Methylation modification in gastric cancer and approaches to targeted epigenetic therapy (Review)

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Abstract. Gastric cancer (GC) is one of the most common cancers and the second leading cause of cancer-related mortality. Increasing discoveries have highlighted aberrant epigenetic modifications actively contribute to the pathogenesis of this fatal disease. Among these epigenetic events, dysregulated methylation is particularly associated with GC progression. Importantly, these aberrant methylation modifications caused by the misregulation of methyltransferases are frequently reversible, which provides opportunities for targeted treatment using specific molecular inhibitors. In the present review, we provide an overview of the current literature on the changes of DNA and histone methylations that alter gene expressions in GC and describe the emerging targeted epigenetic therapy in GC.

Contents

1. Introduction
2. Methylation modifications in cancer progression
3. DNA methylation in GC
4. Protein methylation in GC
5. GC-associated HP and EBV infection in modification of methylation
6. Potential therapy targeting changes in methylation
7. Conclusion

1. Introduction

Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer-related mortality (1). Most GC patients present with advanced-stage disease at the time of diagnosis and have a poor prognosis (2). Genetic and epigenetic aberrations have long been thought to be the two main mechanisms that orchestrate various procedures in the process of GC tumorigenesis. Recent evidence has shown that epigenetic dysregulation plays a crucial role in GC development (3). Among epigenetic alternations, methylation modification is particularly closely associated with GC oncogenesis and progression. These modifications are catalyzed by a class of group-transfer enzymes known as methyltransferases. Although the physiological importance of methylation has been known for many years and has been described in numerous reports, the discovery of enzymes that can reverse methylation has shifted the focus towards the study of methyltransferases, which provides opportunities for targeted treatment using specific inhibitors. The present review highlights the current knowledge of functions of methylation aberrations and may contribute to the understanding of GC mechanisms. We also discuss the importance of methyltransferases and the function of newly emerging molecular inhibitors as anticancer targets in GC.

2. Methylation modifications in cancer progression

Epigenetics is formally defined as a heritable and reversible alterations in gene expression or chromosomal stability without changes in the underlying DNA sequence (4). Epigenetic factors help regulate many processes from development to differentiation and are maintained through multiple cellular cycles. In this way, they play a pivotal role in normal development. Abnormalities in the epigenetic control of the normal processes have been increasingly recognized as one of the mechanisms in cancer initiation and progression (4,5).

In normal cells, DNA methylation occurs predominantly in repetitive genomic regions, maintaining genomic integrity while CpG islands, particularly those associated with promoters, are generally unmethylated. Both hypermethylation and hypomethylation have been observed in all types of cancer cells. Global DNA hypomethylation, particularly in repetitive sequences, plays a vital role across developmental stages and cancer progression. Genome-wide hypomethylation, characterized by the large depletion of methylation, takes place mainly on normally heavily methylated repeat elements such as long interspersed nuclear element (LINE1)
and centromeric satellite repeats (Satα and Sat2). In general, hypomethylation in cancer cells is associated with a series of adverse outcomes, including chromosome instability (CIN), repression of transposable elements, loss of imprinting (LOI) and activation of oncogenes (6-8). Local hypermethylation of genes takes place on the cytosines located in CpG dinucleotides resulting in aberrant gene silencing including tumor-suppressor, cell cycle regulator and DNA-repair genes silencing involved in cellular pathways, such as cell cycle, DNA repair, metabolism, cell adherence, apoptosis and angiogenesis (9,10). Diverse tumor types have a diverse region of gene hypermethylation defined as CpG island methylator phenotype (CIMP). The DNA methylation patterns may be potential prognostic indicators of cancer patients and used as a biomarker (11). DNA methylation is mediated by DNMTs. Histone methylation of cancer mainly focused on H3 and H4. The modification of histone by methylation, causing nucleosomes to tighten into heterochromatin, is generally associated with gene inactivation or silencing. The most extensively studied histone methylation sites include H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20. Histone methyltransferases and demethylases mediate the addition and removal of methyl groups from different lysine residues on histones. Several agents have been identified as ‘capable’ of inhibiting the epigenetic processes. Enzymes that control DNA and histone methylation are targets for numerous drugs. TSG, tumor suppressor gene. The red X denotes blockage of TSG expression by DNA methylation. The grey characters represent histone demethylases. The blue characters represent drugs targeting DNA and histone methylation. The red characters represent drugs proved by FDA.
h3K27 and h4K20 methylations are considered repressive methylations are associated with active genes while h3K9, or me3) (17). A possible explanation is that histone methylation amino acid residues and the range of methylation (me1, me2 with either active or repressive states, depending on the target active transcription, whereas methylation may be associated profiles. Typically, histone acetylation is associated with an active transcription, whereas methylation may be associated with either active or repressive states, depending on the target amino acid residues and the range of methylation (me1, me2 or me3) (17). A possible explanation is that histone methylation does not only alter the charge on the histone tails but also influences the basicity and affinity of different reader proteins to the methylated sites. Generally, H3K4, H3K36 and H3K79 methylation such as the SRA (SET- and RING-associated), cent homology domains (BAh domains) and readers of DNA and methylation of histone lysine residues are the most thoroughly studied histone profiles. Typically, histone acetylation is associated with an active transcription, whereas methylation may be associated with either active or repressive states, depending on the target amino acid residues and the range of methylation (me1, me2 or me3) (17). A possible explanation is that histone methylation does not only alter the charge on the histone tails but also influences the basicity and affinity of different reader proteins to the methylated sites. Generally, H3K4, H3K36 and H3K79 methylation are associated with active genes while H3K9, H3K27 and H4K20 methylations are considered repressive markers leading to inactive genes.

Epigenetic modifications are not standalone processes, instead, are subject to considerable crosstalk among the different types of epigenetic markers. Research in model organisms has shown that there are extensive links and crosstalk between histone modifications and DNA methylation. Key to these links are the readers of histone methylation including plant homeodomains (PHDs), chromodomains and bromo adjacent homology domains (BAH domains) and readers of DNA methylation such as the SRA (SET- and RING-associated), CXXC domain and methyl-CpG-binding domain (MBD). The unifying molecular feature of GC is a profoundly reshaped epigenome characterized by global genomic hypomethylation, gene-specific DNA hyper- or hypo-methylation, aberrant expression of DNMTs and histone methylation enzymes (18).

3. DNA methylation in GC

Global DNA hypomethylation. Global DNA hypomethylation is mostly seen in GC (19-21), even at the early steps of carcinogenesis (22,23). An enhanced hypomethylation was associated with a more invasive and advanced stage type of GC (23). Previous studies have shown that global DNA hypomethylation accumulates with patient age and is associated with copy number alterations in gastrointestinal cancers, possibly due to gradual acquisition of aberrance during DNA methylation and correlates to genomic damage (23). LINE-1 is frequently hypo-methylated in GC (24) and is associated with poor prognosis (25). Moreover, LINE-1 hypomethylation in GC patients is significantly correlated with HP infection (24). Hypomethylation of Alu elements, a member of the short interspersed nuclear element (SINE) family of repetitive elements, was also observed in GC (26). Alu and Sato hypomethylation is induced in gastric mucosae by H. pylori infection during gastric carcinogenesis (21). LOI is another example of an epigenetic alteration related to aberrant hypomethylation (21). LOI of the insulin-like growth factor-2 gene (IGF2) was shown to be associated with increased GC risks (21,27).

Cancer-linked gene-specific DNA hyper- and hypo-methylation. Regions of lower-density methylation near CpG islands (CGIs), defined as ‘shores’, exhibit great variation in methylation, including hypomethylation and hypermethylation, across diverse types of cancers (28). Currently, a number of hypomethylated tumor-promoting genes and hypermethylated tumor-suppressor genes (TSG) have also been found in GC and are associated with oncogene positive transcriptional regulation during a variety of cellular processes (Table I). The DNA methylation of chromatin-modifying enzymes (CMEs) that cause changes in chromatin structure can affect diverse pathways involved in multiple aspects of GC development and progression. For example, SMARCA5 and MGMT (O-6-methylguanine-DNA methyltransferase), SWI/SNF related, are downregulated in GC as a consequence of its promoter methylation (54,55). The methylation HLT, encoding a member of the SWI/SNF family, has been reported in 50% of cases of GC (56). DNA methylation biomarkers of certain genes that are abnormally repressed in GC are appealing in normal gastric epithelium or premalignant lesions as well as in other body samples (e.g. stool and plasma) early in GC development. Alterations in DNA methylation can also influence treatment response in GC. For example, methylation of SULF2 and BMP4 have been linked to chemotherapeutic responses (57,58).

GC-associated alterations in DNA methylation were widespread and tend to localize at CGIs (59). Patients with
widespread gene hypermethylation tend to have poor overall survival (59). However, the methylation profile differs between the intestinal and diffuse types of GC (60). The epithelial cadherin gene CDH1, a tumor suppressor gene which is downregulated in gastric tumors, is hypermethylated more frequently in the diffuse type of GC than in the intestinal type (61,62). Zouridis et al (59) showed that patients with CIMP-positive GC tend to be younger, with less-differentiated tumors, and their cases are associated with diffuse histology and tend to exhibit worse survival outcomes. One potent cause is that genes methylated by CIMP in GC overlap with those corresponding to PRC2-targets in embryonic stem cells, suggesting that it has been present since normal, early development. The strong relationship between CIMP and H. pylori, EBV, and MSI has been emphasized in a meta-analysis, but CIMP cannot be used as a prognostic marker for GC (63).

DNA methyltransferases. Notably, the increased expression of DNMT3A in GC is significantly higher than that of DNMT1 and DNMT3B (64,65). A recent study demonstrated that the poor overall survival rate of GC patients is associated with elevated DNMT3A expression, but not with increased expression of DNMT1 or DNMT3B (66). DNMT3A contributes to the dysregulation of the cell cycle by repressing p16INK4A in a DNA methylation-dependent manner (67). DNA methyltransferase genes have been shown to be mutated in certain cancers. For example, the DNMT3A gene is mutated in acute myelogenous leukemia, myeloproliferative disease and myelodysplastic syndrome (68). Zang et al (69) also observed that GC had mutations in DNMT3A (1/15; 6.7%). In addition, 23.5% allelic loss of DNMT3A was observed in GC (70). Increment of functional polymorphism of DNMT1, DNMT3A and DNMT3B were found in gastric neoplasm and have been found to be associated with development and progression of GC (65,71-73). One meta-analysis showed that DNMT1 rs16999593 and DNMT3A rs1550117 could result in GC and that DNMT3B rs1569686 may be a protective factor against gastric carcinogenesis (73). Furthermore, DNMT1, DNMT3A and DNMT3B proteins are downregulated through overexpression of miR-200b and miR-200c, resulting in the global DNA hypomethylation in GC cell lines (74). In addition, H. pylori infection could increase DNMT activity via upregulation of the epidermal growth factor (EGF) and its receptor or via the release of inflammatory mediators, such as NO (75,76). In particular, overexpression of DNMT1 and DNMT3B was found to be associated with EBV infection in GC (77,78).

### 4. Protein methylation in GC

**Histone methylation and HMT.** Studies conducted on histone methylation of GC have focused mainly on H3 and H4 (56,79). Studies have reported that certain histone methylation markers (H3K4me3, H3K9me3 and H3K27me3) are positively correlated with clinicopathological characteristics in GC, including tumor stage and survival (80-83). As a sequence, specific histone methylation can result in dysregulation of many genes with important roles in GC (Table II). For instance, EZH2 mediates H3K27 trimethylation to maintain transcriptional silencing. EZH2 knockdown represses cell growth, cell proliferation (RUX3 and ANXA6) (90,91),
invasion and migration (E-cadherin and ArgBP2) (92,93), and induces cell cycle arrest in GC cells (p53, p21, p14 and p16) (94,95). In addition, EZH2 promotes the activation of Wnt and MAPK signaling through downregulation of CXXC4 expression, figuring an epigenetic mechanism of wnt signaling activation in GC cells (96,97).

Histone methylation is processed by lysine methyltransferases. A number of HMTs and histone demethylases (HDTs) have been found to mediate the addition and removal of methyl groups from different lysine residues on histones. Indeed, methylation at different lysine residues on histones has been shown to display differential functions (69). A current model suggests that methylated histones are recognized by chromatin effector molecules (readers), leading to the recruitment of other molecules to alter the chromatin and/or transcription states (106). In particular, histone methylation reader proteins such as WD40 repeats, chromo- and bromodomain proteins, and the PHD finger domain were shown to recruit HMTs to their target sites. During the past decade, several HMTs and HDTs have been identified in humans (Fig. 2). All HMTs except KMT4 (DOT1L), contain the SET-domain, which provides interaction and recognition sites for lysine substrates and cofactors for general catalysis. SET-domain-containing protein, a class of HMTs, has been regarded as an important factor in carcinogenesis (107). HDTs include amine oxidase-like (AOL) domain-containing demethylases and Jumonji C (JmjC) domain-containing demethylases (108,109). These HMTs and HDTs have been shown to methylate histones incorporated in chromatin, free histones and non-histone proteins (110).

Non-histone methylation. Beyond alteration of the histone code, HMT interacts with various molecules including DNMT1. For example, EZH2 can recruit and regulate other epigenetic silencing enzymes, such as DNMTs, by influencing the binding capacity between DNMTs and the promoter of target genes (111). DNMT1-mediated promoter methylation is indispensably maintained by EZH2-mediated methylation (112,113). LSD family members contain a SWIRM domain and an AOL domain. The SWIRM domain is present in many chromatin-interacting enzymes and it can interact with DNA. Several studies have shown that the expression of several genes in GC can be regulated by DNA methylation and histone modification simultaneously (83,114,115). For example, gene expression of PRDM5 (PR domain containing 5), a member of the kruppel-like zinc finger family, is downregulated via DNA methylation and H3K27 trimethylation, alleviating the cell-growth-suppressive effect of PRDM5 (116).
The biological and physiological significance of non-histone lysine methylation in human tumorigenesis has been recently explored (117,118). For example, SET and MYND domain-containing protein 2 (SMYD2) was identified as a lysine methyltransferase for K370 of p53 (119), K860 and K810 of Rb (120,121) and K528 of PARP1 (122). SMYD2-dependent methylation of Rb at K810 promotes the cell cycle progression of cancer cells (121). Moreover, methylation of K528 on PARP1 enhances its poly(ADP-ribose) activity in cancer cells (122). Mazur and colleagues (123) found that SMYD3 methylated the lysine 260 of MAP3K2 gene, leading to the activation of the signaling of the Ras/Raf/MEK/ERK in the development of pancreatic ductal adenocarcinoma and lung adenocarcinoma.

5. GC-associated HP and EBV infection in modification of methylation

Inflammation can predispose tissues to cancer, and it is very likely that DNA methylation is involved in this process (124). The stomach has been described as the organ with the highest CpG island hypermethylation frequency which is age-associated and possibly inflammation-mediated (29). From an etiological viewpoint, two pathogens, H. pylori (HP) and Epstein-Barr virus (EBV), are known to participate in gastric carcinogenesis. Chronic inflammation in the gastric mucosa due to HP and EBV infection of gastric epithelial cells has been reported to cause aberrant promoter methylation, which may contribute to the tumorigenic mechanisms of these pathogens.

In the multistep development of gastric carcinogenesis, chronic HP infection progresses over decades, through stages of chronic gastritis, atrophy, intestinal metaplasia, adenoma/dysplasia and cancer. Moreover, global DNA hypomethylation might be implicated in GC associated with HP infection during early stages (125). According to a study that investigated the promoters of 48 genes in the gastric mucosa with or without HP infection, results suggest a possible link between aberrant DNA methylation and HP infection (126). Furthermore, some of these HP-related events (such as hypermethylation) are reversed after HP eradication. However, in patients with preneoplastic lesions, global DNA methylation decreased over time despite eradication of HP infection (125).

HP can induce methylation of multiple CpG islands, especially at sites encoding tumor suppressors such as E-cadherin, MGMT and the eradication has led to a marked decrease in methylation levels of these genes (127). Inflammation triggered by HP infection can induce aberrant DNA methylation, which appears to be a critical process (128). HP may induce aberrant DNA methylation through the release of ROS and nitric oxide (NO) and the activation of DNMT (129). Apart from DNA methylation, HP also regulates histone modifications in human gastric epithelial cells through the ROS and histone methyltransferase. For example, Angrisano et al (130) reported that HP infection is followed by activation of iNOS gene expression, chromatin changes at the iNOS promoter (including decreased H3K9 methylation and increased H3K4 methylation), and selective release of MBD2 from the iNOS promoter in a GC cell line. β-catenin directly binds to JMJD2B (KDM4B) promoter and stimulates JMJD2B expression after HP infection. Increased JMJD2B, together with NF-κB, binds to COX-2 promoter to enhance its transcription by demethylating H3K9me3 locally (131).

EBV+ GC forms a distinct subgroup of GC and exhibits exceptionally high DNA methylation levels, surpassing even CIMP tumors, as described by earlier studies and confirmed by TCGA (132,133). One possible reason is that such hypermethylation may represent a cellular reaction to viral infection. As supported in a previous study, EBV infection can regulate the expression of a panel of genes through promoter methylation, including MINT, TIMP-3, CDH1, p16, ACSS1, FAM3B, IHH and TRABD in GCs (78,134,135). EBV infection-induced hypermethylation of a specific group of silencing genes may favor malignant transformation during development of this unique subtype of GC (136). Such methylation epigenotypes are not a parallel phenomenon to EBV infection but rather caused by the EBV infection itself (135). Matusaka et al (137) classified GC into three epigenotypes, EBV/low methylation, EBV/high methylation and EBV/high methylation, according to the pattern of DNA methylation. EBV-positive GCs exhibited distinct and markedly high levels of methylation (e.g. CXXC4, TIMP2 and PLXND1).

6. Potential therapy targeting changes in methylation

Taking into account the reversibility of epigenetic modification, a new field of therapy focusing on key targets, known as pharmacopeigenomics, has been developed to either block or reverse the aberrant epigenetic modifications at an early stage (138). Compounds in preclinical and clinical stages reported in cancer are summarized in Fig. 1. The present review describes drugs targeting disordered patterns of DNA and histone methylation and their potential efficacy in GC (Table III).

Inhibitors of DNMTs (DNMTi). As mentioned above, it has been well established that many genes are hypermethylated in GC (Table I). Two DNA methylation inhibitors, azacitidine (5-azacitidine) and decitabine (5-aza-deoxycytidine) have been established for the treatment of myeloid malignancies (145,146). Recently, more and more DNMT inhibitors, categorized as nucleoside analogs (decitabine, zebularine, SGI-110 and tetrahydrodruridine) and non-nucleoside compounds (SGI-1027, procainamide, flavonoids, RGI08 and their derivatives), are actively being explored in the clinical and preclinical trials as novel treatments for cancer (Fig. 1) (147).

Azacitidine and decitabine. Azacitidine and decitabine are nucleoside analogs of cytosine that cannot accept a methyl donor at the 5’ position of the pyrimidine ring and depletes cellular DNMT1. Intracellularly, azacitidine can convert to decitabine and subsequently be incorporated into DNA (148). Results showed that azacitidine inhibited the proliferation and decreased the level of DNA methylation in GC cell lines (149). Decitabine treatment resulted in growth suppression and reduced the levels of DNMT3A and DNMT3B accompanied with the demethylation of P16INK4A gene (140). CIMP-positive GC lines appeared to exhibit significant reductions in proliferation after treatment of DNMT inhibitors such as decitabine compared to non-CIMP lines (59). The greatest challenge
Inhibitors of HMTs and HDTs. In contrast to DNMTi, the agents targeting histone methylation is still at a primitive level (156). In 2005, the first study identified the effect of chaetocin, an inhibitor of HMT SUV39H1 (157). Subsequently, more and more inhibitors of various HMTs and HDTs, such as DOT1L (EPZ004777), EZH2 (EPZ-6438 and GSK126, EI1) and G9a (BIX-01294), were investigated as antitumor drugs (Fig. 1). Several inhibitors targeting DOT1L, EZH2 and LSD1, have entered phase 1/2 human clinical trials to assess the safety and maximum tolerated dose (Table IV). For example, EPZ-5676, the most advanced inhibitor for DOT1L that specifically catalyzes the mono-, di- and tri-methylation of H3K79, is currently in clinical trials for MLL-rearranged leukemia (NCT02141828 and NCT01684150). Major clinical studies that have been conducted so far are summarized in Table IV. Here, we summarize some common research targets in cancer, especially in GC.

**EZH2 inhibitors.** The first widely used EZH2 inhibitor, 3-deazaneplanocin A (DZNep), is a cyclopentany1 analog of 3-deazaneplanocine that potently interferes with S-adenosyl-l-homocysteine hydrolase (SAH), which increases cellular SAH levels, repressing the activity of SAH-dependent HMT (158). It can inhibit PRC2 and remove H3K27me3 in EZH2-wild-type and Y641- and A677-mutant EZH2 for the treatment of diffuse large B-cell lymphoma (DLBCL) (161). A recent study has shown that GSK126 suppressed tumor migration and angiogenesis via downregulation of VEGF-A expression in GC cell lines (159). Upon exposure to DZNep, EZH2 is depleted and ubiquitination of wild-type p53 protein is inhibited, resulting in p53 stabilization and activation of downstream p53 pathways involved in apoptosis, cell cycle arrest and senescence in GC cell lines (Table III) (144). However, DZNep has a short half-life and is both non-specific and toxic in animal models (160). GSK126 is an AdoMet-competitive chemical compound targeting Y641- and A677-mutated EZH2 for the treatment of diffuse large B-cell lymphoma (DLBCL) (161). A recent study has shown that GSK126 suppressed tumor migration and angiogenesis via downregulation of VEGF-A expression in GC cell lines and rodent animals (162).

The subsequently developed small-molecule EZH2 inhibitor EPZ005687, has shown dose-dependent inhibition of H3K27me3 in EZH2-wild-type and Y641- and A677-mutant lymphoma cells as well as in cell lines of other cancer types, including breast and prostate cancer. In June 2013, a phase 1/2 clinical trial of EPZ-6438, with better oral bioavailability than EPZ005687 (163), was explored in patients with advanced solid tumors or B cell lymphomas (NCT01897571). EI1, a third SAM-competitive inhibitor, inhibits both wild-type and

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**Table III. Summary of inhibitors targeting the methylation modification in GC.**

<table>
<thead>
<tr>
<th>Targets</th>
<th>Compound</th>
<th>Chemical nature</th>
<th>Mechanism of action</th>
<th>Stage</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT</td>
<td>Vidaza</td>
<td>5-azacitidine</td>
<td>Irreversible covalent link with the enzyme leading to a cellular DNMT depletion</td>
<td>Clinical</td>
<td>(139)</td>
</tr>
<tr>
<td></td>
<td>Decitabine</td>
<td>5-aza-deoxycytidine</td>
<td>Inhibition of DNMTs</td>
<td>Preclinical</td>
<td>(140,141)</td>
</tr>
<tr>
<td></td>
<td>Zebularine</td>
<td>2(1H)-pyrimidinone riboside</td>
<td>Inhibition of DNMT and cytidine deaminase</td>
<td>Preclinical</td>
<td>(142)</td>
</tr>
<tr>
<td>HMT</td>
<td>EZH2</td>
<td>GSK126</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>Preclinical</td>
<td>(143)</td>
</tr>
<tr>
<td></td>
<td>DZNep</td>
<td>3-deazaneplanocin A</td>
<td>SAH hydrolase inhibitor of methyltransferases</td>
<td>Preclinical</td>
<td>(144)</td>
</tr>
</tbody>
</table>

*\(^{4}\)N-((1,2-dihydro-4,6-dimethyl-2-oxo-3-pyridinyl)methyl)-3-methyl-1-((1S)-1-methylpropyl)-6-(6-(1-piperazinyl)-3-pyridinyl)-1H-indole-4-carboxamide.*

**Zebularine.** Compared to other DNMTi, zebularine (2(1H)-pyrimidinone riboside) is a novel DNMT inhibitor with high oral bioavailability, slight toxicity and high stability (154). Several preclinical studies have shown that zebularine can form a tight covalent complex with DNMTs and so reverse the hypermethylation of TSGs in cancer cell lines (155). Treatment with zebularine effectively inhibits GC cells proliferation by inducing cell death, causing apoptosis in a dose-dependent manner. Moreover, zebularine depletes expression of DNMT protein with re-expression of epigenetically silenced genes, such as p16 (142). These results indicated that zebularine is a promising drug for GC therapy, but further exploration is needed.

**Inhibitors of HMTs and HDTs.** More and more inhibitors of various HMTs and HDTs, such as DOT1L (EPZ004777), EZH2 (EPZ-6438 and GSK126, EI1) and G9a (BIX-01294), were investigated as antitumor drugs. Several inhibitors targeting DOT1L, EZH2 and LSD1, have entered phase 1/2 human clinical trials to assess the safety and maximum tolerated dose (Table IV).
Table IV. Clinical trials based on the use of HMTi and HDTi in cancer.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Target</th>
<th>Intervention</th>
<th>Conditions</th>
<th>Phase</th>
<th>Status</th>
<th>Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPZ-5676</td>
<td>DOT1L</td>
<td>Drug: EPZ-5676</td>
<td>AML, ALL, AL</td>
<td>1</td>
<td>Completed</td>
<td>NCT02141828</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug: EPZ-5676</td>
<td>AML, ALL, MDS, MPD</td>
<td>1</td>
<td>Completed</td>
<td>NCT01684150</td>
</tr>
<tr>
<td>EPZ-6438/</td>
<td>EZH2</td>
<td>Drug: EPZ-6438/E7438</td>
<td>B-cell lymphomas (phase 1), advanced solid tumors (phase 1), DLBCL (phase 2), FL (phase 2), transformed FL, PMBL</td>
<td>1/2</td>
<td>Recruiting</td>
<td>NCT01897571</td>
</tr>
<tr>
<td>Tazemetostat</td>
<td>EZH2</td>
<td>Drug: Tazemetostat</td>
<td>DLBCL</td>
<td>1/2</td>
<td>Not yet recruiting</td>
<td>NCT02889523</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug: Rituximab</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Drug: Cyclophosphamide</td>
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<tr>
<td></td>
<td></td>
<td>Drug: Vincristine</td>
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<tr>
<td></td>
<td></td>
<td>Drug: Doxorubicin</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Drug: Prednisolone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EZH2</td>
<td>Drug: Tazemetostat</td>
<td>MRT, RTK, ATRT, selected tumors with rhabdoid features SS, INI1-negative tumors, malignant rhabdoid tumor of ovary, renal MC, epithelioid sarcoma</td>
<td>2</td>
<td>Recruiting</td>
<td>NCT02601950</td>
</tr>
<tr>
<td></td>
<td>EZH2</td>
<td>Drug: Tazemetostat</td>
<td>DLBCL, FL, MRT, RTK, ATRT, SS, ES, mesothelioma, advanced solid tumors</td>
<td>2</td>
<td>Recruiting</td>
<td>NCT02875548</td>
</tr>
<tr>
<td></td>
<td>EZH2</td>
<td>Drug: Tazemetostat</td>
<td>Mesothelioma with BAP1 loss of function</td>
<td>2</td>
<td>Recruiting</td>
<td>NCT02860286</td>
</tr>
<tr>
<td></td>
<td>EZH2</td>
<td>Drug: Tazemetostat</td>
<td>Rhabdoid tumors, INI1-negative tumors, SS, malignant rhabdoid tumor of ovary</td>
<td>1</td>
<td>Recruiting</td>
<td>NCT02601937</td>
</tr>
<tr>
<td>GSK2816126</td>
<td>EZH2</td>
<td>Drug: GSK2816126</td>
<td>Relapsed/refractory DLBCL, transformed FL, other NHL, solid tumors, multiple myeloma</td>
<td>1</td>
<td>Recruiting</td>
<td>NCT02082977</td>
</tr>
<tr>
<td></td>
<td>EZH2</td>
<td>Drug: CPI-1205</td>
<td>B-cell lymphoma</td>
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<td>Recruiting</td>
<td>NCT02395601</td>
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<tr>
<td>DS-3201b</td>
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mutant EZH2 and alters H3K27me2 and H3K27me3 levels in EZH2-mutant DLBCL cells in a SMARCB1-mutant rhabdoid tumor cell line (164). EZH2 silencing promoted growth arrest by low concentrations of doxorubicin in p53 mutant gastric cancer cells (165). The combination of EZH2 inhibitors and doxorubicin may be a potential novel approach to GC treatment.

**LSD1 inhibitors.** LSD1, which demethylates both H3K4 and H3K9 residues, is overexpressed in GCs (Table II) (109). It may be a promising target for GC therapy. Tranylcypromine (TCP), an inhibitor of FAD-dependent monoamine oxidases (MAO), was the first small molecule used to target LSD1 because of the close homology between LSD1 and the MAO (166,167). Recent reports have highlighted the potential efficacy of TCP alone or combination with all-trans-retinoic acid (ATRA) in acute myeloid leukemia (AML) (Table IV). After that, TCP derivatives such as GSK2879552 and ORY-1001 were initiated for patients with relapsed/refractory small-cell lung cancer and AML in clinical trials (Table IV). Although no reports have shown whether these inhibitors can be used in GC, LSD1 inhibitors are worthy of further research into GC.

**7. Conclusion**

Over the past decade, epigenetic regulation has been proven to play a pivotal role in cancer pathogenesis. As an important subgroup, DNA and histone methylation have been reported to control numerous cancer suppressor genes and proto-oncogenes. In GC, hyper- and hypo-methylation events are critical to tumor onset. Of note, histone methylation plays an important role in chromatin regulators and GC tumorigenesis. The many HMTs and HDMs that have been identified can mediate the addition and removal of methyl groups from different lysine residues on histones and display distinct functions. These methylation enzymes have attracted considerable attention as potential targets for GC treatment. Recently, numerous trials have been made to develop and identify molecules targeting these enzymes. In many studies, the use of methylation regulators in combination with already existing chemotherapy results in better outcomes in many studies. However, these regulators still have risks of off-target toxicity and many chromatin-modifying enzymes have more diverse functions. Thus, understanding the underlying epigenetic molecular mechanisms, discovering new targets and agents will be essential to successful targeted epigenetic therapy in the future.

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**References**


