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Short running title: DNA methyltransferases and targeted therapy

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DNA methyltransferases and gastric cancer: insight into targeted therapy

Abstract

Gastric cancer is a major health problem worldwide occupying most frequent causes of cancer-related mortality. In addition to genetic modifications, epigenetic alterations catalyzed by DNA methyltransferases (DNMTs) is a well-characterized epigenetic hallmark in gastric cancer. The reversible nature of epigenetic alterations and central role of DNA methylation in diverse biological processes provides an opportunity for using DNA methyltransferase inhibitors to enhance the efficacy of chemotherapeutics. In this review, we discussed key factors or mechanisms such as SNPs, infections, and genetic modifications that trigger DNMTs level modification in gastric cancer, and their potential roles in cancer progression. Finally, we focused on how inhibitors of the *DNMTs* can most effectively be used for the treatment of gastric cancer with multidrug resistance.

Keywords: DNA methyltransferase (DNMT); epigenetic alterations; gastric cancer; drug resistance; chemotherapy

Introduction

Gastric cancer is caused in part by genetic and epigenetic alterations in oncogenes and tumor suppressor genes (TSGs) [1]. Although epigenetic modifications are regulators and natural phenomenon occurring during normal development, tissue-specific gene expression, and cell functions; aberrant epigenetic modifications can have harmful effects that can contribute to cancer development [2-3]. New and ongoing researches are continuously uncovering the interaction between genes and environment through epigenetic alterations which make a person

susceptible to develop gastric cancer [4-5]. Aberrant epigenetic modifications can dysregulate the transcription level of TSGs and oncogenes through different mechanisms, without any changes in DNA nucleotide sequences of the genes [1]. Epigenetic alterations including histone modification, non-coding RNA, and DNA methylation may initiate and sustain changes which lead to the inactivation of tumor suppressor and other cancer-related genes in gastric cancer [6-8]. Histone proteins determine the chromatin structure and function through a variety of modifications like acetylation and methylation in their N-terminal domain. These modifications have effects on a number of pivotal molecular processes including gene transcription, DNA replication, and nucleosome positioning. Depending on the type of histone modification, transcription is either activated or repressed. Histone hyper-acetylation is linked with transcriptional activation, but the outcome of histone methylation relies completely on the type of modified amino acid residue, and the degree of methylation. For instance, trimethylation of H3K4 may promote gene expression, while trimethylation of H3K9 and H3K27 suppresses gene expression. Interestingly, tumor suppressive and oncogenic miRNAs are well-known epigenetic hallmarks of gastric cancers [3]. One third of miRNAs dysregulation is associated with histone modifications and hypo/hypermethylation of CpG islands which are located in their promoter and 5' regulatory end regions. MiRNAs bind to the 3’ untranslated region of their target genes and cause post-transcriptional gene inactivation [9]. Among the epigenetic processes, DNA methylation is a fundamental mechanism which plays a pivotal role in biological functions responsible for DNA stability preservation such as genomic imprinting, and X-chromosome inactivation. It is not unexpected that aberrant DNA methylation induce the development of numerous malignancies, especially gastric cancer [10-12]. DNA methylation in gastric epithelia can also be influenced by several factors including age, diet, physical activity, chronic inflammation, and infectious agents [13]. Global DNA hypo/hyper methylation frequently occur in gastric tumors, which make contribution to genomic instability

[10, 12]. In fact, aberrant hyperactivation of DNA methyl transferases (DNMTs) is responsible for TSGs silencing or inactivation, and eventually gastric cancer development [14-15].

DNA methyltransferases classification

Based on their structure, DNMTs are composed of three main types, DNMT1, DNMT2, and DNMT3. Maintenance methyltransferase (DNMT1) and *de novo* methyltransferases (DNMT3a, DNMT3b, and DNMT3L) are two major enzyme types with m^5C methyltransferase activity (C-5 cytosine-specific DNA methylase or C5 Mtase), which trigger the transfer of a methyl group to C-5 carbon of cytosines in mammalian DNA [16-17]. DNMT1 is responsible for maintenance of DNA methylation status of newly synthesized daughter strands during replication, leading to full DNA strands methylation. DNMT3 consists of three subtypes comprising DNMT3a, DNMT3b, and DNMT3-Like protein (DNMT3L). DNMT3a and DNMT3b contribute to catalyze DNA methylation pattern throughout the nucleus without distinguishing hemi-methylated from methylated CpG sites during embryogenesis and germ cell development. DNMT3L is known as a regulatory protein for *de novo* methylation. It has been shown that DNMT3L participates in regulating the DNMT3a and DNMT3b activity for *de novo* methylation. DNMT2 is known as a DNA methyltransferase which lacks regulatory domain, but new findings showed the implication of this enzyme in adding methyl groups to tRNA anti-codon loop [18]. In general, the DNMTs consist of a C-terminal catalytic domain and an N-terminal regulatory domain (Fig. 1). The catalytic domain is conserved between DNMTs while the regulatory domain is variable in size and amino acid sequence. These two domains are linked by glycine-lysine repeats (GK)n. The catalytic domain in DNMTs consists of six motifs including I, IV, VI, VIII, IX and X. Motifs I and X are S-adenosylmethionine (SAM) binding site while motifs VIII and IX are implicated in DNA binding. DNMT3L lacks motifs IX and X in its catalytic domain. The N-terminal regulatory region of DNMT1 is

composed of several domains that are DNA methyltransferase associated proteins (DMAP); Proliferating cell nuclear antigen (PCNA) binding domain (PBD), nuclear localization signal (NLS), replication foci targeting sequence (RFTS), cysteine rich, zinc finger DNA-binding motif (CXXC), and bromo-adjacent homology Dmap1-binding region (BAH1 and BAH2). DMAP1 domain is responsible for binding of DNMT1 with the transcriptional repressor DMAP1 [19]. The PBD domain is able to interact with PCNA, and recruits DNMT to the replication foci in S phase. Relatively, the absence of PBD domain delays the methylation after replication [20]. RFTS domain is responsible for replication-coupled DNA methylation at the differentially methylated regions of imprinted genes through binding to SRA domain of UHRF1 (ubiquitin like with PHD and ring finger domains 1) protein that is a replication-coupled methylation factor. In addition, RFTS domain protects the genome against aberrant methylation. It was reported that, truncated DNMT1 which lacks parts of the RFTS domain is unable to perform replication-coupled DNA methylation at the differentially methylated regions of imprinted genes, and can trigger global methylation of the genome as well [21-22]. The CXXC domain binds to unmethylated CpG dinucleotides, and is crucial for DNMT1 enzymatic activity [23]. Both BAH motifs in PBHD domain are essential for the folding of DNMT1 [24-25]. Structurally, the N-terminal region of DNMT3 contains a variable region (280 amino acids in DNMT3a, and 220 amino acids in DNMT3b), and two conserved PWWP (tetrapeptide domain containing proline-tryptophan-tryptophan-proline motif) domains and the ATRX-Dnmt3-Dnmt3L (ADD) domain [26-27]. The PWWP domain that contains 100-150 amino acids is conserved in both DNMT3a and DNMT3b [25]. This domain is responsible for the methylation of major satellite repeats at pericentromeric chromatin, and recognizes the H3K36me3 mark [28-29]. The ADD domain is composed of three cysteine-rich subdomains, and recognizes the unmethylated state of lysine 4 and 9 in histone H3 [30-32].

Targets of DNMT in gastric cancer

Aberrant DNA methylation due to the DNMTs up regulation may trigger tumor progression, invasion, and metastasis through down regulation of genes that have a role in proliferation inhibition and apoptosis-related pathway [33]. Several studies indicate that aberrant expression of DNMTs is closely linked to hypermethylation of TSGs. A series of genes which may become hypermethylated during gastric cancer development are listed in table 1. Many of these genes are involved in cell cycle regulation, apoptosis, proliferation, migration and major cell signaling pathways such as epithelial mesenchymal transition (EMT)), notch, Wnt/β-catenin, and AKT/mTOR. Investigations suggested that deregulation of these types of genes would significantly affect cancer progression. Wnt/β-catenin signaling pathway has a fundamental role in controlling the cellular processes such as cell proliferation, cell cycle control, and migration of epithelial cells of gastric mucosa. Alteration of Wnt signaling has been found to be implicated in gastric cancer development. Hypermethylation of Wnt related inhibitor genes including *sFRP1*, *sFRP2*, *sFRP4*, *sFRP5*, *DKK-1*, *DKK-2*, *DKK-3*, *SOX10*, *SOX17*, *WIF-1*, *NKD1*, *HSULF-1*, *RUNX3*, *PRDM5*, *RASSF10*, *OSR1*, and *APC* at the CpG island contribute to tumor progression through Wnt pathway activation [34-35]. It is known that AKT/mTOR pathway together with other signaling pathways acts as a crucial regulator of cell growth, metabolism, apoptosis, metastasis, and angiogenesis [36]. The alteration of AKT/mTOR pathway is associated with gastric tumor progression. Methylation of the TSGs that are responsible for inhibition of AKT signaling can lead to AKT activation and apoptosis evasion in tumor cells. Phosphatase and tensin homolog (PTEN) and A disintegrin-like and metalloprotease with thrombospondin type 1 motif, 9 (ADAMTS9) which act as suppressors for AKT pathway have been indicated to be silenced in gastric cancer [37-39]. Moreover, it

has been reported that notch signaling is a conserved pathway that plays a critical role in cellular functions vital for cell survival. Notch may act as both an oncogene and a suppressor in gastric cancer [40]. Epigenetic alterations of this pathway may lead to gastric cancer progression. Studies in gastric cancer cell lines demonstrate that promotor methylation of delta-like canonical notch ligand 1 (*DLL1*) which encodes for a ligand of NOTCH1 was restored following treatment with 5-Aza-2'-deoxycytidine (5-Aza-dC), a DNMTs inhibitor [41-42]. Conversely, other investigations show that NOTCH1 can promote tumorigenesis by cyclooxygenase-2 activation [43]. It seems that the function of notch pathway components is ambiguous as they were reported either as suppressor or activator of gastric carcinoma, and their exact effect on different steps of gastric cancer pathogenesis still remains unclear [40, 42, 44-45]. Iroquois homeobox 1 (IRX1) is a member of Iroquois homeobox factors family that is required for embryonic development [46]. Down-regulation of *IRX1* has been reported in several cancers including gastric cancer. Guo et al. have shown that exposure with 5-Aza-dC could restitute IRX1 protein level in gastric cancer cells [47]. Reprimo (RPRM) has been reported to be inhibited by DNMTs and its function was restored by treatment with zebularine, a DNMT inhibitor [48]. Moreover, a possible association between DNMT1 amplification, triggering hypermethylation of CpG islands and down-regulation of TSGs including human MutL homolog 1 (*hMLH1*), thrombospondin 1 (*THBS1*), cadherin 1 (*CDH1*) has been suggested in gastric cancer [49]. Besides, due to adverse effects of chemical drugs, DNMT-targeted inhibition with small interfering ribonucleic acids (siRNAs) for reactivation of silenced genes including cyclin-dependent kinase inhibitor 2A (*CDKN2A*), A-kinase anchoring protein 12 (*AKAP12B*), runt related transcription factor 3 (*RUNX3*), helicase-like transcription factor (*HTLF*), and ras association domain family member 1 (*RASSF1A*) was used as a potential therapeutic strategy [50].

Expression of DNMTs and susceptibility to infections in gastric cancer

Although some clinical evidence show the elevated expression of DNMTs in gastric cancer, and consequent silencing of TSGs during tumorigenesis, little is known about the mechanisms that trigger or cause aberrant methylations [14-15]. *APC* is a TSG that was introduced as a gate keeper, and mutation in this gene plays an important role in gastric cancer as well as in colorectal carcinogenesis [100]. The role of APC in regulating DNMTs level was examined in colorectal cancer, and it was found that full-length APC but not truncated APC is able to suppress DNMT1 activity [101]. Mutations in *APC* gene lead to the production of truncated protein which cannot suppress *DNMT1* expression, and may be responsible for *DNMT1* upregulation in gastric cancer [100]. It has been also reported that exposure of gastric cancer cells to nitric oxide (NO) produced induced DNA methylation by enhancing DNMT enzymatic activity [102-103]. Furthermore, infectious agents can induce epigenetic modifications, and be the most common complications in gastric cancer [104-105]. Infections hijack the host DNA methylation mechanism via increasing DNMTs in order to control host transcription to their benefit. Figure 2 summarizes different ways through which infections increase *DNMTs* expression in gastric cancer. *Helicobacter pylori* and Epstein-Barr virus (EBV) are well-known carcinogenic risk factors in gastric cancer that facilitate DNMTs function and hypermethylation of CpG islands of various cancer-associated genes. Furthermore, several studies have indicated the presence of HPV (Human papillomavirus) in patients with gastric cancer, suggesting its potential role as risk factor for gastric cancer [106-107]. Study on human cervical carcinoma SiHa and CaSki cell lines has shown that *P53* TSG suppresses *DNMT1* expression [108]. *DNMT1* was also transcriptionally suppressed by P53 in non-small cell lung cancer (NSCLC) patients and A549 cell lines. P53 could repress *DNMT1* expression by

interacting with SP1. This complex binds to both P53 and SP1 putative binding sites on *DNMT1* promoter. Unlike P53, SP1 can lead to increased *DNMT1* expression. Thus, mutation in *P53* and overexpression of *SP1* can trigger DNMT1 mediated hypermethylation of TSGs promoters [109]. Study on cervical cancer has shown that HPV-16 E6 protein probably upregulates *DNMT1* expression by repressing P53 [108]. In addition to P53, RB could negatively regulate *DNMT*s expression through interaction with DNMT3a. Murine double minute 2 (*MDM2*) which encodes a nuclear-localized E3 ubiquitin ligase targets both P53 and RB, and therefore causes overexpression of *DNMT*s in cancer cells [110]. Infections increase the *DNMTs* expression by secreting proteins able to activate various signaling pathways [111]. In addition, chronic inflammation which occurs in the presence of infections in cancer cells promotes differentiation and polarization of monocytes into tumor-associated macrophages (TAMs). TAMs, as abundant inflammatory cells in tumor microenvironment, increase *DNMT1* expression by secreting chemokine (C-C motif) ligand 5 (CCL5) [112]. CCL5 a known biomarker in late stage of gastric cancer is upregulated in cells infected by *H. pylori*, EBV, and HPV [113-117]. Therefore, by inducing CCL5 chemokine, these infections may upregulate *DNMTs* in gastric cancer tissue. *H. pylori* infection is the main factor that causes gastric inflammation, and promotes aberrant DNA methylation. DNMT3a was indicated as a poor prognostic hallmark for gastric cancer infected with *H. pylori* [118]. Induction of AKT phosphorylation as the main contributor in AKT-NFκB pathway by *H. pylori* can stimulate *DNMT*s overexpression leading to TSGs hyper-methylation. Promoter hyper-methylation of TSGs is important in tumorigenesis. CagA the important gene of *H. pylori*, could increase AKT phosphorylation by activated PDK1. Phosphorylated AKT (P-AKT) induces *DNMT1* expression by NFκβ activation. Therefore, NFκβ could play a role in up-regulating the expression of *DNMT* by binding directly to its promoter [111]. *H. pylori* infection can also induce IL-1β as an important pro-inflammatory cytokine that has considerable function in

initiating and amplifying the inflammatory response. Increased IL-1β is followed by DNMT induction by activated inducible nitric oxide synthase (iNOS) and NO production which have an important role in aberrant DNA methylation and gastric carcinogenesis [103, 119-120]. *H. pylori* infection may directly effect on gastric cells by inducing macrophages activation, which causes an increase of NO production in gastric cells. Overproduction of NO can activate DNMTs to promote DNA methylation [121]. *H. pylori* infection through increased expression of IL-6 and IL-11 can activate signal transducer and activator of transcription 3 (STAT3) [122]. STAT3 can affect gene expression through epigenetic changes including DNA methylation and chromatin modulation. This transcription factor increases CpG island methylation of TSGs through interacting with DNMT1 [123].

Interestingly, aberrant DNA methylation is strongly induced even in normal tissues by exposure to chronic inflammation due to *H. pylori* infection. Maekita et al. found that some CpG islands regions were significantly highly methylated in gastric mucosae of *H. pylori*-infected individuals in comparison with non-infected individuals [124]. Moreover, methylation levels of three tumor-suppressor miRNAs were found to increase in non-cancerous individuals infected with *H. pylori* in comparison with non-infected healthy subjects [125]. Furthermore, *in vivo* study indicated that inflammations triggered by *H. pylori* infection has a strong potential to induce aberrant DNA methylation in gastric epithelial cells [126]. Inflammation due to *H. pylori* infection induced the expression of chemokine (C-X-C motif) ligand 2 (*CXCL2*), *IL-1b*, *NOS2*, and tumor necrosis factor-alpha (*TNF-α*) genes, in parallel to DNA methylation [126-127]. Accumulation of aberrant DNA methylation in gastric epithelial cells due to inflammatory response associated with *H. pylori* infection may favor cancerogenesis [126].

In addition, TAMs can induce *DNMT1* expression through CCL5/CCR5/STAT3 signaling pathway in gastric cancer cells. Overexpression of *DNMT1* mediated by TAMs also induces gelsolin silencing and may lead therefore to gastric cancer progression [128-131]. The molecular mechanism of aberrant DNA methylation caused by EBV infection is not still clear.

However, at least 2 mechanisms by which EBV can effect DNA methylation have been proposed. One mechanism is that viral latent membrane protein 2A (LMP2A) can increase the phosphorylation of STAT3, which up regulates the *DNMT1* and *DNMT3b* expression and cause epigenetic changes in gastric cells [132-133]. Also, EBV can induce *DNMT1* expression through the viral oncoprotein LMP1 via JNK/AP1-signaling. LMP1 has two main regions called C-terminal activating region (CTAR1 and CTAR2). The YYD domain in CTAR2 can play an important role in activating *DNMT1* gene expression. The YYD domain activates JNK kinase and upon phosphorylation of c-Jun, in turn c-Jun binds to AP1 site of *DNMT1* promoter [134].

Polymorphisms in *DNMTs* and gastric cancer development

Accumulating evidence demonstrate that genetic variations of DNMTs particularly single nucleotide polymorphisms (SNPs), and their haplotype blocks are associated with the occurrence rate of many cancers including gastric cancer [135]. SNPs may be responsible for promoter activity alteration, gene expression modulation, splice site change, transcription factor binding site change, and epigenetic modification [136]. Conspicuously, finding relevant polymorphisms can be used as a potential biomarker for gastric cancer prediction. Wang et al. indicated that the GG genotype of *DNMT3A* rs1550117 variant decreased the death risk of gastric cancer, and may therefore be a potential prognostic marker in gastric cancer [137]. Many studies have described the association of two common polymorphisms rs1569686 (-579G>T) and rs2424913 (-149C>T) on the promoter of *DNMT3B* which alter the promoter activity [138-139]. However, there is conflicting evidence on the contribution of these two SNPs in different cancers [140-143]. Hu et al. [144] and Chen et al. [145] reported the association of rs1569686 with gastric cancer risk in the Chinese population, while Wang et al. [146] and Ahmadi et al. [147] did not observe any significant association in the Chinese and

Iranian population, respectively. Likewise, other studies demonstrated that rs2424913 was irrelevant to the risk of gastric carcinogenesis [144-145, 148-150]. Moreover, haplotype analysis showed that a haplotype block between rs1569686 and rs2424913 in *DNMT3B* locus [145] which carries -149T/-579T increased the susceptibility to gastric cancer. However, few other studies have shown no significant association between *DNMT*s variants and the risk of gastric cancer development. Table 2 summarizes the associated and non-associated variants of *DNMT*s with the risk of gastric carcinogenesis. Some studies have found that *DNMT*s variants can alter the survival rate of gastric cancer. Correspondingly, TG/GG genotypes of *DNMT3B* rs1569686 and AG/AA genotypes of *DNMT3A* rs1550117 were associated with poor survival of gastric cancer [137, 146], while GA/AA genotypes of *DNMT1* rs2228611 were associated with higher rates of gastric cancer survival [151]. The effect of *H. pylori* infection as the main cause of gastric atrophy and gastric cancer, depends on host characteristics such as SNPs of *DMNT*s [152]. However, few studies have investigated the correlation between *H. pylori* infection with *DNMT*s SNPs in gastric cancer occurrence. Relatively, Jiang et al. [152] studied *DNMT1* polymorphisms and demonstrated that the AA genotype of rs2228349 had higher risk of *H. pylori* infection whereas, the GG genotype of rs10420321 and the CC genotype of rs8111085 had lower risk of *H. pylori* infection but a higher risk for gastric atrophy susceptibility [152]. Furthermore, Cao et al. [153] found that the AA genotype of rs1550117 in *DNMT3A* had higher risk of *H. pylori* infection, but they did not observe any association of this SNP with gastric cancer and gastric atrophy. Among the studied SNPs, the 3'UTR SNPs can be recognized by miRNAs. Nevertheless, the key impact of miRNAs on posttranscriptional regulation of gastric cancer regulator genes via binding with their target sites on 3'UTR, and the influence of polymorphisms in miRNAs target sites haven't been studied yet. Therefore, we looked for miRNA target sites alteration through studied *DNMT*s SNP via bioinformatics tools (DIANA-microT v5.0 [159], PolymiRTS Database 3.0 [160], miRNASNP v2.0 [161],

and RNAhybrid v2.1.2 software) to enlighten the molecular mechanisms that underlie the effect of *DNMT*s variants in gastric cancer. Yang et al. reported that *DNMT3A* rs13420827 is associated with gastric cancer risk [150], which was in disagreement with the results of two earlier studies [137, 153]. Bioinformatics tools revealed that rs13420827 is potentially targeted by 3 different miRNAs which are depicted in figure 3. The C allele generates putative miR-24-3p and miR-4263 target sites while it disrupts miR-574-3p target site. MiR-574-3p participates in several cancers including colorectal, breast, lung, liver, and prostate cancer as a tumor suppressor. Su et al. used miRNA microarray and quantitative real-time PCR techniques, and found that miR-574-3p expression was down-regulated in gastric cancer patients at early stage or higher levels of differentiation. They also demonstrated that miR-574-3p suppresses cell proliferation, migration, and invasion of gastric cancer cells [162]. Recently, they also demonstrated that TGF-β1-treated AGS cells show miR-574-3p up-regulation through binding of Mothers against decapentaplegic homolog 4 (SMAD4) to miR-574-3p promoter which might mediate the inhibition of cell proliferation in AGS cells by TGF-β1 [163]. Moreover, mir-24-3p was demonstrated to be involved in gastric cancer inhibition and apoptosis induction, suggesting a tumor suppressive role for this miRNA. [164]. Thanks to the importance of SNPs located in crucial genes like *DNMT*s, further studies might validate their potential effect as a biomarker for early detection of gastric cancer.

Dysregulation of *DNMTs* in gastric cancer

The 5-year relative survival rate for gastric cancer patients is 10–20%, and the detection of the tumor at a late stage could be associated with poor prognosis and metastases in gastric cancer [165]. Mutation in *DNMTs* can cause some inherited diseases. *DNMT1* mutations are associated with autosomal dominant cerebellar ataxia-deafness and narcolepsy (ADCA-DN), and hereditary sensory neuropathy with dementia and hearing loss [166]. ADCA-DN is a nervous system disorder with late onset (30-40 years old) caused by mutations in the C-

terminus end of the *DNMT1* gene. Mutations within the targeting-sequence domain of *DNMT1* cause hereditary sensory neuropathy with dementia and hearing loss [167]. Generally, *DNMT* inhibition prevents DNA replication. Knock down of *DNMT1* dysregulates *P21* (a cyclin dependent kinase inhibitor 1, tumor suppressor) and the apoptosis inducer *BIK* (BCL2-interacting killer) [168]. Another study shows that a cascade of genotoxic stress checkpoint proteins activates and induces cell cycle arrest following knock down of *DNMT1* [168]. Mutations in *DNMT3A* may cause acute myeloid leukemia (AML) by epigenetic reactivation of the leukemogenic factor MEIS1 (myeloid ecotropic viral integration site 1 homolog) [169] Knockdown of *DNMT3a* was shown to inhibit embryonic cardiomyocytes function [170]. Also, depletion of *DNMT3A* may speed up lung tumor progression [171]. Aberrant promoter methylations found during early tumorigenesis are promising biomarkers for screening, early detection, and prognosis of cancer [172]. It has been indicated that hypermethylation of suppressor of cytokine signaling 1 (*SOCS-1*) and death associated protein kinase 1 (*DAPK*) correlated with tumor stage [58, 173-174]. Also, Asada et al. used methylation level of *miR-124a-3*, *EMX1*, and *NKX6-1* to predict the risk of metachronous gastric cancer after endoscopic resection [175]. It seems that a better knowledge of genes involved in epigenetic alterations in gastric cancer can pave the way for designing an informative epigenetic biomarker panel for early detection of gastric cancer, and reduce mortality similar to what was accomplished in the bladder and lung cancers [176-177]. A series of methylated genes and correlation with clinical outcomes in gastric cancer are indicated in figure 4.

DNMTs as target in gastric cancer chemotherapy

One of the main treatment strategies for gastric cancer is chemotherapy. Commonly used chemo drugs for gastric cancer treatment include 5-FU (fluorouracil), cisplatin, irinotecan, and oxaliplatin [178]. The main problem with using this therapeutic approach is drug resistance.

Drug resistance in cancer is related to various factors such as epigenetic changes, mutations, signaling pathways, and microenvironmental alterations. Methylation pattern alterations play important roles in response to treatment, and patient survival [179]. Many evidence showed the positive correlation between *DNMTs* expression levels especially *DNMT1* and *DNMT3b*, with hypermethylation of CpG islands in gastric cancer [14]. Hypermethylated genes involved in chemotherapy resistance in gastric cancer are represented in figure 5. These genes are mainly involved in cell cycle regulation, genomic instability, epithelial-mesenchymal transition, apoptosis, and tumor suppression to eventually escape programmed cell death and acquire chemo-resistance (Fig 5).

Regarding the importance of DNA methylation in gastric cancer progression, anticancer drug sensitivity, and gastric cancer patient survival, DNMT inhibitors (DNMTis) can be useful in promoting the quality of treatment. Correspondingly, *DNMT1* knockdown by short hairpin RNA in gastric cancer cell line led to increased chemo-sensitivity [180]. There are few studies on combining DNMTis with chemotherapy for gastric cancer [181-182]. However, the results are optimistic to accelerate remedy for this cancer by synergistic effect of DNMTis and anticancer drugs. Two clinical trials in phase I and phase II, focus on gastric cancer treatment with DNMTis [183-184]. In these clinical trials, researchers investigated the pretreatment with 5-azacytidine as hypomethylating agent in advanced gastrointestinal cancer [183]. They used 5-azacitidine (V) prior to EOX (epirubicin, oxaliplatin, capecitabine) neoadjuvant chemotherapy in gastric and esophageal adenocarcinoma. This study showed a hypomethylation of tumor-associated loci such as hyperpigmentation progressive 1 (*HPP1*), tissue inhibitor of metalloproteinases 3 (*TIMP3*), *CDKN2A*, estrogen receptor 1 (*ESR1*), and O-6-methylguanine-DNA methyltransferase (*MGMT*). Neoadjuvant VEOX treatment was well-tolerated in all patients with significant clinical and epigenetic responses, with preliminary evidence that priming with V prior to chemotherapy may augment chemotherapy efficacy.

DNA hypomethylation of tumor loci was seen at all dose levels, with greater staining for *HPP1* in the resected specimen compared with the pre-treatment specimen. Moreover, the expression of *HPP1* as TSG marker was induced during neoadjuvant chemotherapy. 6 out of 12 treated patients became disease-free, had complete histologic response, and remained alive. [184]. DNMTis are divided into three general categories which include nucleoside analogs, non-nucleoside analogs, and nucleic acid-based components (Fig 6).

5-azacytidine and 5-aza-2'-deoxycytidine (decitabine) are chemical analogs of cytidine, and important DNMTi drugs approved by FDA for myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and chronic myelomonocytic leukemia (CMML) [185]. Many studies used azacytidine and decitabine as DNMTi for improving the treatment of gastric cancer alone or in combination with chemotherapy drugs such as 5-FU and cisplatin. Results of these studies demonstrate that azacytidine induces apoptosis and inhibits proliferation in gastric cancer cells, and improves the sensitivity of gastric cancer cells to 5-FU. Also, azacytidine upregulates death associated protein kinase 2 (*DAPK2*), *DAPK3*, *RASSF1*, and *THBS1* genes that might be associated with the synergistic effect of chemotherapy. [186-188]. Other types of non-nucleoside analog or nucleic acid-based DNMTi's such as procainamide, procaine [189], hydrazone-gallate [190], genistein [191], microRNA-21 [192], microRNA-335 [193], microRNA-148a [192, 194], and microRNA-155-5p [195] were also used for targeting gastric cancer cells with or without anticancer drug, but further studies are necessary before approving their clinical use. Due to the side effect of chemical drugs, it has been demonstrated that *DNMT*-targeted inhibition with small interfering ribonucleic acids (siRNAs) may be used as a novel approach for reactivation of silenced genes. In support of this claim Jung et al. indicated that *DNMT1* siRNAs inhibited cell proliferation and increased cell death rate in cancer cells without DNA damage, in comparison with azacytidine that increased DNA damage in human gastric adenocarcinoma cell lines [50, 196]. Interestingly, all of these DNMTis

enhanced the chemotherapy efficacy or programmed cell death in gastric cancer in *in vitro* and *in vivo* models. Table 3 summarizes DNMTis and their mechanism of action in gastric cancer.

Radiotherapy is another type of treatment which is commonly used for stage IV gastric cancer. In this method of treatment, the sensitivity and resistance of cells are important. Many studies showed the effects of epigenetic changes, especially methylation in cancer cells radioresistance [202]. Hypermethylation and inactivation of some genes involved in programmed cell death, cell cycle regulation, DNA repair, and TSGs can cause radiotherapy resistance in gastric cancer cells [188]. Treatment with 5-aza-2'-deoxycytidine (5-aza-CdR) showes a positive impact on gastric cancer cells radiosensitivity by increasing the expression of some genes such as *p53*, *RASSF1*, and *DAPK* [203]. Nowadays, combination of routine cancer therapy methods such as radiotherapy or chemotherapy with epigenetic modulation drug is a hot topic in cancer therapy. The epigenetic modulation drugs had a powerful potential to improve cancer treatment. DNA methylation pattern changing can lead to increase or decrease of the expression of genes involved in tumorigenicity, invasion, and programmed cell death. Some clinical and preclinical data detected the toxicity of epigenetic modulating drugs, but other evidence indicate the benefits of epigenetic modulating drugs to cancer remedy. Therefore, in order to achieve an efficient radiotherapy or chemotherapy combination with epigenetic modulating drugs as well as DNMTis, a deeper knowledge of molecular mechanisms, long-term safety, effective dose of usage, limitations, off-targeting, and side effects of these drugs should be attained.

Future Perspective

Gastric cancer remains one of the major causes of morbidity and mortality in the world. Disruption of epigenetic processes can cause abnormal activation of oncogenes or silencing of TSGs as a hallmark of gastric cancer. Studies on epigenetic alterations occurring before or during gastric cancer have been the subject of great interest and could become a starting point for the development of future therapeutics tools in gastric cancer. Among epigenetic modifications, DNA methylation plays an important role in the tumorigenesis of gastric cancer. Emerging evidence shows that cancer cells exhibit aberrant DNA methylation relative to normal cells during gastric cancer development. The DNMTs are responsible for DNA methylation, and have a central role in epigenetic control of gene expression. Up-regulation of *DNMTs* leads to the increased hypermethylation of genes, which contributes to the promotion of tumor growth, invasion, and metastasis. Infections such as EBV, *H. pylori*, and HPV may directly effect tumorigenesis through secretion of proteins to increase the *DNMTs* expression in gastric cancer. Also, infections indirectly via TAMs increase *DNMT1* expression. In addition to infections, some SNPs in *DNMTs* are associated with the survival rate of gastric cancer but little is known about miR-SNPs. Bioinformatics analysis revealed rs13420827 in the 3′-UTR of *DNMT3A* as part of a target site for three miRNAs including miR-24-3p, miR-4263, and miR-574-3p, which miR-24-3p and miR-574-3p have been found to be significantly associated with risk of gastric cancer. Moreover, epigenetic alterations are associated with chemo-resistance in gastric cancer. Interestingly, as DNA methylation is a reversible process, restoration of the aberrant epigenetic changes may represent a promising strategy to overcoming chemo-resistance. Therefore, DNMTis such as shRNAs, miRNAs, 5-azacytidine, and 5-aza-2'-deoxycytidine can be useful in treating gastric cancer multidrug resistance. Another approach for improving efficacy is to combine the DNMTis with chemo drugs. However, epigenetic inhibitors such as 5-azacytidine and 5-aza-2'-deoxycytidine are not a specific inhibitor of DNMTs. CRISPR-Cas9 (clustered regularly interspaced short palindromic

repeats-CRISPR associated nuclease 9) system has found a widespread use in biological and medical research, and becomes a promising strategy for editing DNA methylation. Recently, it has been indicated that CRISPR/Cas9 DNA methyltransferase fusion with a catalytically inactive Cas9 can lead to site-specific induction of DNA methylation [204-207]. Therefore, site-specific silencing of genes that contribute to the development of cancer via CRISPR/Cas9 system can be potentially superior to DNMTis, and appears to be a promising therapy in cancer patients.

Executive Summary

- DNA methyltransferases (DNMTs) consist of a catalytic and regulatory domain; the catalytic domain is conserved while the regulatory domain is variable.
- Aberrant expression of DNMTs is tightly associated with promoter hypermethylation of tumor suppressor genes.
- Infections such as EBV, HPV, and *H. pylori* facilitate DNMTs function by secreting oncogenic proteins or promoting differentiation and polarization of monocytes into tumor-associated macrophages.
- Genetic polymorphisms in DNMTs and their haplotype blocks may be potential biomarkers for gastric cancer prediction.
- Drug resistance in gastric cancer is triggered by hypermethylation of various genes.
- DNA hypomethylation of tumor loci via DNMT inhibitors may facilitate targeting gastric cancer multidrug resistance
- Site-specific DNA methylation through CRISPR/Cas9 system appears to be a promising targeting approach in cancerous cells.

Conflict of interest

Authors declare no conflict of interest.

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