens, increasing invasiveness and malignancy.	[71]
		/	<i>CCM2</i> High	Influences the tumor microenvironment via ROCK-mediated endothelial cell senescence.	[72]
				<i>ICAM</i> High	Enhances colorectal cancer metastasis through the Fusobacterium nucleatum-activated ALPK1/NF- κ B axis.
	GC	TET2	<i>WTIP</i> High	Inhibits the phosphorylation and kinase activity of AKT.	[73]

CRNEN, colorectal neuroendocrine neoplasms; CRC, colorectal cancer; GC, gastric cancer; COAD, colon adenocarcinoma; TREM1, triggering receptor expressed on myeloid cells 1; *ZNF*, zinc finger protein gene; TET2, Ten-eleven-translocation 12; *HADHB*, hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta; FAK, Focal Adhesion Kinase signaling pathway; *SFRP2*, secreted frizzled-related protein 2; *RPRM*, reprimin; DNMT1, DNA methyltransferase 1; *SOX21*, SRY-box transcription factor 21; Lnc, long non-coding; *MAGEA*, melanoma antigen family A; *CCM2*, cerebral cavernous malformation 2; *ICAM*, intercellular adhesion molecule; CD, cluster differentiation; WNT, Wingless-type MMTV integration site family member; CKS2, CDC28 Protein Kinase Regulatory Subunit 2; CCL, C-C motif chemokine ligand; ROCK, Rho-associated coiled-coil containing protein kinase; ALPK1, alpha-protein kinase 1; NF- κ B, nuclear factor kappa-B; WTIP, Wilms tumor protein 1-interacting protein; AKT, protein kinase B.

ing hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta (*HADHB*) low expression to activate the focal adhesion kinase (FAK) signaling pathway and ultimately contribute to CRC progression [67]. While in gastric cancer (GC), hypermethylation of secreted frizzled-related protein 2 (*SFRP2*) leads to aberrant activation of Wingless-type MMTV integration site family member (WNT) signaling pathway, promoting tumor proliferation and hypermethylation of reprimin (*RPRM*) attenuates p53 signaling pathway to accelerate its malignant transformation [68]. Besides, hypermethylation of SRY-box transcription factor 21 (*SOX21*) induces overexpression of the downstream cyclin kinase subunit CDC28 Protein Kinase

Regulatory Subunit 2 (CKS2), thereby driving gastric cancer progression [69] (Table 2, Ref. [65–73]).

4.2 Hypomethylation

In colon adenocarcinoma (COAD), promoter hypomethylation of the long non-coding RNA Lnc00346 upregulates the miR-589-5p/CCL5 axis, promoting tumor progression [70]. In CRC, hypomethylation of melanoma antigen family A (*MAGEA*) results in aberrant expression of cancer-testis antigens, thus increasing tumor invasiveness and malignancy [71] and hypomethylation of cerebral cavernous malformation 2 (*CCM2*) influences the tumor microenvironment via Rho-associated coiled-coil con-

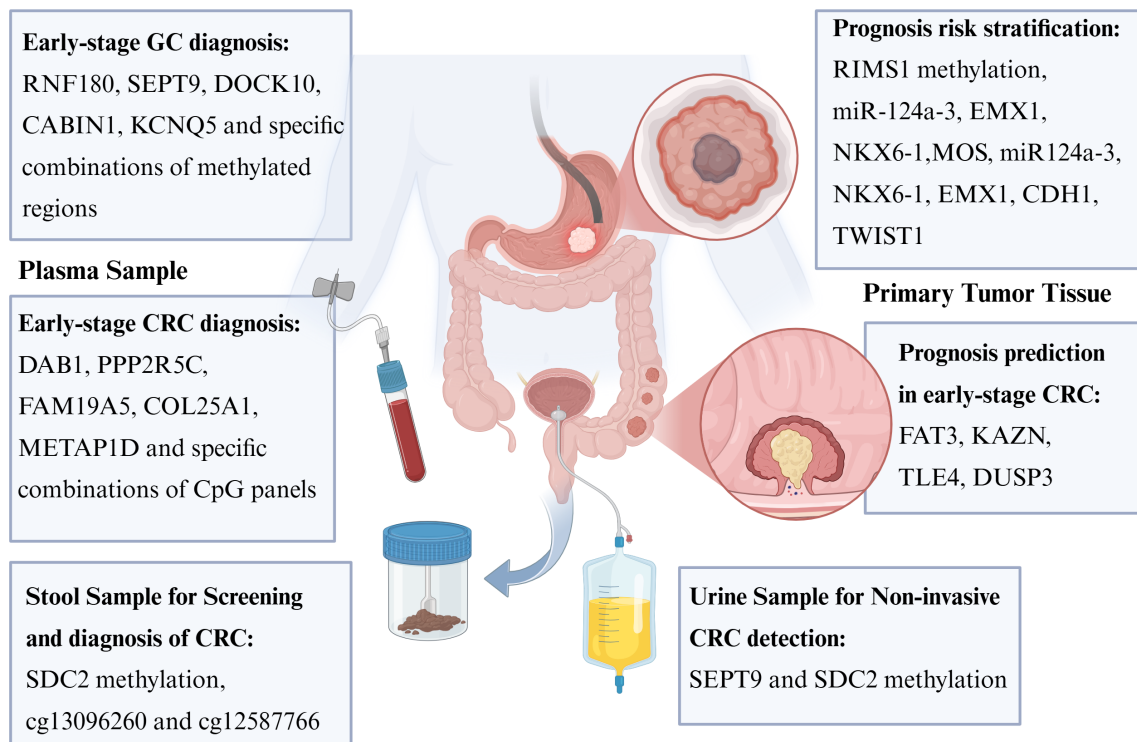


Fig. 4. DNA Methylation in gastrointestinal cancer. DNA methylation plays a significant role in the diagnosis, risk stratification, and prognostic prediction of gastrointestinal tumors. In addition to tumor tissue itself, sources for methylation detection also include plasma samples, stool specimens, and even urine samples. GC, gastric cancer; CRC, Colorectal Cancer. Created in BioRender. (2025) <https://BioRender.com/tnownci>.

taining protein kinase (ROCK)-mediated endothelial cell senescence [72]. At the same time, hypomethylation of intercellular adhesion molecule 1 (ICAM1) enhances the metastatic capability of colorectal cancer through the *Fusobacterium nucleatum*-activated alpha-protein kinase 1/nuclear factor kappa-B (ALPK1/NF- κ B) signaling axis [72]. In GC, TET2 overexpression results Wilms tumor protein 1-interacting protein (WTIP) high expression to inhibit the phosphorylation and kinase activity of protein kinase B (AKT) [73] (Table 2).

5. Clinical Applications of DNA Methylation in Colorectal and Gastric Cancers

Having elucidated the molecular mechanisms underlying DNA methylation and its pivotal role in gene silencing, we now turn our focus to its clinical implications, particularly in the context of colorectal and gastric cancers. The following section synthesizes current evidence and explores the translational potential of DNA methylation biomarkers and therapies in gastrointestinal oncology.

DNA methylation testing has emerged as an ideal biomarker for non-invasive diagnosis and monitoring of gastrointestinal tumors, owing to its high sensitivity, specificity, and stability in body fluids such as peripheral blood [2]. Compared with traditional tissue biopsies, liquid biopsy-based DNA methylation analysis offers distinct ad-

vantages including convenient sampling, high reproducibility, and the capacity to reflect tumor heterogeneity. Furthermore, aberrant DNA methylation frequently occurs during early tumorigenesis, making it an optimal target for early cancer screening [74] (Fig. 4).

Recent advances in high-throughput sequencing technologies and bioinformatics approaches have enabled the identification of numerous methylation markers associated with colorectal and gastric cancers. Corresponding detection techniques such as methylation-specific PCR (MSP), pyrosequencing, and whole-genome bisulfite sequencing have been developed. The clinical application of these technologies is transforming the diagnostic and therapeutic paradigms for colorectal and gastric cancers, providing novel tools for precision medicine [75–77].

The following sections will focus on recent advances in clinical applications of DNA methylation in GC and CRC, encompassing early screening, diagnosis, prognostic evaluation, and treatment monitoring (Table 3, Ref. [78–97]), with the aim of informing clinical practice and future research directions.

5.1 DNA Methylation in Colorectal Cancer

As one of the leading causes of cancer-related mortality worldwide, early detection and precise treatment of CRC are crucial for improving patient outcomes. In recent

years, DNA methylation-based liquid biopsy techniques have demonstrated remarkable progress in CRC screening, recurrence prediction, and treatment guidance [98,99], with their clinical utility validated in multiple high-quality clinical trials.

Circulating tumor DNA (ctDNA) methylation markers have shown exceptional performance in early CRC detection and postoperative recurrence prediction. Gao *et al.* [78] evaluated their independently developed circulating free DNA (cfDNA) methylation detection technology ELSA-seq for screening six common cancers (including CRC), achieving 98.9% specificity and 69.1% sensitivity for CRC detection. Cai *et al.* [79] analyzed 60 CRC cases, 20 advanced adenomas, and 28 normal tissue samples using targeted bisulfite sequencing (PanSeer), identifying 150 differentially methylated regions (DMRs). Validation in 328 plasma samples demonstrated excellent diagnostic performance (AUC = 0.96 for CRC, AUC = 0.86 for advanced adenomas), leading to the development of ColonAiQ, a multiplex quantitative PCR-based blood test that provides a highly sensitive solution for CRC screening and postoperative monitoring. This PCR-based methylation assay is operationally simple, cost-effective, and particularly suitable for routine clinical implementation.

Moreover, methylation marker panels have shown superior performance compared to single markers [80]. Xie *et al.* [81] performed whole-genome methylation profiling of peripheral blood mononuclear cells (PBMCs) using microarray, pyrosequencing, and targeted bisulfite sequencing, identifying five DNA methylation markers. Their multiplex methylation-specific quantitative PCR (multi-msqPCR) assay demonstrated outstanding performance in early CRC diagnosis (AUC = 0.91, sensitivity 81.18%, specificity 89.39%) and could predict CRC risk up to two years in advance. Zhao *et al.* [82] developed ColonSecure, a blood-based test utilizing 149 hypermethylated markers identified through two-stage bisulfite-free sequencing analysis of over 193,000 CpG sites, which showed 86.4% sensitivity (Area Under the Receiver Operating Characteristic Curve (AUROC) = 0.956) for CRC screening, significantly outperforming conventional markers including Carcinoembryonic Antigen (CEA) (45.6%), CRP (39.8%), and CA19-9 (25.2%).

DNA methylation analysis also plays a crucial role in adjuvant chemotherapy decision-making. Mo *et al.* [83] employed a quantitative PCR method targeting six ctDNA methylation markers to analyze blood samples from 299 stage I-III CRC patients. They found that patients with positive ctDNA one month postoperatively had significantly higher recurrence risk (HR = 17.5), with ctDNA monitoring predicting recurrence 3.3 months earlier than imaging. These findings support ctDNA status-guided personalized adjuvant therapy strategies to avoid overtreatment or undertreatment.

Tissue-based DNA methylation profiling has provided abundant markers for clinical applications. Overs *et al.* [84] validated tissue and cell-free DNA samples from 35 CRC patients and 35 healthy controls using methylation-specific digital PCR, demonstrating that a combination of COL25A1 and METAP1D methylation showed 49% sensitivity and 100% specificity (AUC = 1) for CRC detection. In another study based on colonic mucosal tissue, methylation markers of *FAT3*, *KAZN*, *TLE4*, and *DUSP3* genes significantly predicted recurrence risk (HR = 2.35–3.20, $p < 0.001$) [85].

Fecal DNA methylation analysis has also demonstrated advantages in early screening. Kim *et al.* [86] detected *SDC2* methylation in fecal samples from 1124 asymptomatic/high-risk individuals using real-time PCR, achieving 95.0% sensitivity and 81.5% specificity for CRC detection, with a 58.1% detection rate for advanced adenomas, independent of tumor stage ($p = 0.864$). A real-world study further validated the clinical utility of fecal *SDC2* methylation (*mSDC2*) testing, particularly in settings with limited colonoscopy resource [87]. Cao *et al.* [88] analyzed 758 fecal samples (including 62 patients with confounding conditions) and six paired tumor/adjacent tissues using methylation-specific PCR, identifying two specific CpG sites (cg13096260 and cg12587766) whose combined diagnostic model showed significant diagnostic value for CRC and precancerous lesions (AUC = 0.89).

Other body fluids may also serve as potential samples for CRC screening. Bach *et al.* [89] analyzed urine samples from 92 CRC patients and 63 healthy volunteers, demonstrating that a combination of *SEPT9* and *SDC2* methylation markers could detect 70% of CRC cases (86% specificity), providing the first evidence that urine testing could serve as a novel liquid biopsy approach for non-invasive CRC diagnosis.

5.2 DNA Methylation in Gastric Cancer

As the fifth most common cancer and fifth leading cause of cancer death globally, early diagnosis is critical for improving GC patient outcomes. Recent studies have demonstrated significant advantages of DNA methylation markers in GC diagnosis and treatment. Single-cell multi-omics analysis has provided novel insights into GC heterogeneity and DNA methylation characteristics. Bian *et al.* [100] performed single-cell multi-omics sequencing (scTrio-seq3) on 85 samples from 14 GC patients, identifying hypermethylated promoter regions of *TMEM240* and *HAGLROS* genes and hypomethylated *TRPM2-AS* and *HRHI* genes. The “partial reversion” of methylation levels may suppress abnormal transcription of repetitive sequences, thus reducing antigen presentation properties of cancer cells and consequently making these poorly differentiated cancer cells less recognizable and attackable by the immune system. Building upon these fundamental findings, multiple clinical trials have validated their clinical applications [101].

Table 3. Clinical research on DNA methylation in gastrointestinal cancer.

Study	Sample type	Target	Method	Sample size	Clinical application
Gao <i>et al.</i> [78]	Plasma	161,984 CpG sites	Enhanced linear-splinter amplification sequencing	2703 (1693 retrospective and 1010 prospective)	Early detection of six cancers (Including CRC)
Cai <i>et al.</i> [79]	Tumor tissue; Plasma	6 DNA methylation markers	Multiplexed qPCR	108 tissues and 328 plasma samples	Early detection and postoperative surveillance of CRC
Long <i>et al.</i> [80]	Plasma	<i>DAB1, PPP2R5C, FAM19A5</i>	Quantitative methylation-specific PCR	95 CRC patients and 74 healthy controls	Non-invasive CRC diagnosis
Xie <i>et al.</i> [81]	PBMCs	5 DNA methylation markers	Multi-msqPCR	1068 participants	Early-stage colorectal cancer diagnosis
Zhao <i>et al.</i> [82]	Blood	149 hypermethylated markers	Bisulfite-free methylation sequencing	3493 high-risk individuals	CRC screening
Mo <i>et al.</i> [83]	Plasma	6 DNA methylation markers	Multiplex ctDNA methylation qPCR assay	350 patients	Early detection of CRC recurrence
Overs <i>et al.</i> [84]	Plasma	<i>COL25A1</i> and <i>METAP1D</i>	Methylation-specific digital PCR	35 CRC patients and 35 healthy donors	CRC screening and follow-up
Yu <i>et al.</i> [85]	Primary tumor tissues	<i>FAT3, KAZN, TLE4, DUSP3</i>	Infinium MethylationEPIC array	797 patients	Prognosis prediction in early-stage CRC
Kim <i>et al.</i> [86]	Stool samples	<i>SDC2</i> methylation	Real-time PCR	1124 participants	Early CRC detection
Zhao <i>et al.</i> [87]	Stool samples	<i>SDC2</i> methylation	Not mentioned	12,106 populations	Community-based CRC screening
Cao <i>et al.</i> [88]	Stool samples	cg13096260 and cg12587766	Quantitative methylation-specific PCR	758 participants	Screening and diagnosis of precancerous lesions and CRC
Bach <i>et al.</i> [89]	Urine supernatant	<i>SEPT9</i> and <i>SDC2</i>	Quantitative methylation specific PCR	92 CRC patients and 63 healthy volunteers	Non-invasive CRC detection
Qi <i>et al.</i> [90]	Plasma	21 methylated regions	Cell-free methylated DNA immunoprecipitation	150 gastric cancer patients and 100 healthy controls	Early GC detection
Nie <i>et al.</i> [91]	Plasma	<i>RNF180, SEPT9</i>	Specific method not stated	195 GC and 129 controls	Early detection of GC
Ren <i>et al.</i> [92]	Plasma	Mainly <i>DOCK10, CABIN1, KCNQ5</i>	Genome-scale DNA methylation analysis	89 GC patient and 82 controls	Non-invasive detection of GC
Yamada <i>et al.</i> [93]	Biopsy tissue	<i>RIMS1</i> methylation	Pyrosequencing	1624 participants	GC risk stratification
Tanaka <i>et al.</i> [94]	Biopsy tissue	miR-124a-3, <i>EMX1, NKX6-1</i>	Pyrosequencing	71 patients	Predicting metachronous GC risk
Li <i>et al.</i> [96]	Tumor tissue	610 prognosis-related CpG sites	Specific method not stated	406 patients	Prognosis risk stratification for GC
Kim <i>et al.</i> [95]	Noncancerous gastric mucosa	<i>MOS, miR124a-3, NKX6-1, EMX1, CDH1, TWIST1</i>	Quantitative MethyLight assay	245 participants	GC risk assessment in individuals with family history
Chen <i>et al.</i> [97]	FF and FFPE GC tissues	<i>GNAS, FCGBP, and CCDC166</i>	Genome-wide methylation sequencing and qPCR	47 discovery cohort, 302 model development and 130 validation cohort	Lymph node metastasis diagnosis in early GC

qPCR, Quantitative Polymerase Chain Reaction; msqPCR, Multiple Methylation-Specific Quantitative PCR; MethyLight, Fluorescence-based Quantitative Methylation-Specific PCR.

The development of non-invasive detection techniques represents a major breakthrough in early GC screening. Qi *et al.* [90] developed an artificial intelligence-based detection technology using cfDNA methylation, which identified 21 GC-specific methylated regions through whole-genome methylation analysis and achieved 88.38% sensitivity and 94.23% specificity in clinical validation, providing a novel approach for non-invasive early GC screening. Additionally, Nie *et al.* [91] found that even in early gastric cancer, the methylation levels of SEPTIN9 and RNF180 were higher than those in the control group, and the combined panel had a sensitivity of 62.2%, specificity: 84.8%, and AUC of 0.804 for the diagnosis of gastric cancer. Another study [92] employed methylated DNA immunoprecipitation sequencing (MeDIP-seq) to effectively stratify GC patients at different stages, further advancing the clinical application of non-invasive screening technologies. These developments provide valuable data on cfDNA methylation biomarkers for GC and demonstrate the promising potential of cfDNA methylation as a blood-based non-invasive detection method for GC.

Risk stratification following *Helicobacter pylori* eradication presents another clinical challenge. A multicenter prospective study following 1624 participants after *H. pylori* eradication found that high methylation levels of the *RIMS1* gene strongly predicted GC development, with the high methylation group showing a 470% increased risk (HR = 7.7) compared with the low methylation group [93]. Another study revealed correlations between methylation levels of *miR-124a-3* and *EMX1* and gastric lesions following *H. pylori* eradication [94]. A study focusing on familial gastric cancer also found that the methylation level of *CDH1* decreased after Hp eradication [95]. These studies provide important evidence for developing personalized surveillance strategies after *H. pylori* eradication, helping to identify high-risk individuals requiring close follow-up while avoiding unnecessary examinations for low-risk patients.

Although current research on methylation-based prognostic models and treatment response monitoring in GC remains relatively limited, considering the similarities in methylation regulation mechanisms among gastrointestinal tumors, similar approaches may be applicable to GC. In recent years, some studies related to gastric cancer have also revealed the methylation profile of specific gastric cancer types, such as Epstein-Barr Virus-associated gastric cancer [102], and screened out special methylation sites in gastric cancer for subsequent clinical studies [103,104]. Future studies should explore the value of specific methylation markers in predicting responses to neoadjuvant chemotherapy, targeted therapy, and immunotherapy in GC.

5.3 Application Value of DNA Methylation in Gastrointestinal Cancer

DNA methylation has demonstrated multifaceted clinical value in gastrointestinal cancer management, ranging from early screening to prognostic assessment (Table 3). With the approval of commercial testing products, methylation detection is gradually being implemented in clinical practice. However, current research still has several limitations, including the need for large-scale prospective validation of most markers and the lack of standardized operating procedures and cut-off values. Future multicenter studies are needed to validate the clinical utility of existing markers and explore the combined application of methylation testing with other molecular markers to further improve precision medicine in gastrointestinal oncology.

Despite the considerable potential of DNA methylation testing, current research exhibits notable limitations. Significant heterogeneity in the diagnostic accuracy of reported biomarkers across different populations has been observed, which may be attributed to confounding variables such as ethnic background, environmental influences, and variations in study cohort size and design. Furthermore, the integration of methylation assays into routine clinical practice presents multiple challenges, including the absence of standardized protocols, universally accepted cut-off values, and support from large-scale prospective validation. Issues regarding cost-effectiveness, accessibility of automated platforms, and the definitive translation of results into clinical decision-making pathways remain to be adequately addressed. Future efforts should focus on multicenter, real-world studies to verify the robustness of existing biomarkers and explore their combined application with other molecular markers to advance precision oncology.

6. Conclusion

This review provides a systematic elaboration of the chemical mechanisms underlying DNA methylation and demethylation, with a focus on the molecular pathways through which DNA methylation mediates gene silencing. It further summarizes recent clinical research advances and application potential of DNA methylation in the diagnosis of gastrointestinal tumors, highlighting its significant translational value from basic research to clinical practice.

As a crucial epigenetic modification, DNA methylation plays a central role in the initiation and progression of colorectal and gastric cancers. For instance, promoter hypermethylation of key tumor suppressor genes can lead to their transcriptional silencing, thereby promoting malignant transformation, uncontrolled proliferation, and enhanced invasiveness—ultimately increasing tumor aggressiveness. Such epigenetic alterations have emerged as important targets in gastrointestinal cancer research and therapeutic development. From a clinical perspective, DNA methylation-based assays demonstrate considerable value due to their non-invasive nature, high sensitivity, and po-

tential for dynamic monitoring (Table 2). They can be applied in multiple aspects of cancer management, including early detection and prediction, risk stratification, prognostic assessment, postoperative monitoring of minimal residual disease and recurrence, as well as evaluation of therapeutic response to chemotherapy or radiotherapy. These applications provide powerful tools for comprehensive cancer care throughout the disease course.

Furthermore, since DNA methylation is catalyzed by enzymes such as DNMTs, and demethylation is mediated by enzymes including the TET family, targeting these enzymes has become a promising strategy for cancer therapy. For example, DNMT inhibitors (e.g., azacitidine and decitabine, DAC) can reduce global methylation levels, reverse gene silencing, and partially restore tumor suppressor function [105,106]. Similarly, strategies aimed at activating demethylating enzymes (e.g., TET) or their cofactors may help re-establish normal epigenetic states. These interventions seek to restore normal gene expression, suppress malignant phenotypes, and offer novel approaches to overcome chemoresistance and inhibit metastasis [107].

In summary, therapeutic strategies targeting DNA methylation mechanisms are increasingly recognized as vital components of precision oncology, driving the diagnosis of gastrointestinal tumors toward more precise, targeted, and individualized approaches.

Author Contributions

Conceptualization, LB, XY, ZW and ZZ; writing-original draft preparation, LB and XY; writing-review and editing, ZW and ZZ. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors state they have no financial interests or personal relationships that could have influenced the work presented in this paper.

References

- [1] Damiano OM, Stevens AJ, Kenwright DN, Seddon AR. Chronic Inflammation to Cancer: The Impact of Oxidative Stress on DNA Methylation. *Frontiers in Bioscience (Landmark Edition)*. 2025; 30: 26142. <https://doi.org/10.31083/fbl26142>.
- [2] Nishiyama A, Nakanishi M. Navigating the DNA methylation landscape of cancer. *Trends in Genetics: TIG*. 2021; 37: 1012–1027. <https://doi.org/10.1016/j.tig.2021.05.002>.
- [3] Mattei AL, Bailly N, Meissner A. DNA methylation: a historical perspective. *Trends in Genetics: TIG*. 2022; 38: 676–707. <https://doi.org/10.1016/j.tig.2022.03.010>.
- [4] Moore LD, Le T, Fan G. DNA methylation and its basic function. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*. 2013; 38: 23–38. <https://doi.org/10.1038/npp.2012.112>.
- [5] Yang Y, Wang Y, Fan X, Xu X, Wang H, Wang X, *et al*. Role of DNA methylation transferase in urinary system diseases: From basic to clinical perspectives (Review). *International Journal of Molecular Medicine*. 2025; 55: 19. <https://doi.org/10.3892/ijmm.2024.5460>.
- [6] Mohd Murshid N, Aminullah Lubis F, Makpol S. Epigenetic Changes and Its Intervention in Age-Related Neurodegenerative Diseases. *Cellular and Molecular Neurobiology*. 2022; 42: 577–595. <https://doi.org/10.1007/s10571-020-00979-z>.
- [7] Reinisch KM, Chen L, Verdine GL, Lipscomb WN. The crystal structure of HaeIII methyltransferase covalently complexed to DNA: an extrahelical cytosine and rearranged base pairing. *Cell*. 1995; 82: 143–153. [https://doi.org/10.1016/0092-8674\(95\)90060-8](https://doi.org/10.1016/0092-8674(95)90060-8).
- [8] Song J, Rechkoblit O, Bestor TH, Patel DJ. Structure of DNMT1-DNA complex reveals a role for autoinhibition in maintenance DNA methylation. *Science (New York, N.Y.)*. 2011; 331: 1036–1040. <https://doi.org/10.1126/science.1195380>.
- [9] Lyko F. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. *Nature Reviews. Genetics*. 2018; 19: 81–92. <https://doi.org/10.1038/nrg.2017.80>.
- [10] Jeltsch A, Jurkowska RZ. Allosteric control of mammalian DNA methyltransferases - a new regulatory paradigm. *Nucleic Acids Research*. 2016; 44: 8556–8575. <https://doi.org/10.1093/nar/gkw723>.
- [11] Kasai Y, Sato K, Utsumi S, Ichikawa S. Improvement of S_NAr Reaction Rate by an Electron-Withdrawing Group in the Crosslinking of DNA Cytosine-5 Methyltransferase by a Covalent Oligodeoxyribonucleotide Inhibitor. *Chembiochem: a European Journal of Chemical Biology*. 2018; 19: 1866–1872. <https://doi.org/10.1002/cbic.201800244>.
- [12] Wu JC, Santi DV. Kinetic and catalytic mechanism of HhaI methyltransferase. *The Journal of Biological Chemistry*. 1987; 262: 4778–4786.
- [13] Lee YH, Ren D, Jeon B, Liu HW. S-Adenosylmethionine: more than just a methyl donor. *Natural Product Reports*. 2023; 40: 1521–1549. <https://doi.org/10.1039/d2np00086e>.
- [14] Cantoni GL. S-Adenosylmethionine; a new intermediate formed enzymatically from L-methionine and adenosinetriphosphate. *The Journal of Biological Chemistry*. 1953; 204: 403–416.
- [15] Meng H, Cao Y, Qin J, Song X, Zhang Q, Shi Y, *et al*. DNA methylation, its mediators and genome integrity. *International Journal of Biological Sciences*. 2015; 11: 604–617. <https://doi.org/10.7150/ijbs.11218>.
- [16] Smith ZD, Meissner A. DNA methylation: roles in mammalian development. *Nature Reviews. Genetics*. 2013; 14: 204–220. <https://doi.org/10.1038/nrg3354>.
- [17] Davletgildeeva AT, Kuznetsov NA. The Role of DNMT Methyltransferases and TET Dioxygenases in the Maintenance of the

- DNA Methylation Level. *Biomolecules*. 2024; 14: 1117. <https://doi.org/10.3390/biom14091117>.
- [18] Adam S, Klingel V, Radde NE, Bashtrykov P, Jeltsch A. On the accuracy of the epigenetic copy machine: comprehensive specificity analysis of the DNMT1 DNA methyltransferase. *Nucleic Acids Research*. 2023; 51: 6622–6633. <https://doi.org/10.1093/nar/gkad465>.
- [19] Bai J, Zhang X, Hu K, Liu B, Wang H, Li A, *et al.* Silencing DNA methyltransferase 1 (DNMT1) inhibits proliferation, metastasis and invasion in ESCC by suppressing methylation of RASSF1A and DAPK. *Oncotarget*. 2016; 7: 44129–44141. <https://doi.org/10.18632/oncotarget.9866>.
- [20] Panning B, Jaenisch R. DNA hypomethylation can activate Xist expression and silence X-linked genes. *Genes & Development*. 1996; 10: 1991–2002. <https://doi.org/10.1101/gad.10.16.1991>.
- [21] Lauria A, Meng G, Proserpio V, Rapelli S, Maldotti M, Polignano IL, *et al.* DNMT3B supports meso-endoderm differentiation from mouse embryonic stem cells. *Nature Communications*. 2023; 14: 367. <https://doi.org/10.1038/s41467-023-35938-x>.
- [22] Watanabe D, Suetake I, Tada T, Tajima S. Stage- and cell-specific expression of Dnmt3a and Dnmt3b during embryogenesis. *Mechanisms of Development*. 2002; 118: 187–190. [https://doi.org/10.1016/s0925-4773\(02\)00242-3](https://doi.org/10.1016/s0925-4773(02)00242-3).
- [23] Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*. 1999; 99: 247–257. [https://doi.org/10.1016/s0092-8674\(00\)81656-6](https://doi.org/10.1016/s0092-8674(00)81656-6).
- [24] Chen Z, Zhang Y. Role of Mammalian DNA Methyltransferases in Development. *Annual Review of Biochemistry*. 2020; 89: 135–158. <https://doi.org/10.1146/annurev-biochem-103019-102815>.
- [25] Barlas AB, Karaca E. So close yet so far apart: distinct flanking sequence recognition by DNMT3A and DNMT3B. *Communications Biology*. 2025; 8: 1217. <https://doi.org/10.1038/s42003-025-08606-7>.
- [26] Zhang ZM, Lu R, Wang P, Yu Y, Chen D, Gao L, *et al.* Structural basis for DNMT3A-mediated de novo DNA methylation. *Nature*. 2018; 554: 387–391. <https://doi.org/10.1038/nature25477>.
- [27] Sendzikaitė G, Hanna CW, Stewart-Morgan KR, Ivanova E, Kelsey G. A DNMT3A PWWP mutation leads to methylation of bivalent chromatin and growth retardation in mice. *Nature Communications*. 2019; 10: 1884. <https://doi.org/10.1038/s41467-019-09713-w>.
- [28] Kim SH, Park J, Choi MC, Kim HP, Park JH, Jung Y, *et al.* Zinc-fingers and homeoboxes 1 (ZHX1) binds DNA methyltransferase (DNMT) 3B to enhance DNMT3B-mediated transcriptional repression. *Biochemical and Biophysical Research Communications*. 2007; 355: 318–323. <https://doi.org/10.1016/j.bbrc.2007.01.187>.
- [29] Chedin F, Lieber MR, Hsieh CL. The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99: 16916–16921. <https://doi.org/10.1073/pnas.262443999>.
- [30] Hata K, Okano M, Lei H, Li E. Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development* (Cambridge, England). 2002; 129: 1983–1993. <https://doi.org/10.1242/dev.129.8.1983>.
- [31] de Mendoza A, Bonnet A, Vargas-Landin DB, Ji N, Li H, Yang F, *et al.* Recurrent acquisition of cytosine methyltransferases into eukaryotic retrotransposons. *Nature Communications*. 2018; 9: 1341. <https://doi.org/10.1038/s41467-018-03724-9>.
- [32] Kim GD, Ni J, Kelesoglu N, Roberts RJ, Pradhan S. Cooperation and communication between the human maintenance and de novo DNA (cytosine-5) methyltransferases. *The EMBO Journal*. 2002; 21: 4183–4195. <https://doi.org/10.1093/emboj/cdf401>.
- [33] Viré E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, *et al.* The Polycomb group protein EZH2 directly controls DNA methylation. *Nature*. 2006; 439: 871–874. <https://doi.org/10.1038/nature04431>.
- [34] Kikuchi A, Onoda H, Yamaguchi K, Kori S, Matsuzawa S, Chiba Y, *et al.* Structural basis for activation of DNMT1. *Nature Communications*. 2022; 13: 7130. <https://doi.org/10.1038/s41467-022-34779-4>.
- [35] Kamel EM, Ali MAM, Allam AA, Ahmed NA, Abalkhail A, Aba Alkhayl FF, *et al.* Disrupting the epigenetic alliance: structural insights and therapeutic strategies targeting DNMT1-UHRF1. *Functional & Integrative Genomics*. 2025; 25: 194. <https://doi.org/10.1007/s10142-025-01708-9>.
- [36] Yarychivska O, Shahabuddin Z, Comfort N, Boulard M, Bestor TH. BAH domains and a histone-like motif in DNA methyltransferase 1 (DNMT1) regulate *de novo* and maintenance methylation *in vivo*. *The Journal of Biological Chemistry*. 2018; 293: 19466–19475. <https://doi.org/10.1074/jbc.RA118.004612>.
- [37] Kubo N, Uehara R, Uemura S, Ohishi H, Shirane K, Sasaki H. Combined and differential roles of ADD domains of DNMT3A and DNMT3L on DNA methylation landscapes in mouse germ cells. *Nature Communications*. 2024; 15: 3266. <https://doi.org/10.1038/s41467-024-47699-2>.
- [38] Uehara R, Au Yeung WK, Toriyama K, Ohishi H, Kubo N, Toh H, *et al.* The DNMT3A ADD domain is required for efficient de novo DNA methylation and maternal imprinting in mouse oocytes. *PLoS Genetics*. 2023; 19: e1010855. <https://doi.org/10.1371/journal.pgen.1010855>.
- [39] Cho CC, Huang HH, Jiang BC, Yang WZ, Chen YN, Yuan HS. Histone modification-driven structural remodeling unleashes DNMT3B in DNA methylation. *Science Advances*. 2025; 11: eadu8116. <https://doi.org/10.1126/sciadv.adu8116>.
- [40] Vincenzetti L, Leoni C, Chirichella M, Kwee I, Monticelli S. The contribution of active and passive mechanisms of 5mC and 5hmC removal in human T lymphocytes is differentiation- and activation-dependent. *European Journal of Immunology*. 2019; 49: 611–625. <https://doi.org/10.1002/eji.201847967>.
- [41] Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. *Nature Reviews. Molecular Cell Biology*. 2019; 20: 590–607. <https://doi.org/10.1038/s41580-019-0159-6>.
- [42] Schübeler D. Function and information content of DNA methylation. *Nature*. 2015; 517: 321–326. <https://doi.org/10.1038/nature14192>.
- [43] Wu X, Zhang Y. TET-mediated active DNA demethylation: mechanism, function and beyond. *Nature Reviews. Genetics*. 2017; 18: 517–534. <https://doi.org/10.1038/nrg.2017.33>.
- [44] Kohli RM, Zhang Y. TET enzymes, TDG and the dynamics of DNA demethylation. *Nature*. 2013; 502: 472–479. <https://doi.org/10.1038/nature12750>.
- [45] He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, *et al.* Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science (New York, N.Y.)*. 2011; 333: 1303–1307. <https://doi.org/10.1126/science.1210944>.
- [46] Wu H, Zhang Y. Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. *Genes & Development*. 2011; 25: 2436–2452. <https://doi.org/10.1101/gad.179184.111>.
- [47] Cortellino S, Xu J, Sannai M, Moore R, Caretti E, Cigliano A, *et al.* Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. *Cell*. 2011; 146: 67–79. <https://doi.org/10.1016/j.cell.2011.06.020>.
- [48] Joshi K, Liu S, Breslin S J P, Zhang J. Mechanisms that regulate the activities of TET proteins. *Cellular and Molecular Life Sciences: CMLS*. 2022; 79: 363. <https://doi.org/10.1007/s00018-022-04396-x>.

- [49] Ko M, An J, Bandukwala HS, Chavez L, Aijö T, Pastor WA, *et al.* Modulation of TET2 expression and 5-methylcytosine oxidation by the CXXC domain protein IDAX. *Nature*. 2013; 497: 122–126. <https://doi.org/10.1038/nature12052>.
- [50] Zhang L, Lu X, Lu J, Liang H, Dai Q, Xu GL, *et al.* Thymine DNA glycosylase specifically recognizes 5-carboxylcytosine-modified DNA. *Nature Chemical Biology*. 2012; 8: 328–330. <https://doi.org/10.1038/nchembio.914>.
- [51] Weber AR, Krawczyk C, Robertson AB, Kuśnierczyk A, Vågbo CB, Schuermann D, *et al.* Biochemical reconstitution of TET1-TDG-BER-dependent active DNA demethylation reveals a highly coordinated mechanism. *Nature Communications*. 2016; 7: 10806. <https://doi.org/10.1038/ncomms10806>.
- [52] Prasad R, Beard WA, Wilson SH. Studies of gapped DNA substrate binding by mammalian DNA polymerase beta. Dependence on 5'-phosphate group. *The Journal of Biological Chemistry*. 1994; 269: 18096–18101.
- [53] Beard WA, Wilson SH. Structure and mechanism of DNA polymerase β . *Biochemistry*. 2014; 53: 2768–2780. <https://doi.org/10.1021/bi500139h>.
- [54] Hardeland U, Bentele M, Jiricny J, Schär P. The versatile thymine DNA-glycosylase: a comparative characterization of the human, *Drosophila* and fission yeast orthologs. *Nucleic Acids Research*. 2003; 31: 2261–2271. <https://doi.org/10.1093/nar/gkg344>.
- [55] Martisova A, Holcakova J, Izadi N, Sebuyoya R, Hrstka R, Bartosik M. DNA Methylation in Solid Tumors: Functions and Methods of Detection. *International Journal of Molecular Sciences*. 2021; 22: 4247. <https://doi.org/10.3390/ijms22084247>.
- [56] Kaluscha S, Domcke S, Wirbelauer C, Stadler MB, Durdu S, Burger L, *et al.* Evidence that direct inhibition of transcription factor binding is the prevailing mode of gene and repeat repression by DNA methylation. *Nature Genetics*. 2022; 54: 1895–1906. <https://doi.org/10.1038/s41588-022-01241-6>.
- [57] Kreibich E, Kleinendorst R, Barzaghi G, Kaspar S, Krebs AR. Single-molecule footprinting identifies context-dependent regulation of enhancers by DNA methylation. *Molecular Cell*. 2023; 83: 787–802.e9. <https://doi.org/10.1016/j.molcel.2023.01.017>.
- [58] Du Q, Luu PL, Storzaker C, Clark SJ. Methyl-CpG-binding domain proteins: readers of the epigenome. *Epigenomics*. 2015; 7: 1051–1073. <https://doi.org/10.2217/epi.15.39>.
- [59] Schmidt A, Zhang H, Cardoso MC. MeCP2 and Chromatin Compartmentalization. *Cells*. 2020; 9: 878. <https://doi.org/10.3390/cells9040878>.
- [60] Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, Kouzarides T. The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *The Journal of Biological Chemistry*. 2003; 278: 4035–4040. <https://doi.org/10.1074/jbc.M210256200>.
- [61] de Ruijter AJM, van Gennip AH, Caron HN, Kemp S, van Kuilenburg ABP. Histone deacetylases (HDACs): characterization of the classical HDAC family. *The Biochemical Journal*. 2003; 370: 737–749. <https://doi.org/10.1042/BJ20021321>.
- [62] Yang XJ, Seto E. HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene*. 2007; 26: 5310–5318. <https://doi.org/10.1038/sj.onc.1210599>.
- [63] Padeken J, Methot SP, Gasser SM. Establishment of H3K9-methylated heterochromatin and its functions in tissue differentiation and maintenance. *Nature Reviews. Molecular Cell Biology*. 2022; 23: 623–640. <https://doi.org/10.1038/s41580-022-00483-w>.
- [64] Scoumanne A, Chen X. Protein methylation: a new mechanism of p53 tumor suppressor regulation. *Histology and Histopathology*. 2008; 23: 1143–1149. <https://doi.org/10.14670/HH-23.1143>.
- [65] Guo H, Zheng G, Li J, Yao S, Jia Q, Tan J, *et al.* DNA methylation regulates TREM1 expression to modulate immune responses and drive progression in colorectal neuroendocrine neoplasm as a potential therapeutic target. *Discover Oncology*. 2025; 16: 1624. <https://doi.org/10.1007/s12672-025-03468-1>.
- [66] Zhou J, Ye D, Li Y, Lai X, Cui W, He W, *et al.* Combining the DNA methylation markers of circulating tumor cells with immune infiltrating cells to assess recurrence and prognosis and to suggest a therapeutic strategy in stage III-IV colorectal cancer. *Frontiers in Immunology*. 2025; 16: 1607548. <https://doi.org/10.3389/fimmu.2025.1607548>.
- [67] Li L, Jiang M, Wang W, Cao X, Ma Q, Han J, *et al.* DNA demethylase TET2-mediated reduction of HADHB expression contributes to cadmium-induced malignant progression of colorectal cancer. *Ecotoxicology and Environmental Safety*. 2024; 280: 116579. <https://doi.org/10.1016/j.ecoenv.2024.116579>.
- [68] Dede S, Şakalar Ç, Yılmaz B, Çubukçı G, Acar M, Yavuz A, *et al.* SFRP2 and RPRM as methylation based serum biomarkers for the detection of gastric cancer. *Discover Oncology*. 2025; 16: 1606. <https://doi.org/10.1007/s12672-025-03472-5>.
- [69] Wei J, Xue S, Du X, Dai Y, Ji Y, He G. DNMT1 blocks SOX21-repressed CKS2 transcription to promote gastric cancer progression. *BMC Cancer*. 2025; 25: 1182. <https://doi.org/10.1186/s12885-025-14577-z>.
- [70] Chen J, Lin H, Huang S, Wang Y, Huang M, Fu Y, *et al.* Hypomethylation-mediated LINC00346 facilitates cell phenotype progression of colon adenocarcinoma via modulating miR-589-5p/CCL5. *Process Biochemistry*. 2023; 130: 492–502. <https://doi.org/10.1016/j.procbio.2023.05.004>.
- [71] Poojary M, Jishnu PV, Kabekkodu SP. Prognostic Value of Melanoma-Associated Antigen-A (MAGE-A) Gene Expression in Various Human Cancers: A Systematic Review and Meta-analysis of 7428 Patients and 44 Studies. *Molecular Diagnosis & Therapy*. 2020; 24: 537–555. <https://doi.org/10.1007/s40291-020-00476-5>.
- [72] Xia L, Wang J, Gao J, Cui X, Song M, Ding W, *et al.* Integrative multi-omics analysis identifies key genes and colocalized signals associated with colorectal cancer risk. *BMC Cancer*. 2025; 25: 1372. <https://doi.org/10.1186/s12885-025-14798-2>.
- [73] Guo Y, Yu H, Li J, Liu K, Han M, Tang Y, *et al.* DNA-methylation eraser TET2 activates WTIP expression to suppress an AKT-dependent chemoresistance of gastric cancer. *Neoplasia (New York, N.Y.)*. 2025; 65: 101166. <https://doi.org/10.1016/j.neo.2025.101166>.
- [74] Müller D, Györfy B. DNA methylation-based diagnostic, prognostic, and predictive biomarkers in colorectal cancer. *Biochimica et Biophysica Acta. Reviews on Cancer*. 2022; 1877: 188722. <https://doi.org/10.1016/j.bbcan.2022.188722>.
- [75] Gong T, Borgard H, Zhang Z, Chen S, Gao Z, Deng Y. Analysis and Performance Assessment of the Whole Genome Bisulfite Sequencing Data Workflow: Currently Available Tools and a Practical Guide to Advance DNA Methylation Studies. *Small Methods*. 2022; 6: e2101251. <https://doi.org/10.1002/smtd.202101251>.
- [76] Lee AV, Nestler KA, Chiappinelli KB. Therapeutic targeting of DNA methylation alterations in cancer. *Pharmacology & Therapeutics*. 2024; 258: 108640. <https://doi.org/10.1016/j.pharmthera.2024.108640>.
- [77] Yousefi PD, Suderman M, Langdon R, Whitehurst O, Davey Smith G, Relton CL. DNA methylation-based predictors of health: applications and statistical considerations. *Nature Reviews. Genetics*. 2022; 23: 369–383. <https://doi.org/10.1038/s41576-022-00465-w>.
- [78] Gao Q, Lin YP, Li BS, Wang GQ, Dong LQ, Shen BY, *et al.* Unintrusive multi-cancer detection by circulating cell-free DNA methylation sequencing (THUNDER): development and independent validation studies. *Annals of Oncology: Official Journal*

- nal of the European Society for Medical Oncology. 2023; 34: 486–495. <https://doi.org/10.1016/j.annonc.2023.02.010>.
- [79] Cai G, Cai M, Feng Z, Liu R, Liang L, Zhou P, *et al.* A Multilocus Blood-Based Assay Targeting Circulating Tumor DNA Methylation Enables Early Detection and Early Relapse Prediction of Colorectal Cancer. *Gastroenterology*. 2021; 161: 2053–2056.e2. <https://doi.org/10.1053/j.gastro.2021.08.054>.
- [80] Long Z, Gao Y, Han Z, Yuan H, Yu Y, Pei B, *et al.* Discovery and Validation of Methylation Signatures in Circulating Cell-Free DNA for the Detection of Colorectal Cancer. *Biomolecules*. 2024; 14: 996. <https://doi.org/10.3390/biom14080996>.
- [81] Xie Y, Li P, Sun D, Qi Q, Ma S, Zhao Y, *et al.* DNA Methylation-Based Testing in Peripheral Blood Mononuclear Cells Enables Accurate and Early Detection of Colorectal Cancer. *Cancer Research*. 2023; 83: 3636–3649. <https://doi.org/10.1158/0008-5472.CAN-22-3402>.
- [82] Zhao F, Bai P, Xu J, Li Z, Muhammad S, Li D, *et al.* Efficacy of cell-free DNA methylation-based blood test for colorectal cancer screening in high-risk population: a prospective cohort study. *Molecular Cancer*. 2023; 22: 157. <https://doi.org/10.1186/s12943-023-01866-z>.
- [83] Mo S, Ye L, Wang D, Han L, Zhou S, Wang H, *et al.* Early Detection of Molecular Residual Disease and Risk Stratification for Stage I to III Colorectal Cancer via Circulating Tumor DNA Methylation. *JAMA Oncology*. 2023; 9: 770–778. <https://doi.org/10.1001/jamaoncol.2023.0425>.
- [84] Overs A, Peixoto P, Hervouet E, Molimard C, Monnier F, Durand J, *et al.* COL25A1 and METAP1D DNA methylation are promising liquid biopsy epigenetic biomarkers of colorectal cancer using digital PCR. *Clinical Epigenetics*. 2024; 16: 146. <https://doi.org/10.1186/s13148-024-01748-1>.
- [85] Yu H, Wang X, Bai L, Tang G, Carter KT, Cui J, *et al.* DNA methylation profile in CpG-depleted regions uncovers a high-risk subtype of early-stage colorectal cancer. *Journal of the National Cancer Institute*. 2023; 115: 52–61. <https://doi.org/10.1093/jnci/djac183>.
- [86] Kim CW, Kim H, Kim HR, Won DD, Nam WJ, Min BS, *et al.* A Stool DNA-Based SDC2 Methylation Test for the Early Detection of Colorectal Cancer in an Asymptomatic, High-Risk Population: A Multicenter Prospective Randomized Trial. *The American Journal of Gastroenterology*. 2025; 120: 614–622. <https://doi.org/10.14309/ajg.0000000000003044>.
- [87] Zhao S, He Z, Sui X, Zhang S, Li Z, Bai Y, *et al.* Real-World Stool-Based Syndecan-2 Methylation Test Improved Detection of Advanced Colorectal Neoplasia for Colorectal Cancer Screening: A Prospective, Multicenter, Community-Based Study. *Gastroenterology*. 2024; 167: 611–614.e7. <https://doi.org/10.1053/j.gastro.2024.04.019>.
- [88] Cao Q, Dan Z, Hou N, Yan L, Yuan X, Lu H, *et al.* Discovery and validation of colorectal cancer tissue-specific methylation markers: a dual-center retrospective cohort study. *Clinical Epigenetics*. 2024; 16: 122. <https://doi.org/10.1186/s13148-024-01735-6>.
- [89] Bach S, Paulis I, Sluiter NR, Tibbesma M, Martin I, van de Wiel MA, *et al.* Detection of colorectal cancer in urine using DNA methylation analysis. *Scientific Reports*. 2021; 11: 2363. <https://doi.org/10.1038/s41598-021-81900-6>.
- [90] Qi J, Hong B, Wang S, Wang J, Fang J, Sun R, *et al.* Plasma cell-free DNA methylome-based liquid biopsy for accurate gastric cancer detection. *Cancer Science*. 2024; 115: 3426–3438. <https://doi.org/10.1111/cas.16284>.
- [91] Nie Y, Gao X, Cai X, Wu Z, Liang Q, Xu G, *et al.* Combining methylated SEPTIN9 and RNF180 plasma markers for diagnosis and early detection of gastric cancer. *Cancer Communications (London, England)*. 2023; 43: 1275–1279. <https://doi.org/10.1002/cac2.12478>.
- [92] Ren J, Lu P, Zhou X, Liao Y, Liu X, Li J, *et al.* Genome-Scale Methylation Analysis of Circulating Cell-Free DNA in Gastric Cancer Patients. *Clinical Chemistry*. 2022; 68: 354–364. <https://doi.org/10.1093/clinchem/hvab204>.
- [93] Yamada H, Abe S, Charvat H, Ando T, Maeda M, Murakami K, *et al.* Precision risk stratification of primary gastric cancer after eradication of *H. pylori* by a DNA methylation marker: a multicentre prospective study. *Gut*. 2025; 74: 1410–1418. <https://doi.org/10.1136/gutjnl-2025-335039>.
- [94] Tanaka I, Ono S, Watanabe Y, Yamamoto H, Oikawa R, Matsumoto S, *et al.* Long-term changes in aberrant DNA methylation and gastritis after *Helicobacter pylori* eradication focused on metachronous gastric cancer. *Helicobacter*. 2022; 27: e12915. <https://doi.org/10.1111/hel.12915>.
- [95] Kim HJ, Kim N, Kim HW, Park JH, Shin CM, Lee DH. Promising aberrant DNA methylation marker to predict gastric cancer development in individuals with family history and long-term effects of *H. pylori* eradication on DNA methylation. *Gastric Cancer: Official Journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association*. 2021; 24: 302–313. <https://doi.org/10.1007/s10120-020-01117-w>.
- [96] Li T, Chen X, Gu M, Deng A, Qian C. Identification of the subtypes of gastric cancer based on DNA methylation and the prediction of prognosis. *Clinical Epigenetics*. 2020; 12: 161. <https://doi.org/10.1186/s13148-020-00940-3>.
- [97] Chen S, Yu Y, Li T, Ruan W, Wang J, Peng Q, *et al.* A novel DNA methylation signature associated with lymph node metastasis status in early gastric cancer. *Clinical Epigenetics*. 2022; 14: 18. <https://doi.org/10.1186/s13148-021-01219-x>.
- [98] Jung G, Hernández-Illán E, Moreira L, Balaguer F, Goel A. Epigenetics of colorectal cancer: biomarker and therapeutic potential. *Nature Reviews. Gastroenterology & Hepatology*. 2020; 17: 111–130. <https://doi.org/10.1038/s41575-019-0230-y>.
- [99] Chen XQ, Lian K, Chen ZW, Zhang X, Li T, Wu T, *et al.* Multi-Omics Characteristics of Ferroptosis Associated with Colon Adenocarcinoma Typing and Survival. *Frontiers in Bioscience (Landmark Edition)*. 2024; 29: 13. <https://doi.org/10.31083/j.fb12901013>.
- [100] Bian S, Wang Y, Zhou Y, Wang W, Guo L, Wen L, *et al.* Integrative single-cell multiomics analyses dissect molecular signatures of intratumoral heterogeneities and differentiation states of human gastric cancer. *National Science Review*. 2023; 10: nwad094. <https://doi.org/10.1093/nsr/nwad094>.
- [101] Kalra A, Meltzer SJ. The Role of DNA Methylation in Gastrointestinal Disease: An Expanded Review of Malignant and Nonmalignant Gastrointestinal Diseases. *Gastroenterology*. 2025; 168: 245–266. <https://doi.org/10.1053/j.gastro.2024.07.001>.
- [102] Preston-Alp S, Caruso LB, Su C, Keith K, Soldan SS, Maestri D, *et al.* Decitabine disrupts EBV genomic epiallele DNA methylation patterns around CTCF binding sites to increase chromatin accessibility and lytic transcription in gastric cancer. *mBio*. 2023; 14: e0039623. <https://doi.org/10.1128/mbio.00396-23>.
- [103] Cai M, Guo H, Wang D, Zhao T, Liang X, Li J, *et al.* Expression, DNA methylation pattern and transcription factor EPB41L3 in gastric cancer: a study of 262 cases. *Cell Communication and Signaling: CCS*. 2024; 22: 470. <https://doi.org/10.1186/s12964-024-01849-7>.
- [104] Muhammad JS, Manzoor S, Cui ZG, Khoder G. DNA Methylation-Mediated Overexpression of *CXCL1* in *Helicobacter pylori*-Induced Gastric Cancer: In Silico- and In Vitro-Based Identification of a Potential Biomarker for Carcinogenesis. *International Journal of Molecular Sciences*. 2023; 24: 795. <https://doi.org/10.3390/ijms24010795>.
- [105] Nishikawa J, Iizasa H, Yoshiyama H, Nakamura M, Saito M,

- Sasaki S, *et al.* The Role of Epigenetic Regulation in Epstein-Barr Virus-Associated Gastric Cancer. *International Journal of Molecular Sciences*. 2017; 18: 1606. <https://doi.org/10.3390/ijms18081606>.
- [106] Tampakis A, Tampaki EC, Nebiker CA, Kouraklis G. Histone deacetylase inhibitors and colorectal cancer: what is new? *Anti-cancer Agents in Medicinal Chemistry*. 2014; 14: 1220–1227. <https://doi.org/10.2174/1871520614666140919095828>.
- [107] Wang J, Yang J, Li D, Li J. Technologies for targeting DNA methylation modifications: Basic mechanism and potential application in cancer. *Biochimica et Biophysica Acta. Reviews on Cancer*. 2021; 1875: 188454. <https://doi.org/10.1016/j.bbcan.2020.188454>.