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REVIEW

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Insight into the role of DNA methylation in prognosis and treatment response prediction of gastrointestinal cancers

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ABSTRACT

Gastrointestinal (GI) cancers impose a significant disease burden, underscoring the critical importance of accurate prognosis prediction and treatment response evaluation. DNA methylation, one of the most extensively studied epigenetic modifications, has gained prominence due to its reliable measurement across various sample types. Numerous studies have reported that DNA methylation was linked to the diagnosis, prognosis and treatment response in malignancies, including GI cancers. While its diagnostic role in GI cancers has been comprehensively reviewed. Recent research has increasingly highlighted its potential in prognosis prediction and treatment response evaluation. However, no existing reviews have exclusively focused on these two aspects. In this review, we retrieved relevant studies and included 230 of them in our discussion, thereby providing an overview of the clinical applicability of aberrant DNA methylation in these two fields among patients with esophageal, gastric, colorectal, pancreatic cancers, and hepatocellular carcinomas. Additionally, we discuss the limitations of the current literature and propose directions for future research. Specifically, we emphasize the need for standardized DNA methylation methodologies and advocate for the integration of gene panels, rather than single genes, to address tumor heterogeneity more effectively.

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1. Introduction

Gastrointestinal (GI) cancers, which affects the gastrointestinal tract and accessory organs, account for approximately 4.78 million new cases and 3.23 million deaths annually worldwide, presenting a major clinical challenge [1]. This underscores the need for strategies to reduce mortality through precise survival predictions and more effective treatments. The tumor-node-metastasis (TNM) staging system, established by the American Joint Committee on Cancer (AJCC), is acknowledged as the standard prognostic tool [2]. The TNM staging system classifies tumors based on the depth of invasion (T), lymph node involvement (N), and the presence of distant metastases (M) [2]. Nevertheless, survival outcomes among patients within the same TNM stages vary greatly, highlighting the need for additional prognostic indicators. Moreover, therapeutic efficacy remains limited when applied broadly, emphasizing the importance of identifying biomarkers that can predict treatment response.

Epigenetic changes, including DNA methylation and histone modifications, are heritable changes in gene expression that do not cause permanent alteration of the underlying DNA sequences [3–5]. DNA methylation, the most extensively studied epigenetic change, has been reported to be involved in the initiation and progression of cancers, by mediating the transcriptional silencing of tumor suppressor genes through hypermethylation, and resulting in the aberrant activation of oncogenes via hypomethylation [6]. Recently, it has garnered significant interest in clinical practice, including diagnosis, prognosis prediction and treatment response evaluation, owing to its advantage of easily accessibility [7]. Its utility in early detection, based on tissue or noninvasive samples, has been comprehensively studied and reviewed, and some DNA methylation-based biomarkers, such as *SEPT9* methylation in plasma, have been approved by the U.S. Food & Drug Administration (FDA) for the early detection of colorectal cancer (CRC), and by the National Medical Products Administration (NMPA) for the early detection of gastric cancer (GC) [8–10]. Recently, numerous studies have reported that aberrant DNA methylation, especially CpG island methylator phenotype (CIMP), is associated with the prognosis and treatment response of GI cancers. However, some ambiguous conclusions exist, and to our knowledge, no relevant reviews have comprehensively summarized these two aspects.

In this review, we focus on DNA methylation in GI cancers, including esophageal, gastric, colorectal, pancreatic cancers, as well as hepatocellular carcinoma, and provide an overview of the potential of DNA methylation signatures for prognosis prediction and treatment response evaluation. Moreover, we

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Article highlights

- Gastrointestinal (GI) cancers present clinical challenges, requiring precise survival predictions and better treatments. TNM staging, though standard, shows variability in outcomes, necessitating additional markers. Broad therapies often fail, highlighting the need for predictive biomarkers.
- Epigenetic changes, including DNA methylation, involve heritable gene expression alterations without modifying DNA sequences. DNA methylation, widely studied, plays a key role in cancer pathogenesis and has gained clinical interest due to its accessibility.
- Aberrant DNA methylation includes regional DNA hypermethylation and global DNA hypomethylation. DNA hypermethylation usually results in decreased expression of relevant genes, particularly tumor suppressor genes. On the other hand, DNA hypomethylation is linked to the increased gene expression, leading to chromosomal instability (CIN) and inappropriate activation of oncogenes.
- DNA methylation frequently occurred at the 5′-position of cytosine residues (5mC or 5-methyl cytosine) within a CpG dinucleotide sequence. Some regions are characterized by a particularly high CpG content, known as 'CpG islands'. In normal cells, CpG islands are typically unmethylated; however, hypermethylation of these CpG islands is a characteristic feature of the CpG Island Methylator Phenotype (CIMP).
- The utility of DNA methylation in early detection is well-studied, with recent research emphasizing its role in prognosis and treatment response prediction in GI cancers.
- Several methods are available for measuring aberrant DNA methylation. The most widely applied technique for assessing CIMP status is MSP/qMSP, although some studies utilized COBRA and other methods. For detecting the methylation status of single genes, various methods have been employed, which might partly lead to discrepancy in conclusion.
- In the context of prognosis prediction in GI cancers, CIMP status has been extensively studied. Research has shown that CIMP-positive status is associated with poor survival outcomes in patients with HCC. However, findings for CRC patients have been inconsistent, partly due to variations in the gene panels used to assess CIMP status and differences in the definition of CIMP-high across studies. Moreover, some aberrant DNA methylation gene loci have also been reported to be linked to prognosis and show a promise for future clinical application: *LINE-1* hypomethylation in CRC patients; *CDKN2A/p16*, *MLH1*, *CDH1* in GC patients; and *DIRAS1*, *EPB41L3*, *LINE-1*, *APC* in ESCC patients.
- For treatment response evaluation, inconsistent results were obtained toward the CIMP status and chemotherapy efficacy, might largely be due to the ambiguous conclusions regarding its association with cancer prognosis. Additionally, the aberrant methylation of certain genes, such as *MGMT* and *TFAP2E* in CRC; *DAPK*, *CDKN2A* and *RPRM* in GC, have emerged as promising biomarkers for evaluation treatment response.
- Advances in high-throughput technologies have streamlined the assessment of DNA methylation, enhancing its utility. As a result, DNA methylation analysis shows great promise as a key biomarker for prognosis and treatment response in precision medicine.
- The CIMP status is expected to be implemented in the future for prognosis prediction and treatment response evaluation, pending the standardization of DNA methylation panels, a clear definition of CIMP, and subsequent external validation.

discuss the current limitations and future prospects of DNA methylation research. We hope that this review will help translate these findings on DNA methylation in GI cancers into clinical practice.

2. Methods

PubMed, Cochrane Library, Embase, and Google Scholar were initially searched to retrieve studies reporting data on DNA methylation from January 2000 to July 2024. The following Medical Subject Heading (MeSH) terms were used alone or in combination with the logical operators "OR" or "AND" to obtain the maximum number of articles: "Stomach Neoplasms," "Colorectal Neoplasms," "Esophageal Neoplasms," "Liver Neoplasms," "Pancreatic Neoplasms," "DNA methylation," "Epigenomics" "Prognosis," "Treatment Outcome." We excluded repetitive as well as non-English studies. After an initial screening of titles and abstracts, full-text copies of each article were retrieved. Each relevant article was subsequently reviewed, and 230 representative scientific papers were finally selected. The inclusion and exclusion criteria are provided in Table S1, and the flow diagram is available in Figure S1.

3. DNA methylation and cancer

DNA methylation, the addition of a methyl group to the specific DNA region by DNA methyltransferases (DNMTs), is one of the most commonly studied epigenetic alteration in malignancies, including GI cancers, and plays an indispensable role in the genome integrity maintenance and its relevant regulation [4,11–14]. DNA methylation is predominantly found at the 5′-position of cytosine residues (5mC or 5-methyl cytosine) within a CpG dinucleotide sequence. Some regions in the genome are characterized by a particularly high CpG content, known as 'CpG islands' [15]. In normal cells, these CpG islands are typically unmethylated. However, CpG islands are frequently hypermethylated in cancer cells, participating in the initiation and progression of cancer. A subset of tumors is characterized by widespread hypermethylation of CPG islands in the promotor region of genes, defined as CIMP [11]. In malignancies, including GI cancers, global DNA hypomethylation and aberrant regional DNA hypermethylation are observed [16]. DNA hypermethylation usually results in decreased expression of relevant genes, particularly tumor suppressor genes [17]. On the other hand, DNA hypomethylation is linked to increased gene expression, commonly occurring in the highly repetitive DNA sequences, such as Long Interspersed Nucleotide Element (*LINE-1*), Short Interspersed Nuclear Element (*SINE*), Intracisternal A Particle (*IAP*) and Alu elements, leading to chromosomal instability (CIN) and inappropriate activation of oncogenes [18–20].

Several methods are available for measuring aberrant DNA methylation, which can be broadly classified into two major categories: single-gene techniques and whole-genome techniques [21]. Single-gene techniques include bisulfite conversion-based detection [such as Methylation-Specific PCR (MSP)/Quantitative Methylation-Specific PCR (qMSP), Pyrosequencing, Methylation-Specific High-Resolution Melting (MS-HRM), combined bisulfite restriction analysis (COBRA)], and methylation-sensitive restriction enzyme (MSRE)-based techniques [such as MSRE PCR, Methylation-Sensitive Multiplex Ligation-Dependent Probe Amplification (MS-MPLA)]. The whole-genome techniques encompass methods for determining global DNA methylation (such as Mass Spectrometry) and genome-wide DNA methylation profiling [such as DNA Methylation Arrays, Next-Generation Sequencing (NGS)] [21]. The most widely applied technique

for assessing CIMP status is MSP/qMSP, although some studies utilized COBRA and other methods. For detecting the methylation status of single genes, various methods have been employed, which might partly lead to discrepancy in conclusion. Therefore, standardization of the measurement methods is warranted. Among the detection methods, MSP and COBRA exhibit favorable performance in terms of sensitivity and specificity, yet each presents unique advantages and disadvantages. For instance, MethyLight (a form of MSP), excels in terms of high specificity but is limited by its expense and inability to discern methylation heterogeneity. Consequently, investigators might select the most suitable methodology based on the specific context of application, considering each method's distinct benefits and limitations (Table S7). Furthermore, samples used for detecting DNA methylation status include tumor tissue and body fluids (such as peripheral blood and peritoneal washes). Studies have shown that the methylation status of DNA in tumor tissue is highly correlated with that in plasma DNA [22–24]. Circulating tumor DNA (ctDNA), which carries cancer-specific genetic and epigenetic signatures, offers the potential for dynamic monitoring of DNA methylation in plasma to evaluate treatment efficacy and survival outcomes [22,23].

In terms of prognosis prediction in GI cancers, particularly CRC and GC, CIMP status and aberrantly methylated genes have been subjects of extensive study. The majority of the published studies have reported that CIMP-positive status is correlated with dismal survival outcome in patients with hepatocellular carcinoma (HCC), while inconsistent conclusions have been drawn for patients with CRC, limiting its clinical applicability [25–27]. This discrepancy might partly stem from variations in the gene panels used to quantify CIMP and difference in the definition of CIMP-high across studies. For instance, Shuji Ogino et al. defined CIMP status based on the methylation of eight CIMP specific promoters (*CACNA1G*, *CDKN2A (p16)*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3* and *SOCS1*), characterizing CIMP-high as more than 6 out of 8 methylated markers, CIMP-low as 1 to 5 out of 8 methylated markers, and CIMP- negative as 0 out of 8 methylated markers [28]. Besides, the CIMP panel described by Vedeld et al. includes *CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3* and *SOCS1*, with samples showing a PMR value ≥10 for ≥3 out of 5 markers considered CIMP high, no DNA methylation as CIMP negative, and all others as CIMP-low [26]. Moreover, some aberrant DNA methylation gene loci have also been reported to be linked to prognosis and show promise for future clinical application: *LINE-1* (hypomethylation) in CRC patients [29]; *CDKN2A/p16*, *MLH1*, *CDH1* in GC patients [30–32]; and *DIRAS1*, *EPB41L3*, *LINE-1*, *APC* in esophageal squamous cell carcinoma (ESCC) patients [33–36].

For treatment response evaluation, most studies have focused on the role of CIMP status in chemotherapy, though further exploration in the context of targeted therapies is warranted. CIMP status has been reported to be associated with chemotherapy efficacy, but inconsistencies exist, likely due to the ambiguous conclusions regarding its association with cancer prognosis. Further investigations are needed, including the unification of CIMP definition, analysis of homogenous participants, and standardized treatment regimes. Mechanically, studies have reported that CIMP-high patients exhibit higher level of intracellular folate (a substance positively correlated with 5-FU efficacy) and lower level of g-glutamyl hydrolase (an enzyme negatively associated with folate concentration), compared to those with CIMP- low [37]. Additionally, the aberrant methylation of certain genes, such as *MGMT* and *TFAP2E* in CRC [38,39]; *DAPK*, *CDKN2A* and *RPRM* in GC [30,40,41], has emerged as promising biomarkers for evaluating treatment response. External validation of previously reported DNA methylation signatures in large cohorts, along with further exploration of additional DNA methylation biomarkers, is anticipated. Establishing standardized measurement methods and analyzing homogenous population groups will be crucial prerequisites for these advancements.

4. DNA methylation in colorectal cancer

Epigenetic instability, such as the CIMP subclass of CRC, along with other factors including CIN, microsatellite instability (MSI), altered tumor microenvironments, and metabolic changes, plays a significant role in the molecular pathogenesis of CRC [42–44]. CIMP, a molecular subclass first identified in 1999, is implicated in the pathogenesis of 15–20% of CRC cases. Individuals with CIMP high status exhibit elevated methylation levels in the gene promotor region [45,46]. Several gene panels have been applied to designate CIMP, with the most acknowledged one is a panel consisting of five genes: *CACN3A1G*, *IGF2*, *NEUROG1*, *RUNX3*, and *SOCS1* [47]. CIMP-positive status is more commonly observed among female patients with proximal tumor location, MSI-high, and *KRAS*/*BRAF* mutations [48,49]. The molecular classification of CRC, widely accepted for its translational potential, incorporates CIMP status and categorizes CRC patients into four subtypes: CMS1 through CMS4. Of these, CMS1 is characterized by global hypermethylation (CIMP-high), while the other subtypes exhibit intermediate or low methylation patterns (CIMP-low) [50]. Patients classified as CMS1 comprise approximately 14% of CRC cases and tend to have worse survival outcomes after relapse compared to other subtypes [50]. Furthermore, the hypermethylation or hypomethylation of specific DNA regions has also been reported to have prognostic significance.

4.1. Prognosis prediction

Among DNA methylation signatures, CIMP status is widely regarded as one of the most promising prognostic indicators in CRC and has been extensively studied [6]. Nevertheless, discrepancies exist regarding the prognostic role of CIMP in CRC among study results; the majority of studies have reported that CIMP correlates with poor survival outcomes, including a cohort of 1035 CRC patients by Vedeld et al. [hazard ratio (HR) = 1.89, 95% confidence interval (*CI*): 1.34–2.65, $p < 0.001$] and in another study conducted by Barault et al. (HR = 2.90, 95% *CI*: 1.53–5.49, $p = 0.001$) [25,26]. Nevertheless, the study by Ogino et al. reported a contrary result in patients with stage I-IV colon cancer, showing reduced mortality (HR = 0.44, 95% *CI*: 0.22–0.88), whereas other studies found no correlation between CIMP status and patients' survival outcomes [28,51,52]. Studies pointed out

that the unfavorable prognosis of patients with CIMP-positive status might result from the co-existing V600E *BRAF* mutations, which is recognized as an indicator of poor prognosis [53,54]. Additionally, MSI status is considered as another confounding factor, as differences in prognosis have been observed between CIMP-positive patients with MSI-high versus those with microsatellite stable (MSS) tumors [5-year overall survival (OS) rate: 80.5% *vs*. 45.2%] [55]. Nevertheless, a meta- analysis of 33 studies involving 10,635 individuals concluded that CIMP positive patients frequently exhibit an unfavorable survival outcome toward OS and disease-free survival (DFS), regardless of MSI status [56]. The discrepancy across studies might be partly due to co-existing prognostic factors, such as MSI and *BRAF* status. Therefore, the prognostic value of CIMP status warrants further investigation in large cohorts, with subsequent subgroup analyses in terms of prognostic baseline clinical-pathological variables. Moreover, the varied criteria used to classify CIMP status, such as the number of methylated markers, might also contribute to the conflicting results. For instance, one study categorized patients based on the number of the methylated markers as CIMP-P1 (5–6 markers), CIMP-P2 (7–8 markers), and CIMP-negative (0–4 markers), the survival analysis showed that patients with CIMP-negative or CIMP-P2 present a favorable survival outcome, compared to patients with CIMP-P1 [57]. Overall, the conflicting conclusions regarding the prognostic role of CIMP in CRC may be attributed to several factors, including the gene panels utilized to define CIMP, the criteria for classifying CIMP-high status, and baseline characteristics of the study participants, such as MSI and *BRAF* status.

On the other hand, the prognostic value of CIMP in rectal cancer has been less frequently studied, and the conclusions drawn are consistent, indicating a negative correlation between CIMP status and patient survival [58,59].

In addition to CIMP status, the aberrant methylation of specific DNA loci has been reported to be associated with the development of CRC and has the potential to act as a prognostic marker [13]. Hypermethylation in promotor regions leads to the inactivation and dysfunction of relevant genes, including tumor suppressor genes such as *CDKN2A (p16)*, *PTCH1* and *E-cadherin*; DNA repair genes such as *MLH1* and *MGMT*; and apoptosis-related genes such as *Apaf1*, *Bcl2*, and *p53* [60–63]. The most widely investigated gene is *CDKN2A (p16)*, a tumor suppressor gene that acts as a cyclin-dependent kinase inhibitor, blocking the transition from the G1 to S phase [25,64,65]. Hypermethylation of this gene has been reported as an indicator of unfavorable survival outcome in several independent studies and a subsequent meta-analysis [64–68]. Furthermore, several other aberrantly methylated gene loci have been reported to be prognostic, but remain in the early stages of investigation owing to small sample sizes, heterogenous methodologies, or a lack of external validation. In addition to tissue-based analyses, aberrantly methylated genes such as *SEPT 9*, *HLTF*, *TAC1* and others, detected in blood-based samples, have also been reported to have prognostic value [69,70]. For example, methylated *SEPT 9* in peripheral blood has been reported as a risk factor of cancer-specific mortality among patients with stage I–III CRC (HR = 2.69, 95%*CI*: 1.26–5.73, $p < 0.05$) [69]. Apart from that, the dynamic changes in methylation levels could serve as a prognostic indicator. Barault et al. reported that a decrease in DNA methylation levels of five genes (*EYA4*, *GRIA4*, *ITGA4*, *MAP3K14-AS1*, and *MSC*) was associated with prolonged progression-free survival (PFS) in stage IV CRC patients (HR = 0.48, 95%*CI*: 0.17–0.87, $p = 0.039$) [71]. In another study, an increase in the levels of hypermethylated *WIF1* and *NPY* in cell-free DNA (cfDNA) was observed among patients with localized CRC who experienced relapse, as well as among those with metastatic CRC who exhibited progressive disease (PD) [72]. Moreover, a DNA methylation-based risk model established by incorporating several genes showed favorable performance in detecting recurrence [Area Under the Curve (AUC) = 0.825, 95%*CI*: 0.680–0.970], which indicates that comprehensively considering the methylation status of multiple genes might provide more accurate information than provided by a single gene. Additionally, individuals with a high methylation risk score exhibited poorer survival than those with low risk score (HR = 4.349, 95%*CI*: 1.783–10.61, $p = 0.002$) [73]. This indicates that a comprehensive assessment of the methylation profiles across multiple genes demonstrates superior performance and exhibits potential for clinical utility.

Moreover, DNA hypomethylation has also been reported to be prognostic. Ogino et al. reported that patients with *LINE-1* hypomethylation had worse survival outcome compared to their counterparts (HR = 1.85, 95%*CI*: 1.25–2.75, $p = 0.002$) [74]. A consistent result was obtained by Inamura et al. in a large population, particularly among those with MSI-high status [29]. A meta-analysis further confirmed a negative association between *LINE-1* hypomethylation and the prognosis of CRC patients by pooling data from 13 independent studies involving 3620 patients [75]. Recently, a meta-analysis quantitatively synthesized the prognostic impact of previously reported DNA methylation biomarkers and further evaluated their prognostic value in an external validation cohort of 2,303 patients. Nine genes were confirmed with consistent results compared to previous studies, among these, *CDKN2A*, *WNT5A*, *MLH1*, and *EVL* were identified as the most promising DNA methylation biomarkers for prognostication [76] (Table S2).

Accumulating evidence supports the prognostic value of aberrant DNA methylation signatures in patients with CRC. Among these, CIMP status, *CDKN2A, MLH1* and *LINE-1* are considered as promising biomarkers expected to be applied in the future, following the standardization of measurement methods, the selection of homogenous participants, the unification of CIMP-high definitions and large cohort-based external validation.

4.2. Treatment response evaluation

CIMP, the most widely studied prognostic DNA methylation signature, has also shown potential in predicting chemotherapy response in CRC. Some studies have reported that CIMP-positive patients do not benefit from and may even experience worse survival outcome after receiving 5-Fluorouracil (5-FU)-based adjuvant chemotherapy [67,77]. However, one study has reported an opposing conclusion [78]. This inconsistency might predominantly derived from the discrepancy in

its prognostic role. Moreover, CIMP status has been identified as a predictive marker for adjuvant irinotecan (with a 5-year OS rate of 69% in the irinotecan/5-FU arm *vs*. 56% in the 5-FU arm) and oxaliplatin (HR = 1.46, 95% *CI*: 1.15–1.87, $p = 0.002$) [79,80]. While CIMP shows promise in predicting treatment efficacy, it is crucial to first verify its prognostic significance.

Moreover, *MGMT* hypermethylation has been associated with a favorable response to adjuvant 5-FU in advanced CRC tumors and preoperative chemoradiotherapy in patients with advanced rectal cancers (particularly to dacarbazine) [81,82]. *MGMT* hypermethylation also predicts a better outcome in the treatment of metastatic CRC patients with alkylating agents (median PFS: 2.1 *vs*. 1.8 months, $p = 0.008$) [38]. Additionally, aberrant methylation of genes such as *TFAP2E*, *COD1*, *SRBC*, etc. has been linked to the treatment response of cytotoxic agents, including docetaxel, gemcitabine, and 5-FU in CRC patients [83–85]. Regarding targeted therapy, which is another vital treatment option for CRC patients, relevant studies are relatively scarce. However, *HPP1* methylation in plasma is considered as a predictor of non-responsiveness to bevacizumab-containing therapy [86]. Furthermore, *CREB1* methylation might serve as an indicator of the efficacy of radiotherapy in patients with rectal cancer [87]. Moreover, among stage IV CRC patients, a decrease in the average methylation level of a gene panel in cfDNA near the time of radiological assessment was reported to be associated with partial response (PR) or stable disease (SD), while no indicator of PD was observed [71] (Table S2).

Overall, CIMP status is the most extensively investigated DNA methylation biomarker for evaluating treatment response in CRC patients. We believe it is worthwhile to further validate the predictive role of CIMP status in chemotherapy and to explore its potential in targeted treatment through larger, prospective studies. Additionally, *MGMT* hypermethylation and *TFAP2E* hypermethylation could also be considered potential biomarkers for evaluating the treatment response [81,85].

5. DNA methylation in gastric cancer

The Cancer Genome Atlas (TCGA) classification, a molecular classification system developed based on factors such as mutation patterns, DNA methylation, and biological pathway dependencies, plays a critical role in precise survival prediction and the selection of appropriate therapeutic agents in GC. The TCGA classification system stratified patients into four subtypes: the Epstein Barr virus (EBV) subtype, the MSI subtype, the CIN subtype, and the genome stable subtype [88]. The EBV subtype, associated with the presence of the EBV, accounts for approximately 9% of GC cases [6]. This subtype exhibits genome-wide increases in DNA methylation, known as 'CIMP', and is considered to have the highest level of DNA methylation [89]. The MSI subtype, which occurs in approximately 22% of cases, is characterized by a microsatellite unstable genome caused by the hypermethylation of DNA repair genes such as *MLH1*, *MGMT*, *PMS2*, and *MSH2* [18]. This subtype is also noted for its high tumor mutation burden and a hypermethylated CIMP epigenome, albeit to a lesser extent than the EBV subtype [18]. The CIN subtype (approximately 50% of cases) and the genome stable subtype (around 20% in frequency) are defined by relative genetic stability [18]. In these latter two subtypes, epigenetic alteration plays a dominant role in the pathogenesis of GC by activating oncogenes and suppressing tumor suppressor genes [18].

Etiological factors of GC including chronic inflammation (such as *Helicobacter pylori* or EBV), smoking and age lead to aberrant DNA methylation changes [90,91]. *Helicobacter pylori* infection is linked to regional DNA hypermethylation and hypomethylation of Alu repeat elements in human gastric mucosae [92,93]. The complete or partial reversal of methylation at cancer-related genes such as *CDH1*, *MGMT* and *COX2* has been observed in patients receiving *Helicobacter pylori* eradication treatment [94–96]. In contrast, EBV-induced hypermethylation is thought to result from direct pathogen infection, leading to modulation of host *DNMT1* activity and downregulation of the *TET2* (a demethylase), rather than through inflammation-related intermediate pathways [90,97].

5.1. Prognosis prediction

The widely studied prognosis-related genes in GC include *CDKN2A*, which is methylated in all EBV subtype GC; *MLH1*, which is methylated in the majority of MSI GC and mediates the MSI phenotype; and *CDH1*, which is commonly methylated in diffuse GC and in GC arising from hereditary diffuse GC syndrome [6,98,99]. Shi et al. analyzed the prognostic value of DNA methylation markers in 119 GC cases and reported that the methylation of *CDKN2A*, along with *MGMT*, *RASSF2* and *FLNc*, associated with poor survival outcome [30]. Studies by Shigeyasu et al. reported that hypermethylation of *MLH1* is an indicator of improved overall survival in GC ($p = 0.026$) [31]. Aberrant methylation of *CDH1* was reported to be linked to worse survival in GC [32].

Moreover, several other genes have also been reported to be prognostic in GC. Aberrant methylation of *THBS1*, a gene involved in the transition of cancer cells to the mesenchyme, can be detected in peritoneal lavage fluid, serum and tissue, and is associated with peritoneal metastasis and adverse survival outcome in GC (tissue: HR = 4.377, 95%*CI*: 1.880–10.190, $p = 0.001$; serum: HR = 3.838, 95%*CI*: 1.691–8.71, $p = 0.001$; peritoneal lavage fluid: HR = 4.015, $p < 0.001$) [100]. Teng et al. observed that methylation of *PRDM5*, a transcription factor, is associated with tumor proliferation and poor prognosis in GC [101]. Xu et al. examined the prognostic role of *BCL6 B* methylation in 309 GC patients from two independent study cohorts and reported that the *BCL6 B* methylation is linked to worse OS (RR = 2.14, 95% *CI*:1.36–3.36, $p = 0.001$) [102]. Aberrant methylation of *DAPK* was reported to confer dismal survival [40]. Another study echoed this observation by investigating the prognostic value of *DAPK* and *TMS1* (*TMS1*: $p = 0.0123$; *DAPK*: $p = 0.0464$) [103]. Methylation of *HOXA11*, considered a candidate tumor suppressor gene, was detected in GC tissue and was highly correlated with worse survival: the survival time of those without *HOXA11* promoter methylation was 2 times longer than patients who have methylated *HOXA11* promoter (HR = 2.4, 95%*CI*:1.19–4.86, $p = 0.015$) [104]. In addition to the genes mentioned above, the aberrant methylation of other genes

has also been reported to correlate with patients' survival outcomes.

CIMP has also been detected in GC tissue and reported to be associated with GC survival, although it has been studied less frequently compared to CRC [105]. There is a discrepancy in the prognostic effect of CIMP across studies in GC. Some studies have observed a positive correlation between CIMP positivity and GC survival, while others have reported the opposite correlation [31,106]. A meta-analysis that included 10 studies with a total of 918 patients failed to observe a significant prognostic role of CIMP status in GC, highlighting the need for prospective large cohort studies [107] (Table S3).

Aberrant methylated genes, such as *CDKN2A*, *MLH1*, and *CDH1*, have been investigated in several studies and are considered to be associated with patient prognosis. However, since most of these studies were conducted with relatively small sample size, their external applicability is limited. CIMP status has also been investigated in GC, but the inconsistency in study results indicates the necessity of further research in large cohorts.

5.2. Treatment response evaluation

The DNA methylation signatures with the capacity to predict treatment response have been less frequently studied, and relevant research has primarily focused on its utility in the chemotherapy setting for GC. Sugita et al. reported that methylation of either *BNIP3* or *DAPK* predicted lower response to 5-FU-based chemotherapy by analyzing 80 stage I-IV GC cases ($p = 0.0007$) [40]. Methylation of *DAPK*, along with *TMS1*, was also reported to correlate with a poor response to 5-FU in patients with recurrent GC [103]. Additionally, aberrant methylation of *RPRM* and *CDKN2A* has been reported to be associated with low response to 5-FU or 5-FU-based chemotherapy [41,108]. (TableS3)

To date, some aberrantly methylated genes, including *DAPK*, *CDKN2A* and *RPRM*, have been reported to be linked to treatment response in GC. However, the applicability of these findings is restricted by limited sample size, underscoring the need for further external validation in large cohorts. The exploration of novel DNA methylation biomarkers is expected to contribute to the development of precise medicine.

6. DNA methylation in esophageal squamous cell carcinoma

Esophageal Cancer (EC) includes two prominent histological subtypes: ESCC, the most prevalent subtype accounting for more than 85% of EC cases, and esophageal adenocarcinoma (EAC) [109,110]. In this review, we focus on ESCC. Although the methylation status of several genes has been reported as prognostic, DNA methylation markers that can be used to predict treatment response remain scarce.

6.1. Prognosis prediction

Similar to other malignancies, several aberrantly methylated genes have been reported as prognostic markers. Zhu et al. reported that reduced expression of *DIRAS1*, a tumor suppressor gene in ESCC, is associated with poor survival ($p = 0.002$) [33]. The hypermethylation induced reduction in the expression of other tumor suppressor genes, such as *EPB41L3*, *LINE-1*, has also been reported as an indicator of worse survival in ESCC [34]. Moreover, the hypermethylation of *APC*, *RASSF2*, *CDH1*, *DENND2D,CLK1*, *ZEB2* and *DACT1/2* were also reported to be linked to poor survival [111–114]. Additionally, a panel consisting of several genes can be used to indicate the prognosis of ESCC. Xi et al. reported that four CpG sites, identified by differentially methylated CpG sites between cancerous tissue and adjacent normal tissue, have the capacity to distinguish high-risk individuals [115]. Besides DNA hypermethylation, the hypomethylation of *LINE-1* has been associated with shorter DFS, particularly in stage I ESCC (overall population: $p < 0.001$; stage I: $p = 0.0006$; stage II-III: $p = 0.11$) [35]. Others studies have echoed this conclusion [116]. CIMP positivity was reported to be associated with poor survival in ESCC [117]. Furthermore, a risk score that integrates the methylation status of multiple genes has been reported to effectively stratify individuals at high risk with poor survival outcomes [118] (Table S4).

The CIMP status, along with the hypermethylation of genes, such as *DIRAS1*, *EPB41L3*, *LINE-1*, *APC*, etc., has been reported to be associated with patient survival outcomes and is expected to be applied in the future after validation in large cohorts.

6.2. Treatment response evaluation

Studies exploring DNA methylation signatures in prediction treatment efficacy have primarily focused on chemoradiotherapy, the fundamental treatment option in ESCC. A risk score based on a six-CpG panel of DNA methylation biomarkers, including *IFNGR2*, *KCNK4*, *NOTCH4*, *NPY*, *PAX6* and *SOX17*, has been reported to predict response to chemoradiation therapy in ESCC, demonstrating a strong predictive capacity with an AUC value of 0.930 [119]. Hamilton et al. reported that the mean methylation levels of several genes, including *p16*, *Reprimo, MGMT*, *TIMP-3*, and *HPP1*, were lower in responders to chemoradiotherapy compared to non-responders; notably, *Reprimo* hypermethylation was linked to poor response to chemoradiation ($p = 0.037$) [120]. Moreover, *PAX-5* methylation has been identified as an indicator of poor response to cisplatin-based chemotherapy (univariate analysis: HR = 3.16, 95%*CI*:1.55–6.59, $p = 0.002$; multivariate analysis: HR = 3.12, 95%*CI*:1.48–6.70, $p = 0.003$) [121]. Furthermore, the methylation of *ZNF695* and *FGF5* has been reported as an indicator of favorable response to chemoradiotherapy [122,123]. Additionally, it has been reported that CHFR hypermethylation correlates with enhanced sensitivity to docetaxel and paclitaxel treatments, potentially serving as a predictive biomarker for the selection of chemotherapeutic agents [124] (Table S4). Overall, the prognosis and treatment response-related DNA methylation signatures are less commonly studied in ESCC. Further exploration of novel DNA methylation-based biomarkers and validation of previously reported ones in prospective, large-sample cohorts are warranted.

7. DNA methylation in hepatocellular carcinoma

The etiological factors of HCC, including hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol, and genotoxins, are

considered to induce epigenetic alterations, primarily DNA methylation, thereby participating in the pathogenesis and progression of HCC [125,126]. DNA methylation markers in HCC have been investigated in several studies for their role in prognosis prediction; however, studies assessing DNA methylation signatures in relation to treatment response are relatively limited.

7.1. Prognosis prediction

CIMP status has also been detected in HCC and is considered a risk factor for the prognosis of HCC. Li et al. reported that patients with CIMP-high exhibit the worst outcomes, compared to those with CIMP-low or CIMP-medium ($p = 0.00054$) [27]. This conclusion has been supported by other relevant studies and a meta-analysis incorporating 568 patients from seven studies [127,128]. Aberrant methylation of several other genes has been reported as prognostic among patients with HCC. The hypermethylation of *LCAT* and hypomethylation of *SPP1* have been identified as significant prognostic factors, with a risk model incorporating these two aberrantly methylated genes demonstrating robust risk stratification capabilities (HR = 2.81, $p < 0.001$) [129]. The hypermethylation-induced reduction of *FES* was reported to indicate worse OS and PFS among patients undergoing hepatectomy [130]. Additionally, *BMP4* hypermethylation is associated with poor survival outcomes (HR = 3.431, 95%*CI*:1.333–8.833, $p = 0.011$) [131]. In some cases, the prognostic value of specific methylation sites preferentially exists in HCC with corresponding etiology. For instance, the methylation status *SOCS3* negatively impacts the prognosis of patients with HBV-induced HCC, but is not relevant to the survival outcome in general HCC cases [132]. Thus, epigenetic changes might be closely correlated with the etiology of HCC and considering the etiological factors when examining the prognostication of aberrant DNA methylation might provide valuable information.

Moreover, hypomethylation of specific genes can also indicate the prognosis of HCC. For instance, the increased expression of *K19*, induced by hypomethylation, is a survival risk factor ($p = 0.025$) [133]. Cao et al. reported that hypomethylation-induced high expression of *FOXK1* is an indicator of poor survival in stage I-IV HCC patients, based on the analysis of TCGA dataset [134]. *LINE-1* hypomethylation has also been detected in HCC, with patients exhibiting *LINE-1* hypomethylation having inferior survival than those with LINE-1 normomethylation ($p = 0.007$) [135]. Furthermore, the extent of hypomethylation was also reported to be prognostic. A study comparing survival difference between patients with weak and strong hypomethylation of CCAAT/enhancer-binding protein-beta (*C/EBPβ*) concluded that those with strong hypomethylation frequently present with worse survival outcomes (HR = 4.404, $p = 0.0026$) [136]. This study underscores the pivotal importance of consistent definitions across studies, as unifying these definitions is essential for reliable conclusions.

The methylation level of a set of genes have also been reported to be associated with prognosis and can be applied to predict survival outcome. Xu et al. constructed a model integrating TNM staging and a combined prognosis score (cp-score) generated by comprehensively analyzing 8-methylation ctDNA panel, which showed encouraging prognosis prediction capacity with an AUC value of 0.79 in the training dataset [22]. (Table S5)

In summary, CIMP status is a potential biomarker for prognosis prediction in HCC. Although published studies have reported its negative impact on HCC patients' survival outcome, external validation is required due to limited sample size across studies. Further investigation is also needed to validate the prediction role of remaining aberrantly methylated genes.

7.2. Treatment response evaluation

The role of aberrant DNA methylation in treatment response has been studied in the context of transarterial chemoembolization (TACE) and targeted therapies, both of which are foundational treatments for HCC. The methylation of *SOCS1* has been reported to be associated with poor response to TACE in 246 hCC patients (poor response rate in the methylated group *vs*. the unmethylated group: 43.9% *vs*. 28.1%, $p = 0.011$) [137]. Saeki, I., et al. reported that patients with methylated *SEPT9* in serum exhibited worse survival outcomes after receiving first-line treatment with lenvatinib or sorafenib ($p = 0.022$ and $p = 0.010$ in the training and validation cohorts, respectively) [138]. Additionally, the investigators constructed a score that incorporates AFP levels, methylated *SEPT9*, and ECOG performance status, which can be used to predict survival in patients receiving molecular targeted regimes [138]. Further studies are warranted to explore the association between DNA methylation marker and treatment efficacy in HCC (Table S5).

Overall, the use of DNA methylation biomarkers in evaluating treatment response in HCC is still in its early stages, and more research is needed.

8. DNA methylation in pancreatic adenocarcinoma

In this review, we focused on pancreatic adenocarcinoma (PANCA), the most common subtypes of pancreatic cancer (PC), accounting for approximately 90% of all PC cases [139]. The epigenetic alterations occurred in PANCA are less comprehensively studied, compared to CRC and GC. However, by integrating DNA methylation signatures, copy number variations, and other factors, PANCA can be classified into different subtypes, providing a pathway toward precise medicine [140].

Owing to its aggressive nature, the majority of patients (>80%) are diagnosed at an advanced stage, leading to dismal survival outcomes regardless of the treatment applied. Consequently, there have been relatively few studies focused on identifying biomarkers that signify patient survival and treatment efficacy, and those that have been published often involve small sample sizes.

8.1. Prognosis prediction

A study reported that the methylation of two genes, *TNFRSF10C* and *ACIN1*, in whole blood samples was an indicator of worse OS ($p = 0.023$ and $p = 0.012$) [141]. Sato et al. observed that patients with RPRM methylation frequently

exhibited unfavorable OS in a cohort of 87 patients ($p = 0.007$) [142]. Moreover, the hypermethylation of *MUC1* and *MUC4* was reported to be associated with favorable survival [143]. In contrast, the hypermethylation-induced reduction in the expression of *RELN* was reported to be linked to unfavorable survival outcomes among patients with survival times exceeding 16 months (HR = 3.7, 95%*CI*: 1.13–12.25, $p = 0.03$), but failed to predict survival in all patients when not stratified by survival time (HR = 1.4, 95%*CI*: 0.86–2.14, $p = 0.19$) [144]. This highlights the necessity of considering the survival times in prognosis analysis of high aggressive tumor, such as PANCA. The hypomethylation-induced increased expression of *MET* and *ITGA2* in PANCA tissue was reported to be associated with worse survival [145]. Garcia-Ortiz et al. reported that the methylation of *NPTX2* in plasma was associated with dismal outcome among patients with stage IV PANCA ($p = 0.0067$) [146]. To date, no studies reporting the CIMP status and its prognostication were available in PANCA [6]. In most studies regarding DNA methylation-related prognostic analysis in PANCA, the sample sizes are small, ranging from 30 to 118, which hinders the ability to draw definitive conclusions (Table S6).

8.2. Treatment response evaluation

Currently, studies investigating DNA methylation markers related to treatment response are scarce. Among metastatic pancreatic cancer patients receiving targeted therapy, high methylation of *NPTX2* in cell-free DNA fragment was reported to be linked to PD, whereas undetectable or low *NPTX2* methylation is more frequently detected in patients with SD [23].

In aggregate, the clinical utility of aberrantly methylated genes is still in the early stage of investigation, and their translational potential is expected to be further explored in the near future.

9. Limitations of current studies

Despite extensive research, several limitations persist in CRC studies. Some studies have not incorporated or mentioned the consideration of co-existing prognostic factors, such as MSI status and *BRAF* mutations, when examining the prognostication of CIMP status. Moreover, the gene panels applied to designate CIMP status and definition of high or low CIMP status varied across studies, which leads to confusing conclusions obtained from studies. Moreover, the role of DNA methylation in evaluating treatment response has been investigated across studies with diverse therapeutic regimens and lack external validation, which limits its clinical applicability.

In GC-related studies, several challenges remain. Variability in the gene panels used to define CIMP status results in discrepancies between studies and the inconclusive results of a meta-analysis regarding its prognostic value. Additionally, there is a scarcity of studies examining its role in treatment response assessment, with the existing literature limited by small sample sizes.

The role of DNA methylation in prognosis prediction and treatment response evaluation has been less extensively studied in other GI cancers. In ESCC, there is a scarcity of data on the prognostic and predictive capacity of DNA methylation, particularly regarding its utility in treatment response evaluation. In HCC, studies on the prognostic value of CIMP status are hindered by the absence of large-scale external validation, and the development of DNA methylation biomarkers for treatment response is still in nascent stages. In PANCA, research on DNA methylation-based biomarkers for prognosis assessment is constrained by small sample sizes, and studies exploring their predictive value in treatment response are exceedingly rare.

Moreover, the majority of DNA methylation markers were examined using tissue samples, the dynamic monitoring of which is restricted, and investigation of DNA methylation biomarkers based on peripheral blood are encouraged.

10. The prognostic role of DNMTs

DNA methylation, catalyzed by DNMTs, includes DNMT1, which primarily maintains existing methylation patterns, and DNMT3A and DNMT3B, which act as de novo methyltransferases targeting unmethylated CpG sites to establish new methylation marks [147]. Previous studies have reported a close association between DNMT1 mRNA expression levels and CIMP status in CRC and GC [148]. In GC, DNMT expression levels were notably high, with 81.6% for DNMT1 and DNMT3A, and 68.4% for DNMT3B, respectively [149]. A meta-analysis further confirmed the association of these elevated DNMT expression levels with an increased risk of GC [150]. Overexpression of DNMT1 is correlated with poor differentiation, lymph node metastasis, and dismal survival outcomes in GC patients [149,151–153]. Besides, the expression of DNMT3A in GC is also a prognostic indicator, whereas DNMT3B is not [154–156]. Among patients with CRC, high expression of DNMT1 and DNMT3B is reported to be associated with worse survival, as analyzed from the TCGA database [157,158]. In ESCC, the expression level of DNMT1 is 65%, and moreover, the overexpression of DNMT3B is reported to be an independent risk factor of patients' prognosis in multivariate analysis (OS: OR = 4.28, $p < 0.001$; DFS: OR = 2.79, $p < 0.001$) [159,160]. Relevant studies have indicated that DNMT1 is overexpressed in HCC compared to normal liver tissue (43% vs 0%), and patients with positive DNMT1 expression have been observed to have shorter OS and recurrence-free survival (RFS) ($p < 0.001$) [161]. Another study reported the consistent elevation of DNMT expression (DNMT1: 14%, DNMT3A: 60%, DNMT3B: 9%) in HCC tissue, with dismal survival observed among patients with high DNMT3A and DNMT3B expression levels [162]. In PANCA, the expression of DNMTs is also elevated and has been reported to be prognostic [163–165]. Higher expression levels of DNMT1 (78.7%), DNMT3A (23.9%), and DNMT3B (77.3%) are observed, and patients with positive DNMT1 exhibited shorter OS than those with negative expression (8.2 months *vs*. 11.6 months) [163]. Another study confirmed the prognostic role of DNMT proteins in PANCA, and moreover, the high expression of DNMT3B remained an independent prognosticator in multivariate analysis (HR = 3.142, 95% *CI*: 1.559–6.331, $p = 0.001$) [164]. Collectively, DNMT expression is elevated in GI cancers and linked to poor prognosis, providing a rationale for exploring DNMT inhibitors as

therapeutic agents to enhance the efficacy of anticancer treatments [4].

11. Conclusions

Advances in high-throughput measurement technologies have greatly facilitated the convenient and efficient assessment of DNA methylation, enhancing its applicability. Therefore, DNA methylation analysis holds significant potential as a vital biomarker for indicating prognosis and treatment response in the field of precision medicine.

To now, no DNA methylation signature with prognosis prediction capacity or with treatment response evaluation ability in GI cancers has been approved by the FDA or NMPA. Figure 1 summarizes some of these promising DNA methylation biomarkers. Published studies have yielded heterogeneous conclusions and less convincing due to small sample size, varying eligibility criteria and differing methodologies used to detect DNA methylation status, etc. Among them, the prognostic value of CIMP status has been the most widely investigated and is anticipated to be implemented in future for prognosis prediction and treatment response evaluation, following the standardization of DNA methylation panels, its definition and subsequent external validation.

12. Future perspectives

Overall, with the increasing application of genome-wide methylation analyses, the external validation of previously reported DNA methylation signatures and the identification of additional DNA methylation markers is anticipated to deal with the complexity of tumor biology. Firstly, in the realm of biomarker validation and exploration, the deployment of highly sensitive analytical technologies, in conjunction with rigorous, bias-minimizing optimization and methodological standardization, is of paramount importance. Secondly, considering the multi-clonal origins of tumors and the substantial intratumoral heterogeneity of malignancies, the DNA methylation signatures derived from a single gene may be insufficient to capture the entire landscape. Thus, a comprehensive analysis of the DNA methylation signatures across a panel of

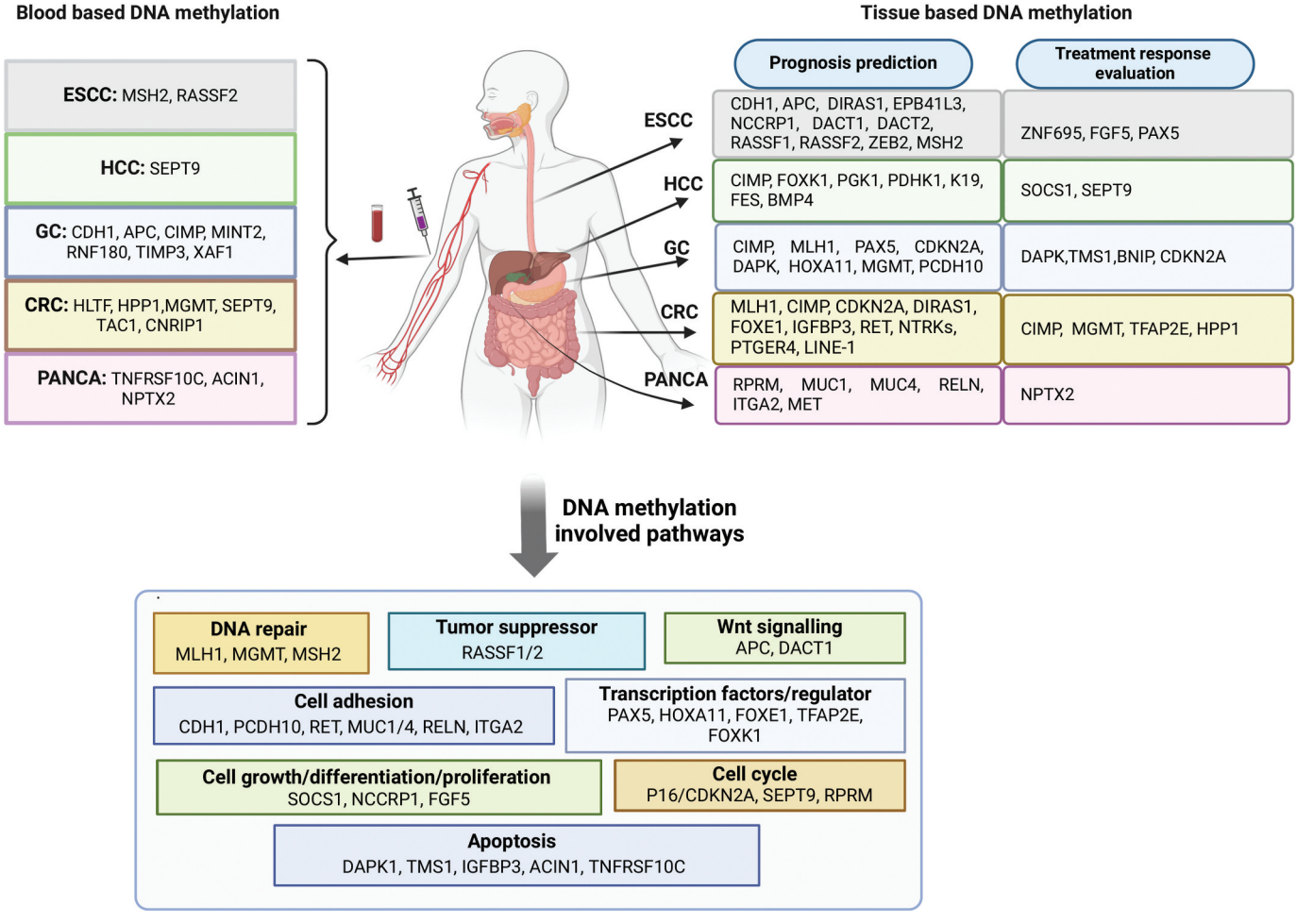

Figure 1. The aberrant DNA methylation biomarkers in GI cancers in prognosis prediction and treatment response evaluation.
(Created in BioRender. Abudurousuli, R. (2024) https://BioRender.com/b37e265).

genes is advocated. Additionally, the majority of DNA methylation markers have been investigated in tissue samples, constraining the capacity for dynamic monitoring of disease progression. Given the high concordance between DNA methylation profiles detected in tissue and body fluids, the clinical utility of detecting DNA methylation signatures in plasma or blood emerges as a promising focus for future research endeavors. Lastly, artificial intelligence (AI) and machine learning are extensively advancing across various research domains, demonstrating their potential to enhance the clinical utility of DNA methylation signatures in GI cancers.

Moreover, the transition of DNA methylation biomarkers into clinical practice is largely constrained by two major obstacles. One lies in the limited sensitivity and specificity of most existing DNA methylation biomarkers, and the other lies in the high cost of currently applied DNA methylation detection methods. Addressing these issues will help facilitate the translation of DNA methylation biomarkers into routine clinical practice. In summary, DNA methylation biomarkers are anticipated to play a significant role in the prognostic prediction and treatment response evaluation in GI cancers.

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Author contributions

Abudurousuli Reyila drafted the manuscript. Xianchun Gao revised the manuscript, and Jun Yu commented on the study. Yongzhan Nie designed and supervised the study.

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