Epigenetic changes in gastrointestinal cancers

Hironobu Shigaki[1], Yoshifumi Baba[1], Kazuto Harada[1], Naoya Yoshida[1], Masayuki Watanabe[2], Hideo Baba[1]

[1]*Department of Gastroenterological Surgery, Graduate School of Medical Science, Kumamoto University, Kumamoto 860-8556, Japan.*
[2]*Department of Gastroenterological Surgery, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo 135-8550, Japan.*

Correspondence to: Dr. Hideo Baba, Department of Gastroenterological Surgery, Graduate School of Medical Science, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan. E-mail: hdobaba@kumamoto-u.ac.jp

ABSTRACT

Epigenetic alterations, including DNA methylation, histone modification, loss of genome imprinting, chromatin remodeling and non-coding RNAs, are associated with human carcinogenesis. Among them, DNA methylation is a fundamental epigenetic process to modulate gene expression. In cancer cells, altered DNA methylation includes hypermethylation of site-specific CpG island promoter and global DNA hypo-methylation. Detection of aberrant gene promoter methylation has been applied to the clinic to stratify risk in cancer development, detect early cancer and predict clinical outcomes. Environmental factors associated with carcinogenesis are also significantly related to aberrant DNA methylation. Importantly, epigenetic changes, including altered DNA methylation, are reversible and thus, used as targets for cancer therapy or chemoprevention. An increasing number of recent studies reported DNA methylation level to be a useful biomarker for diagnosis, risk assessment and prognosis prediction for gastrointestinal (GI) cancers. This review summarized the accumulated evidence for clinical application to use aberrant DNA methylation levels in GI cancers, including colorectal, gastric and esophageal cancer.

Key words: Colorectal cancer, DNA methylation, epigenetic alterations, esophageal cancer, gastric cancer

Introduction

Epigenetics refers to heritable changes in gene expression that, unlike mutations, are not attributable to alterations in genomic DNA sequences. Epigenetic changes, such as DNA methylation, histone modifications, and altered expression of microRNAs, can regulate gene expression through mechanisms other than changes in genomic DNA sequence. Among them, genomic DNA methylation is a major epigenetic mechanism to mediate the X-chromosome inactivation, imprinting and repression of endogenous retroviruses.[1-4] DNA methylation is the covalent post-replicative addition of a methyl group (-CH_3) to the 5-carbon of the cytosine ring in CpG dinucleotides. CpG dinucleotides are non-uniformly distributed throughout the human genome.[2-4] Regions of the genome that are rich in sequences of a cytosine preceding a guanine (CpG dinucleotide) are known as CpG islands, which in particular, exist in the promoter regions of approximately half of all coding genes.

Altered DNA methylation in human cancers includes hypermethylation of site-specific CpG island promoter and global DNA hypo-methylation.[1-4] DNA methylation in gene promoter CpG islands results in its transcriptional inactivity and silence of protein expression. Thus, hypermethylation of a gene promoter is now recognized as a means of silencing tumor suppressor genes with effects similar to those of mutation or allelic loss in the development of cancer or other diseases.[3] Another DNA methylation alteration in human cancer is genome-wide DNA hypo-methylation.[5] Genome-wide DNA hypo-methylation appears to play an important role in genomic instability, leading to cancer development.[6-8] Previous experimental studies demonstrated that DNA hypo-methylation of repetitive sequences, that is, short interspersed transposable elements (SINE or Alu elements) or long interspersed transposable elements (LINEs) may predispose cells to chromosomal defects and rearrangements, resulting in genetic instability.[6] As *LINE-1* constitutes a substantial portion (approximately 17%) in the human genome, levels of *LINE-1* methylation are regarded to be surrogate markers for global DNA methylation.[9] Thus, epigenetic regulation of gene expression has emerged as a fundamental way in pathogenesis of numerous malignancies, including cancers of the digestive system.

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In fact, many exciting discoveries in epigenetics have emerged from the study of gastrointestinal (GI) cancers. In this review, we summarized the accumulated evidence supporting the clinical application of DNA methylation level in diagnosis of esophageal, gastric and colorectal cancers.

Altered DNA Methylation in Esophageal Cancer

Esophageal cancer can be classified into two histological types, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Their incidences vary notably by geographic distribution. ESCC accounts for approximately 90% of the esophageal cancers in East Asian countries,[10,11] whereas the highest number of EAC is found in Northern and Western Europe, North America and Oceania.[12] These two subtypes also have different epigenetic alterations. Growing evidence suggests that there is a field of epigenetic changes in esophageal cancer[13-15] by particularly emphasized significance of promoter hypermethylation of 14 specific genes (*SFRP1*, *SFRP2*, *DCC*, *APC*, *p16*, *p14*, *APBA1*, *APBA2*, *APBA3*, *CACNA1G*, *PTGS2*, *DAPK1*, *MLH1* and *MGMT*) in non-cancerous mucosae from ESCC patients *vs*. mucosae from healthy volunteers,[13] indicating that aberrant methylation or these 14 gene promoters in esophageal mucosae is associated with ESCC development. An overview of different previous studies of clinical implications of DNA methylation in esophageal cancer is provided in Table 1. Aberrant promoter methylation of tumor suppressor genes has also been used to predict clinical outcomes following curative ESCC resections. For example, promoter methylation of *APC* has been associated with reduced survival of ESCC patients after esophagectomy.[16] Ling *et al*.[17] showed that *MSH2* promoter hypermethylation in circulating tumor DNA was a valuable predictor of disease-free survival of ESCC patients after esophagectomy. Aberrant methylation of *FHIT* was also reported to be associated with exposure to tobacco smoking and individuals with early-stage ESCC whose tumors exhibited *FHIT* hypermethylation had poor prognoses.[18] CDH1 hypermethylation was detected in 14-61% of ESCC, which was associated with recurrence of early-stage ESCC.[19] Moreover, aberrantly methylated gene promoters were also detected in plasma or sera of ESCC patients. Hibi *et al*.[20] showed that *p16* promoter methylation in ESCC specimens had this same methylation change in their serum DNA in 23% the of patients, which implied that detection of serum DNA *p16* promoter methylation could serve as a tumor marker. However, few studies have addressed or detected DNA hypo-methylation in ESCC. *LINE-1* methylation is regarded as a surrogate marker for global DNA methylation. To better understand DNA methylation in ESCC tissues, our group measured their *LINE-1* methylation using the pyrosequencing technology. Chronic tobacco smoking and heavy alcohol drinking are established as risk factors for ESCC development.[21-25] *LINE-1* hypo-methylation is significantly associated with tobacco smoking, which supports its plausibility as a surrogate marker for an epigenetic field defect.[26] *LINE-1* methylation is highly variable among ESCC specimens (25-92%) and its hypo-methylation is strongly associated with poor ESCC prognosis.[27] Moreover, loss of insulin-like growth factor 2 (*IGF2*) imprinting has been found in ESCC and loss of *IGF2* methylation is associated with shorter survival of patients.[28]

Table 1: Association of gene promoter methylation with clinical outcomes of esophageal cancer patients

Gene	Histological type	Correlation with clinical outcomes	Reference
DNA hypermethylation			
APC	ESCC	Associated with poor prognosis	[16]
CDH1	ESCC	Associated with poor prognosis	[19]
p16	ESCC	Associated with poor prognosis, serum promoter methylation	[20,94]
Claudin-4	ESCC	Associated with poor prognosis	[95]
FHIT	ESCC	Associated with poor prognosis and tobacco/alcohol consumption	[18,96]
Integrin α4	ESCC	Associated with poor prognosis	[19]
MGMT	ESCC	Association with lymph node metastasis	[97]
MSH2	ESCC	Associated with poor prognosis	[17,98]
AKAP12	Barrett/BAC	Progression prediction in Barrett's esophagus	[31]
CDH13	Barrett/BAC	Progression prediction in Barrett's esophagus	[31]
p16	Barrett/BAC	Progression prediction in Barrett's esophagus	[31,99]
HPP1	Barrett/BAC	Progression prediction in Barrett's esophagus	[31,99]
NELL1	Barrett/BAC	Progression prediction in Barrett's esophagus	[31]
RUNX3	Barrett/BAC	Progression prediction in Barrett's esophagus	[31,99]
SST	Barrett/BAC	Progression prediction in Barrett's esophagus	[31]
TAC1	Barrett/BAC	Progression prediction in Barrett's esophagus	[31]
DNA hypomethylation			
IGF2	ESCC	Associated with poor prognosis	[28]
LINE-1	ESCC	Associated with poor prognosis and tobacco consumption	[26,27]

ESCC: Esophageal squamous cell carcinoma; Barrett/BAC: Barrett's esophageal adenocarcinoma

In EAC, methylation patterns of promoter CpG islands in several genes, such as tumor suppressor genes (*APC*, *TIMP3*, *SFRP1*, *SFRP2*, *WIF1*, *AKAP12*, *RUNX3*, *SOCS1 and SOCS3)* and DNA repair genes (*MGMT*), have been reported previously.[29] In Barrett's esophagus, a pre-malignant condition that can lead to EAC development, aberrant DNA methylation has also been shown to occur in promoters of tumor suppressor genes, adhesion molecules and DNA repair genes (*AKAP12*, *APC*, *CDH13*, *DAPK1*, *GPX*, *GST*, *MGMT*, *NELL1*, *REPRIMO/RPRM*, *p16*, *SFRP*, *SOCS*, *SST*, *TAC1*, *TIMP3* and *WIF1*).[30] Jin *et al.* reported that promoter hypermethylation of eight genes (*p16*, *RUNX3*, *HPP1*, *NELL1*, *TAC1*, *SST*, *AKAP12* and *CDH13*) could predict neoplastic progression risk in Barrett's esophagus.[31] However, in the study of DNA hypo-methylation in Barrett's EAC (BAC), Alvarez *et al.* reported a predominance of DNA hypo-methylation rather than DNA hyper-methylation in early-stage of BAC carcinogenesis. They also detected DNA hypo-methylation in a series of genes associated with the immune system such as chemokines (*CXCL1* and *CXCL3*).[32]

Altered DNA Methylation in Gastric Cancer

Gastric cancer is the fourth most frequently diagnosed cancer and the second leading cause of cancer-related deaths in the world.[33] Gastric adenocarcinoma accounts for 90-95% of gastric cancer and has two histological subtypes (intestinal and diffuse) based on microscopic observation and tumor growth patterns, which differ widely in molecular pathogeneses.[34] Nonetheless, epigenetic alterations play important roles in the development of both gastric carcinoma types. Gene promoter methylation has been reported to associate with gastric cancer development, such as *CDKN2A*, *CDK2AP2*, *CDH1*, *MGMT*, *RASSF1*, *RUNX3*, *DLC1*, *ITGA4*, *ZIC1*, *PRDM5*, *PCDH10*, *TFPI2*, *RUNX3*, *SPINT2*, *BTG4*, *SFRP2*, *hMLH1*, *DKK-3*, *TCF4*, *GRIK2*, *RAR*, *CHFR*, *BNIP3*, *RASSF1A*, *LRP1B* and *SFRP5*, promoter of which was more frequently methylated in gastric cancer tissues than those of the corresponding normal gastric tissue.[35,36] Furthermore, promoter methylation of many genes with different biological functions has been associated with the clinicopathological characteristics and prognosis of gastric cancer [Table 2].[37] Of these genes, promoter hypermethylation of *CDH1*[38] and *MGMT*[39,40] was associated with worse outcomes of gastric cancer patients after surgery. However, patients with hypermethylated *IGF2* in blood leukocyte DNA reportedly had a significantly better survival rate than those with hypo-methylated *IGF2*.[41] Additionally, DNA methylation of detected in body fluids that can be obtained non-invasively, such as serum and gastric washes, may have a clinical application for gastric cancer; for example, detection of aberrant DNA methylation of *CDH1*, *DAPK*, *GSTP1*, *p15*, *p16*, *RARβ*, *RASSF1A*, *RUNX3* and *TFPI2* in serum may be a useful biomarker for gastric cancer.[42]

Environmental factors also significantly affect DNA methylation. Etiological studies have closely associated two distinct infectious agents, *Helicobacter*

Table 2: Association of gene promoter methylation with clinical outcomes of gastric cancer

Gene	Correlation with clinical outcomes	References
DNA hypermethylation		
BNIP3	Association with poor prognosis	[100,101]
CACNA2D3	Correlation with lymph node metastasis	[102]
CDH1	Association with poor prognosis, *H. pylori* infection, and EBV infection	[38,46,49-51]
DAPK	Correlation with cell differentiation, lymph node metastasis	[100,103]
FLNc	Association with poor prognosis	[104]
GPX3	Correlation with lymph node metastasis	[105,106]
HAI-2/SPINT2	Correlation with cell differentiation, lymph node metastasis	[107]
HoxD10	Association with poor prognosis	[108]
LOX	Association with poor prognosis and *H. pylori* infection	[45]
MGMT	Association with poor prognosis	[103,104,109]
MLH1	Association with poor prognosis	[104]
p15	Association with EBV infection	[49-51]
p16	Association with poor prognosis, *H. pylori* infection and EBV infection	[38,46,49-51,102,104]
p73	Association with EBV infection	[52]
PAX6	Association with poor prognosis	[100]
RASSF1A	Association with poor prognosis	[100,103]
RASSF2	Association with poor prognosis	[104]
RUNX3	Correlation with TNM stage and *H. pylori* infection	[110,111]
DNA hypormetylation		
LINE-1	Association with poor prognosis and *H. pylori* infection	[55,56]
SURF	Association with poor prognosis	[57]

H. pylori: Helicobacter pylori; EBV: Epstein-Barr virus

pylori and Epstein-Barr virus (EBV) with gastric carcinogenesis.[43,44] Previous prospective studies showed that *H. pylori* infection had an essential role in gastric carcinogenesis[43] and the mechanisms, underlying gastric carcinogenesis due to *H. pylori*-induced DNA methylation, had been indicated. *H. pylori* infection induced aberrant promoter methylation in tumor-suppressor genes, such as *RUNX3*, *p16*, *LOX* and *CDH1*.[45,46] Furthermore, *IL-1β* is thought to be especially significant as a specific single-nucleotide polymorphism of *IL-1β* in association with increases in both gastric cancer risk and incidence.[47,48] EBV infection occurs at a very early-stage in cancer development and plays an important role in gastric carcinogenesis. Aberrant methylation of tumor suppressor genes, such as *CDH1*, *p15*, *p16* and *p73*, is frequently observed in EBV-associated gastric cancer but is less frequently detected in adjacent non-neoplastic mucosa,[49-52] which suggests that aberrant methylation is a critical mechanism of EBV-related gastric tumorigenesis. Regarding the molecular mechanisms underlying host DNA methylation during early-stage EBV infection in gastric epithelium, *LMP2A* expression was upregulated through STAT3 phosphorylation, which further induced DNA methyltransferases during EBV infection.[53]

However, few studies addressed or detected DNA hypo-methylation in gastric cancer. In gastric cancer, global genomic hypo-methylation has been found in premalignant stages of the disease.[54] In our previous study that assessed 203 resected gastric cancer specimens, we found gastric cancer tissues had significantly lower *LINE-1* methylation levels than that of their matched normal gastric mucosa. *LINE-1* hypo-methylation in gastric cancer was also associated with shorter survival of patients.[55] Moreover, *LINE-1* hypo-methylation of non-cancerous gastric mucosae in gastric cancer patients significantly correlated with *H. pylori* infection.[56] Hur *et al*. reported that gastric cancer tissues had conspicuously higher expression of *SULF1* regulated by promoter hypo-methylation than that of the normal mucosa. *SULF1* is also an independent prognostic factor, and LN is a metastasis predictive factor in gastric cancer patients.[57]

Altered DNA Methylation in Colorectal Cancer

Aberrant DNA methylation was reported as an important hallmark of colorectal cancer. Colorectal cancer is a heterogeneous disease and molecularly, it can be classified into three major molecular subtypes, that is, microsatellite instability (MSI), chromosomal instability and CpG island methylator phenotype (CIMP).[58] In 1999, Baylin and Issa *et al*. coined the term "CpG island methylator phenotype" or CIMP, in which promoter of tumor suppressor genes was methylated to contribute to tumorigenesis at least in theory through progressive genetic silence, possibly even in the absence of any genetic mutations.[59] According to epigenetic and clinical profiles, primary colorectal cancer is divided into three distinct subclasses, that is, CIMP1, CIMP2 and CIMP-negative. CIMP1 tumor often shows mutations of MSI (80%) and *BRAF* (53%) while CIMP2 tumor often shows *K-RAS* mutation (92%) but rarely shows MSI or *BRAF* or *TP53* mutations. Non-CIMP tumor has a high frequency of *TP53* mutations (71%).[60] CIMP1 has a favorable prognosis, whereas CIMP2 is associated with poor prognosis.[60] Cancer CIMP status has been assessed as a predictive marker for 5-FU responsiveness.[61]

Colorectal cancer with CIMP is distinct from those with chromosomal instability, and there are two forms of nuclear derangement represented alternative pathways for colorectal cancer development,[62,63] which overlap somewhat as hypermethylation can occur in *APC* and is part of the chromosomal instability pathway,[64] or in the *MLH1* gene, triggering MSI.[65] *MLH1* accounts for approximately 40% of the cases of the hereditary colorectal cancer and Lynch syndrome.[66] Detection of *MLH1* methylation is currently used to discriminate between sporadic colorectal cancer with MSI and familial forms (Lynch syndrome).[67] Methylation of *MGMT* promoter also occurs during colorectal cancer progression in either pathway and may facilitate the accumulation of point mutations as tumors evolve.[65]

The CpG island methylation affects a number of genes in colon cancer, and significance of these epigenetic alterations in colon cancer pathogenesis has been widely reported.[68,69] Hundreds of gene promoters have been found to be aberrantly methylated in the average colorectal cancer genome and their number is ever-growing, including genes of the Wnt signaling pathway such as *APC*, *AXIN2*, *DKK1*, *SFRP1*, *SFRP2* and *WNT5A*, the DNA repair genes *MGMT*, *hMLH1* and *hMLH2*, cell cycle-related genes such as *p14*, *p15* and *p16*, RAS signaling genes *RASSF1A* and *RASSF1B* and many more.[70,71]

Several DNA methylation markers have been proposed as useful early biomarkers for colorectal cancer early detection and prediction of prognosis. For instance, methylation of *MLH1* can be detected in colorectal cancer tissue samples[72] or blood[73] to help interpret MSI because its presence helps to exclude diagnosis of Lynch syndrome. The presence of aberrantly methylated *SEPT9* (which encodes a GTPase that is involved in dysfunctional cytoskeletal organization) in plasma is a valuable and minimally invasive blood-based polymerase chain reaction test with a sensitivity of almost 70% and a specificity of 90% in colorectal cancer detection.[74-78] In fact, an assay that detects hypermethylated *SEPT9* is now being commercialized and offered in some parts of Europe to screen colorectal cancer. Moreover, detection of aberrant methylation of vimentin in fecal DNA was reported in colorectal cancer when compared with normal control;[79] the sensitivity and specificity of methylated vimentin for colorectal cancer were 88%

and 87%, respectively.[80] Kamimae *et al*. have recently shown that detection of DNA methylation in mucosal wash fluid from patients undergoing colonoscopy may be a good molecular marker for predicting invasiveness of colorectal tumors.[81]

Promoter hypermethylation of *MLH1*, *MGMT* and *HIC1* can be detrimental and lead to cancer progression.[82-85] Seven additional genes (*TIMP3*, *CXCL12, ID4, IRF8, CHFR*, *IGFBP3* and *CD109*) were frequently methylated in late-stage colorectal cancer and could have a role in colorectal cancer progression and metastasis.[71,86,87] Yi *et al*. observed that colorectal cancers that have silenced (methylated) genes in the extracellular matrix-remodeling pathway, such as *IGFBP3, EVL, CD109* and *FLNC*, showed worse survival, suggesting that methylation of this pathway-related genes might represent a prognostic signature for colorectal cancer patients.[87] Moreover, hypo-methylation of the IGF2 differentially methylated region in colorectal tumors was associated with poor prognosis.[88] However, all of these possible markers need to be further validated before they are used clinically.

Global hypo-methylation may influence tumor progression by making chromosomes more susceptible to breakage and causing disruption of normal gene structure and function, leading to reactivating previously silenced retrotransposons.[89-91] Most recent research on *LINE-1* methylation levels in GI cancers has focused on colorectal cancer; Ogino *et al.* reported *LINE-1* methylation levels widely occurred and approximately normally distributed (range: 23.1-90.3%) in a cohort of 869 colorectal cancer patients.[92] *LINE-1* hypo-methylation was inversely associated to the MSI and CIMP;[92,93] these findings suggest that CIMP/MSI and genomic hypo-methylation represent different pathways in colorectal cancer development. A summary of reported gene methylation in stool, blood and tissue samples of patients with colorectal cancer is shown in Tables 3 and 4.

Table 3: Association of gene promoter methylation with diagnosis of colorectal cancer

Gene	Specimen type	Correlation with clinical outcomes	References
DNA hypermetylation		Diagnosis	
AGTR1	Stool	Diagnosis of CRC	[112]
ALX4	Blood	Diagnosis of colorectal adenomas and cancers	[113]
APC	Blood	Diagnosis of CRC	[114]
BMP3	Stool	Diagnosis of colorectal adenomas and cancers	[115]
BMP3	Tissue	Diagnosis of colorectal adenomas and cancers	[112]
CNIP1	Stool	Diagnosis of CRC	[116]
DAPK	Blood	Diagnosis of CRC	[117]
FBN1	Stool	Diagnosis of CRC	[116]
GATA-5	Stool	Diagnosis of CRC	[118]
IGFBP7	Cells	Diagnosis of CRC	[119]
INA	Stool	Diagnosis of CRC	[116]
MAL	Stool	Diagnosis of CRC	[116]
MGMT	Blood	Diagnosis of CRC	[114]
MLH1	Blood, cells	Diagnosis of sporadic MSI CRC	[73]
NDRG4	Stool	Diagnosis of CRC	[120]
NDRG4	Stool	Diagnosis of colorectal adenomas and cancers	[115]
NEUROG1	Blood	Diagnosis of CRC	[121]
NGFR	Blood	Diagnosis of CRC	[74]
p16	Blood	Diagnosis of CRC	[122]
RASSF2	Stool	Diagnosis of CRC, distinction from gastric cancer	[123]
RASSF2A	Blood	Diagnosis of CRC	[114]
RUNX3	Blood	Diagnosis of CRC	[124]
SDC2	Blood	Diagnosis of CRC	[125]
SEPT9	Blood	Diagnosis of CRC	[74,75]
SFRP2	Stool, blood, tissue	Diagnosis of CRC, distinction from gastric cancer	[123]
SLIT2	Stool	Diagnosis of CRC	[112]
SNCA	Stool	Diagnosis of CRC	[116]
SPG20	Stool	Diagnosis of CRC	[116]
TFPI2	Stool	Diagnosis of colorectal adenomas and cancers	[115]
TMEFF2	Blood	Diagnosis of CRC	[74]
Vimentin	Stool, blood	Diagnosis of colorectal adenomas and cancers	[126]
WIF1	Blood	Diagnosis of CRC	[114]
WNT1	Stool	Diagnosis of CRC	[112]

CRC: Colorectal cancer; MSI: Microsatellite instability

Table 4: Association of gene promoter methylation with prognosis of colorectal cancer

Gene	Specimen type	Correlation with clinical outcomes	References
DNA hypermetylation		Prognosis	
APC	Tissue	Associated with poor prognosis	[127]
CD109	Tissue	Associated with poor prognosis	[87]
EVL	Tissue	Associated with poor prognosis	[87]
FLNC	Tissue	Associated with poor prognosis	[87]
HLTF	Blood	Associated with poor prognosis	[128]
HOPX-β	Tissue	Worse prognosis of stage III CRC	[129]
HPP1	Blood	Associated with poor prognosis	[128]
IGFBP3	Tissue	Associated with poor prognosis	[87]
MLH1	Blood	Associated with favorable prognosis	[130]
p16	Tissue	Associated with poor prognosis	[127]
RASSF2A	Tissue	Associated with poor prognosis	[131]
TFPI2	Blood	Associated with poor prognosis	[132]
DNA hypomethylation		Prognosis	
IGF2	Tissue	Associated with prognosis	[88]
LINE-1	Tissue	Associated with worse OS	[133]

CRC: Colorectal cancer; OS: Overall survival

Conclusion

In this review, we have summarized the main epigenetic alterations in GI cancer-global DNA hypo-methylation and site-specific CpG island promoter hypermethylation with clinical characteristics in patients with GI cancers. Epigenetic signatures have a potential usefulness in early diagnosis, screening, monitoring and prediction of prognoses or therapy responses for GI cancer patients. Further investigation in this field would increase our knowledge of epigenetic alterations of GI cancer and help to develop novel therapeutic strategies for GI cancers.

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Conflicts of interest

There are no conflicts of interest.

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